

THE ESSENTIAL 'HOW TO..., WHEN TO...' GUIDE FOR CLINICIANS

OXFORD HANDBOOK OF CLINICAL AND LABORATORY INVESTIGATION

EDITED BY Drew Provan

Provides both a patient-centred and specialty-centred approach to investigation of symptoms and signs, with advice on all relevant tests and the pitfalls of interpreting results

Thoroughly revised, with latest investigations and tests added

Cross-referenced with the Oxford Handbook of Clinical Medicine for more detailed clinical management advice



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Clinical and
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Oxford Handbook of Tropical Medicine 4e
Oxford Handbook of Urology 3e

Oxford Handbook of Clinical and Laboratory Investigation

Fourth Edition

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To Richard and Fraser.

Foreword

This book fills an important gap in the market, being a comprehensive guide to the requesting and interpretation of a wide range of diagnostic tests. The authors have crammed a huge amount of information into a relatively small volume. Its size, scope, and relevance mean that it is likely to be used daily as a quick reference and aide-memoire. This fourth edition, which has been entirely updated, covers conditions from the very common, such as nausea and joint pain, to those seen less often. The fact that it is written by experienced clinicians, including trainees, is evident from its practical approach and focus on the patient.

This book highlights the importance, often forgotten, of diagnostic tests in almost all patient care pathways. Its use will ensure that the right investigations are done first time, reducing unnecessary testing and enabling faster and more accurate diagnosis. I am particularly pleased that it contains a section on collecting specimens and how to avoid laboratory errors.

No medical student or junior doctor should be without this book (it is ideal for revision); in fact, any doctor at any stage of their career will find it useful. The appropriate requesting and interpretation of clinical and laboratory investigations is vital for maximizing the value of healthcare and improving the quality of care for patients.



Suzy Lishman
President of The Royal College of Pathologists
2018

Preface to the fourth edition

Six years have elapsed since the third edition of this book was published and during that time there have been advances in investigative techniques, both laboratory-based and clinical. My own specialty, haematology, has seen refinements in diagnostic tests for conditions such as leukaemias and lymphomas, but there have also been developments in the red cell and clotting arenas. My colleagues in other clinical specialties have also enjoyed advances within their own disciplines, and in order to make the book truly contemporary, we have had to update all sections of the book bringing in all of these new techniques.

As before, I have had the privilege to work with leaders in all branches of medicine who have given up their time to update their chapters, bringing them right up-to-date, and I am immensely grateful to them.

I am also indebted to Oxford University Press for their tireless work on this Oxford Handbook which has been used by clinicians worldwide for 14 years. It has grown from 600 pages to almost 1000 in that time! If this small book has helped in the diagnosis of patients, then I feel we have achieved our task. Special thanks go to Michael Hawkes, Elizabeth Reeve, and many others who have helped bring this book to publication.

Being an edited text, I take responsibility for errors or omissions in the book and welcome any comments readers may have. As ever, this book is meant to be used at the bedside and in the clinic, and its usability relies on input from readers. Please contact me at drewprovan@mac.com if you have any suggestions or spot any errors in the book.

Drew Provan
2018

Preface to the first edition

With the increasing complexity of modern medicine, we now have literally thousands of possible investigative techniques at our disposal. We are able to examine our patient's serum and every other body fluid down to the level of individual nucleotides, as well as being able to perform precise imaging through CT, MRI, and other imaging technologies. The problem we have all faced, especially as senior medical students or junior doctors is: Which test should we use in a given setting? What hazards are associated with the tests? Are there any situations where specific tests should not be used or are likely to produce erroneous results? As medical complexity increases, so too does cost; many assays available today are highly expensive and, wherever possible, we would ideally like to use a test that is cheap, reliable, reproducible, and right for a given situation.

Such knowledge takes many years to acquire and it is a fact of life that senior doctors (who have attained such knowledge) are not usually those who request the investigations. In this small volume, we have attempted to distil all that is known about modern tests, from blood, urine, and other body fluids, along with imaging and molecular tests. The book is divided into two principal parts: the first deals with symptoms and signs in *The patient* section, because that is how patients present. We have tried to cover as many topics as possible, discussing these in some detail and have provided differential diagnoses where possible. We also try to suggest tests that might be of value in determining the cause of the patient's symptom or sign. The second part of the book *Investigations* is specialty-specific and is more relevant once you know roughly what type of disease the patient might have. For example, if the symptom section suggests a likely respiratory cause for the patient's symptoms, then the reader should look to the *Respiratory medicine* chapter in order to determine which tests to carry out or how to interpret the results.

The entire book is written by active clinicians, rather than scientists, since we wanted to provide a strong clinical approach to investigation. We have tried, wherever possible, to cross-refer to the *Oxford Handbook of Clinical Medicine*, Oxford University Press, which provides the clinical detail omitted from this handbook. The symbol  is used to highlight a cross-reference to OHCM, in addition to cross-referencing within this book.

We would value feedback from readers since there will doubtless be tests omitted, errors in the text, and many other improvements we could, and will, make in future editions. All contributors will be acknowledged individually in the next edition. We would suggest you e-mail us directly.

Drew Provan
Andrew Krentz
2002

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Symbols and abbreviations

~	approximately
α	alpha
β	beta
δ	delta
γ	gamma
κ	kappa
λ	lambda
→	leading to
↻	cross-reference
↑	increased
↓	decreased
↔	normal
🌐	website
►	important
►►	very important
>	greater than
≥	equal to or greater than
>>	much greater than
<	less than
≤	equal to or less than
=	equal to
+ve	positive
-ve	negative
°	degree
°C	degree Celsius
1°	primary
2°	secondary
♂	male
♀	female
↑↑	greatly increased
±	plus or minus
2D	two-dimensional
3D	three-dimensional
β-TG	beta-thromboglobulin
γGT	gamma-glutamyl transpeptidase
5HIAA	5-hydroxyindole acetic acid

μg	microgram
μL	microlitre
μmol	micromole
$^{99\text{m}}\text{Tc}$	technetium-99m
AA	aortic arch
AAA	abdominal aortic aneurysm
AAFB	acid- and alcohol-fast bacilli
ABG	arterial blood gas
ABO	ABO blood groups
ABPA	allergic bronchopulmonary aspergillosis
AC	air conduction
ACD	anaemia of chronic disease
ACE	angiotensin-converting enzyme
ACEI	angiotensin-converting enzyme inhibitor
ACh	acetylcholine
AChE	red cell cholinesterase
AChRAb	acetylcholine receptor antibodies
ACL	anticardiolipin antibody
ACPA	anti-cyclic citrullinated peptide antibodies
ACR	albumin:creatinine ratio
ACS	acute coronary syndrome
ACTH	adrenocorticotrophic hormone
ADA	American Diabetes Association
ADC	apparent diffusion coefficient
ADH	antidiuretic hormone
ADP	adenosine 5-diphosphate
AF	atrial fibrillation
AFP	alpha-fetoprotein
AICD	automatic intracardiac defibrillation device
AIDS	acquired immune deficiency syndrome
AIH	autoimmune hepatitis
AIHA	autoimmune haemolytic anaemia
AIP	autoimmune profile
AKI	acute kidney injury
ALD	adrenoleucodystrophy
ALL	acute lymphoblastic leukaemia
ALP	alkaline phosphatase
ALT	alanine transaminase
AMA	antimitochondrial antibodies
AML	acute myeloid leukaemia

ANA	antinuclear antibodies
ANAE	alpha-naphthyl acetate esterase
ANCA	antineutrophil cytoplasmic antibody
ANNA	anti-neuronal nuclear antibodies
AP	anteroposterior; action potential
APCR	activated protein C resistance
APS	antiphospholipid syndrome
APTR	activated partial thromboplastin time ratio
APTT	activated partial thromboplastin time
APVD	anomalous pulmonary venous drainage
ARB	angiotensin II receptor blocker
ARDS	acute respiratory distress syndrome
ARF	acute renal failure
ARMS	amplification refractory mutation system
ASAS	Assessment of SpondyloArthritis International Society
ASCA	antibodies to <i>Saccharomyces cerevisiae</i>
ASIS	anterior superior iliac spine
ASMA	anti-smooth muscle antibodies
ASO	anti-streptolysin
ASOT	anti-streptolysin O titre
AST	aspartate transaminase
AT	antithrombin
AT-II	angiotensin II
ATN	acute tubular necrosis
AV	atrioventricular; arteriovenous
AVM	arteriovenous malformation
AVN	avascular necrosis
AVP	arginine vasopressin
AVPD	anomalous pulmonary venous drainage
AXR	abdominal X-ray
AZT	zidovudine
BAEP	brainstem auditory evoked potential
BAER	brainstem auditory evoked response
BAL	bronchoalveolar lavage
BC	bone conduction
BCG	bacillus Calmette–Guérin
bd	bis die (twice daily)
bDNA	branched-chain deoxyribonucleic acid
BIPLED	bihemispheric periodic lateralized epileptiform discharge
BIRADS	breast imaging and reporting data system

BJP	Bence–Jones protein
BM	bone marrow
BMI	body mass index
BMT	bone marrow transplant
BOLD	blood oxygen level
BP	blood pressure
bpm	beat per minute
BSAEP	brainstem auditory evoked potential
BSG	British Society of Gastroenterology
BSL	Biosafety level
BW	bronchial washing
C&S	culture and sensitivity
Ca ²⁺	calcium
Ca 19-9	carbohydrate antigen 19-9
Ca-125	cancer antigen 125
CAD	computer-assisted detection
CAH	congenital adrenal hyperplasia
cAMP	cyclic adenosine monophosphate
c-ANCA	cytoplasmic antineutrophil cytoplasmic antibody
CaSR	calcium-sensing receptor
CBC	complete blood count
CBD	common bile duct
CC	craniocaudal
CCF	congestive cardiac failure
CCK	cholecystokinin
CCP	cyclic citrullinated peptide
CCTA	coronary computed tomography angiography
CCU	coronary care unit
CD	cluster differentiation
CEA	carcinoembryonic antigen
cf.	compare with
CF	complement fixation
CFA	cryptogenic fibrosing alveolitis
CFU	colony-forming unit
CGMS	continuous glucose monitoring systems
CHD	coronary heart disease
Cho	choline
Chr	reticulocyte
CINCA	chronic infantile neurologic, cutaneous and articular syndrome
CJD	Creutzfeldt–Jakob disease

CK	creatinine kinase
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
Cl ⁻	chloride
CLL	chronic lymphocytic/lymphatic leukaemia
CLO	<i>Campylobacter</i> -like organism
cm	centimetre
CMAP	compound motor action potential
cmH ₂ O	centimetre of water
CML	chronic myeloid leukaemia
CMR	cardiovascular magnetic resonance
CMV	cytomegalovirus
C3Nef	C3 nephritic factor
CNS	central nervous system
CO	carbon monoxide
CO ₂	carbon dioxide
COHb	carboxyhaemoglobin
COPD	chronic obstructive pulmonary disease
cP	centipoise
CPAP	continuous positive airway pressure
CPE	carbapenemase-producing <i>Enterobacteriaceae</i>
CPK	creatinine phosphokinase
CPPD	calcium pyrophosphate disease
CPS	complex partial seizure
Cr	creatine
CrAg	cryptococcal antigen
CrC	creatinine clearance
CRC	colorectal carcinoma
CREST	calcinosis, Raynaud's syndrome, oesophageal motility dysfunction, sclerodactyly, and telangiectasia
CRH	corticotropin-releasing hormone
CRP	C-reactive protein
CSF	cerebrospinal fluid
CSU	catheter specimen of urine
CT	computed tomography
CTC	computed tomography colonography
CT-IVP	computed tomography intravenous pyelography
CTLp	cytotoxic T-lymphocyte precursor
CTPA	computed tomography pulmonary angiography
CTU	computed tomography urography

CVA	cerebrovascular accident (stroke)
CVD	cardiovascular disease
CVID	common variable immunodeficiency
CVP	central venous pressure
CVS	cardiovascular system; chorionic villus sampling
CW	continuous wave
CXR	chest X-ray
CyF	cystic fibrosis
DAT	direct antibody test
dB	decibel
DBCE	double contrast barium enema
DCCT	Diabetes Control and Complications Trial
DEC	diethylcarbamazine
DESS	dual-echo steady state
DEXA	dual-energy X-ray absorptiometry
DFa	direct fluorescein-labelled monoclonal antibody
DFA	direct fluorescent antibody
DHEAS	dehydroepiandrosterone sulfate
DI	diabetes insipidus
DIC	disseminated intravascular coagulation
DIDMOAD	diabetes insipidus, diabetes mellitus, optic atrophy, and deafness
DIF	direct immunofluorescence
DIP	distal interphalangeal joint
DJ	duodenojejunal
DKA	diabetic ketoacidosis
dl	decilitre
DLCO	diffusing lung capacity for carbon monoxide
DM	diabetes mellitus
DMSA	dimercaptosuccinic acid
DNA	deoxyribose nucleic acid
DOAC	direct-acting anticoagulant
DPLD	diffuse parenchymal lung disease
DRVVT	dilute Russell viper venom test
DSA	digital subtraction angiography
ds-DNA	double-stranded DNA
DSI	dimensionless severity index
DTI	diffusion tensor imaging
DTPA	diethylenetriaminepentaacetic acid

DU	duodenal ulcer
DVT	deep vein thrombosis
DWI	diffusion-weighted imaging
DXA	dual-energy X-ray absorptiometry
EBB	endobronchial biopsy
EBUS	endobronchial ultrasound
EBV	Epstein–Barr virus
ECG	electrocardiogram
EDC	estimated date of confinement
EDH	extradural haemorrhage
EDTA	ethylenediamine tetra-acetic acid
EEG	electroencephalogram
EF	ejection fraction
eGFR	estimated glomerular filtration rate
EGFR	epidermal growth factor receptor
EGPA	eosinophilic granulomatosis with polyangiitis
EIA	enzyme-linked assay
ELISA	enzyme-linked immunosorbent assay
EM	electron microscopy
EMA	endomysial antibody
EMG	electromyogram/electromyography
EMU	early morning urine
ENA	extractable nuclear antigen
EOG	electro-oculography
EP	evoked potential
Epo	erythropoietin
EQA	external quality assurance
ER	evoked response
ERCP	endoscopic retrograde cholangiopancreatography
ERF	established renal failure
ESA	erythropoiesis-stimulating agent
ESR	erythrocyte sedimentation rate
ESS	Epworth sleepiness scale
ETT	endotracheal tube
EUS	endoscopic ultrasound
Fab	antibody fragment
FACS	fluorescence-activated cell sorter
FAP	familial polyposis syndrome
FBC	full blood count
FBHH	familial benign hypocalciuric hypercalcaemia

FCHL	familial combined hyperlipidaemia
FDG	fluorodeoxyglucose
FDP	fibrin degradation product
FeNO	exhaled nitric oxide fraction
FEV	forced expiratory volume
FEV ₁	forced expiratory volume in 1 second
FFP	fresh frozen plasma
FGF	fibroblast growth factor
FH	familial hypercholesterolaemia
FiO ₂	inspired oxygen concentration
FISH	fluorescence <i>in situ</i> hybridization
fL	femtolitre
FLAIR	fluid attenuation inversion recovery
fMRI	functional magnetic resonance imaging
FNA	fine-needle aspirate/aspiration
FNH	focal nodular hyperplasia
FOB	faecal occult blood
FPG	fasting plasma glucose
Fr	French
FRC	functional residual capacity
FSH	follicle-stimulating hormone
FT4	free T4
FTA-ABS	fluorescent treponemal antibody absorption
FUO	fever of unknown origin
FVC	flow–volume curve; forced vital capacity
FVL	factor V Leiden
g	gram
GABA	gamma-aminobutyric acid
GAD	glutamic acid decarboxylase
GA _n	general anaesthetic
GBM	glomerular basement membrane
GBS	Guillain–Barré syndrome
GC	gas chromatography
GCA	giant cell arteritis
GC-MS	gas chromatography mass spectrometry
GCS	Glasgow Coma Scale
Gd	gadolinium
GFR	glomerular filtration rate
GGT	gamma glutamyl transpeptidase
GH	growth hormone

GHRH	growth hormone-releasing hormone
GI	gastrointestinal
GIT	gastrointestinal tract
GLC	gas-liquid chromatography
GM-CSF	granulocytic macrophage colony-stimulating factor
GnU	genitourinary
GORD	gastro-oesophageal reflux disease
GPA	granulomatosis with polyangiitis
GPC	gastric parietal cell
G6PD	glucose-6-phosphate dehydrogenase
GPI	glycosyl phosphatidyl inositol
GRA	glucocorticoid remediable aldosteronism
G&S	group and save
GT	glucose tolerance
GTN	glyceryl trinitrate
GTT	glucose tolerance test
GU	gastric ulcer
GvHD	graft-versus-host disease
h	hour
HAART	highly active antiretroviral therapy
HACEK	<i>Haemophilus</i> species, <i>Actinobacillus actinomycetemcomitans</i> , <i>Cardiobacterium hominis</i> , <i>Eikenella corrodens</i> , and <i>Kingella</i> species
HAV	hepatitis A virus
Hb	haemoglobin
HbA _{1c}	haemoglobin A _{1c}
HbC	haemoglobin C
HbD	haemoglobin D
HbE	haemoglobin E
HbF	fetal haemoglobin
HbH	haemoglobin H
HbO	oxyhaemoglobin
HbS	sickle haemoglobin
HbSC	haemoglobin SC
HBV	hepatitis B virus
HC	haemoglobin content
hCG	human chorionic gonadotrophin
HCO ₃ ⁻	bicarbonate
Hct	haematocrit
HCV	hepatitis C virus
HD	Hodgkin's disease

HDFN	haemolytic disease of the fetus and newborn
HDL	high-density lipoprotein
HDN	haemolytic disease of the newborn
HELLP	haemolysis, elevated liver enzymes, and low platelet count
HEMPAS	hereditary erythroblastin multinuclearity with positive acidified serum lysis test
HEV	hepatitis E
HHT	hereditary haemorrhagic telangiectasia
HHV	human herpesvirus
Hib	<i>Haemophilus influenzae</i> type B
HIE	hypoxic–ischaemic encephalopathy
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HLH	haemophagocytic lymphohistiocytosis syndrome
HMPAO	hexamethyl-propylene-amine-oxime
HMSN	hereditary motor sensory neuropathy
HNA	heparin neutralizing activity
HNF	hepatic nuclear factor
HNPPCC	hereditary non-polyposis colorectal carcinoma
HNPP	hereditary neuropathy with liability to pressure palsies
HONK	hyperosmolar non-ketotic
Hp	haptoglobin
HPA	hybridization protection
HPFH	hereditary persistence of fetal haemoglobin
HPLC	high-performance liquid chromatography
HPOA	hypertrophic pulmonary osteoarthropathy
HPV	human papillomavirus
HRC	hypochromic red cell
HRCT	high-resolution computed tomography
HRP-2	histidine-rich protein 2
HRT	hormone replacement therapy
hsTnI	high-sensitivity troponin I
hsTnT	high-sensitivity troponin T
HSV	herpes simplex virus
HTLV	human T-lymphotropic virus
HU	Hounsfield unit
HUS	haemolytic uraemic syndrome
HUVS	hypocomple mentemic urticarial vasculitis syndrome
Hz	hertz
IABP	intra-aortic balloon pump

IAT	indirect antiglobulin test
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
ICA	islet cell antibodies
ICD	internal cardioverter–defibrillator
ICM	insertable cardiac monitor
ICP	intracranial pressure
ICPMS	inductively coupled plasma mass spectroscopy
ICU	intensive care unit
IDA	iminodiacetic acid
IDDM	insulin-dependent (type 1) diabetes mellitus
IDMS	isotope dilution mass spectrometry
IEF	isoelectric focusing
IF	intrinsic factor
IFA	intrinsic factor antibodies
IFCC	International Federation of Clinical Chemistry
IFG	impaired fasting glucose
IFT	immunofluorescence test
Ig	immunoglobulin
IgA	immunoglobulin A
IgD	immunoglobulin D
IgE	immunoglobulin E
IGE	idiopathic generalized epilepsy
IGF	insulin-like growth factor
IgG	immunoglobulin G
IgM	immunoglobulin M
IGRA	interferon gamma release assays
IGT	impaired glucose tolerance
IHD	ischaemic heart disease
IIH	intracranial hypertension
IL	interleukin
ILD	interstitial lung disease
ILR	implantable loop recorder
IM	intramuscular
IMN	idiopathic membranous nephritis
INR	international normalized ratio
INR/PT	international normalized ratio/prothrombin time
IPSS	inferior petrosal sinus sampling
IQ	intelligence quotient
ITP	idiopathic thrombocytopenic purpura

ITT	insulin tolerance test
ITU	intensive therapy unit
IU	international unit
IUP	intrauterine pregnancy
IV	intravenous
IVC	inferior vena cava
IVI	intravenous infusion
IVP	intravenous pyelography
IVU	intravenous urogram
JME	juvenile myoclonic epilepsy
JVP	jugular venous pressure; jugular venous pulse
K ⁺	potassium
kBq	kilobecquerel
KCCT	kaolin cephalin clotting time
KCO	transfer coefficient for carbon monoxide
kDa	kilodalton
KDIGO	Kidney Disease Improving Global Outcomes
kg	kilogram
kPa	kilopascal
KSHV	Kaposi's sarcoma-associated herpesvirus
kU	kilounit
KUB	kidney, ureter, bladder (X-ray)
kV	kilovoltage
L	litre
LA	lactic acidosis
LADA	latent autoimmune diabetes of adults
LAN	local anaesthetic
LAP	leucocyte alkaline phosphatase; left atrial pressure
LAt	left atrial
Ib	pound
LC	liver cytosol
LCM	left costal margin
LC-MS/MS	liquid chromatography tandem-mass spectrometry
LC/Q-TOF-MS	liquid chromatography/quadrupole time-of-flight mass spectrometry
LCR	ligase chain reaction
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LDST	low-dose dexamethasone suppression test
LEMS	Lambert–Eaton myasthenic syndrome

LFT	liver function test
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
LIF	left iliac fossa
LKM	liver–kidney microsomal
LMN	lower motor neurone
LOC	loss of consciousness
LP	lumbar puncture
LPL	lipoprotein lipase
LSCC	lateral semicircular canal
LTOT	long-term oxygen therapy
LUQ	left upper quadrant
LV	left ventricle
LVEDP	left ventricular end-diastolic pressure
LVF	left ventricular failure
m	metre
MAA	macroaggregated albumin
MAG	myelin-associated glycoprotein
MAG3	mercaptoacetyl-triglycine
MAHA	microangiopathic haemolytic anaemia
MAIPA	monoclonal antibody immobilization of platelet antigens
MALDI	matrix-assisted laser desorption/ionization
MALDI-TOF MS	matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MALT	mucosa-associated lymphoid tumour
MAOI	monoamine oxidase inhibitor
MARS	Molecular Absorbance Recirculating System
MBq	megabecquerel
MCH	mean cell haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCP	metacarpophalangeal
M,C&S	microscopy, culture, and sensitivity
MCUG	micturating cystourethrogram
MCV	mean cell volume
MCVm	mutated citrullinated vimentin
MD	myotonic dystrophy
MDMA	methylene dioxymethamphetamine or ecstasy
MDR	multi-drug-resistant
MDRD	Modification of Diet in Renal Disease
MDR-TB	multi-drug-resistant tuberculosis

MDS	myelodysplastic syndrome
MELAS	mitochondrial myopathy, lactic acidosis, and stroke-like episodes
MEN	multiple endocrine neoplasia
MEP	motor evoked potential
mEq	milliequivalent
MERRF	myoclonic epilepsy and ragged red fibres
MERS-CoV	Middle East respiratory syndrome coronavirus
MetHb	methaemoglobin
mg	milligram
Mg ²⁺	magnesium
MG	myasthenia gravis
MGUS	monoclonal gammopathy of undetermined significance
MHC	major histocompatibility complex
MHz	megahertz
MI	myocardial infarction
MIBG	iodine-131-meta-iodobenzylguanide
MIC	minimum inhibitory concentration
min	minute
m-In	myo-inositol
mlU	milli-international unit
mL	millilitre
MLC	mixed lymphocyte culture
MLO	mediolateral oblique
mm	millimetre
mmHg	millimetre of mercury
M-mode	motion-mode
mmol	millimole
MND	motor neurone disease
MoAb	monoclonal antibody
MODY	maturity-onset diabetes of the young
mOsmol	milliosmole
MP	metacarpophalangeal
mPa	millipascal
MPD	myeloproliferative disease
MPHR	max predicted heart rate
MPI	myocardial perfusion imaging
MPO	myeloperoxidase
MPV	mean platelet volume
MR	magnetic resonance

MRA	magnetic resonance angiogram
MRC	Medical Research Council
MRCP	magnetic resonance cholangiopancreatography
MRD	minimal residual disease
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRS	magnetic resonance spectroscopy
MRSA	meticillin-resistant <i>Staphylococcus aureus</i>
MRV	magnetic resonance venography
ms	millisecond
MS	multiple sclerosis
MSD	mean sac diameter
MSU	midstream urine
mSv	millisievert
MTP	metatarsophalangeal
mU	milliunit
MUGA	multigated radionuclide angiography
MUP	motor unit potential
MuSK	muscle-specific kinase
mV	millivolt
Na ⁺	sodium
NAA	<i>N</i> -acetyl-aspartate
NAC	<i>N</i> -acetylcysteine
NAG	<i>N</i> -acetyl-D-glucosaminidase
NAP	neutrophil alkaline phosphatase
NASBA	nucleic acid sequence-based amplification
NCS	nerve conduction studies
NCSE	non-convulsive status epilepticus
NEC	necrotizing enterocolitis
NET	neuroendocrine tumour
ng	nanogram
NH ₃	ammonia
NH ₄ ⁺	ammonium
NHL	non-Hodgkin's lymphoma
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIDDM	non-insulin-dependent diabetes mellitus
NK	natural killer
nm	nanometre
nmol	nanomole

NMS	neuroleptic malignant syndrome
NO	nitric oxide
NOGG	National Osteoporosis Guideline Group
NPA	nasopharyngeal aspirate
NPH	normal pressure hydrocephalus
NPSA	National Patient Safety Agency
NREM	non-rapid eye movement
NSAID	non-steroidal anti-inflammatory drug
NSF	nephrogenic systemic fibrosis
NSTEMI	non-ST-elevation myocardial infarction
O ₂	oxygen
OA	osteoarthritis
Oc	calculated osmolality
OCB	oligoclonal band
OCP	oral contraceptive pill
OGD	oesophagogastroduodenoscopy
OGTT	oral glucose tolerance test
OHCM	<i>Oxford Handbook of Clinical Medicine</i>
Om	measured osmolality
OMIM	Online Mendelian Inheritance in Man
OSA	obstructive sleep apnoea
OTC	over the counter
PA	pernicious anaemia
P-A	posteroanterior
PABA	para-amino benzoic acid; <i>N</i> -benzoyl- <i>L</i> -tyrosol <i>p</i> -aminobenzoic acid
P _a CO ₂	partial pressure of carbon dioxide in arterial blood
PACS	picture archiving and communication systems
PAD	peripheral arterial disease
PAN	polyarteritis nodosa
p-ANCA	perinuclear antineutrophil cytoplasmic antibody
P _a O ₂	arterial oxygen tension
PAS	periodic acid-Schiff
PB	peripheral blood
PBC	primary biliary cirrhosis
PC	protein C; provocation concentration
PCH	paroxysmal cold haemoglobinuria
PCI	percutaneous coronary intervention
PCNA	proliferating cell nuclear antigen
PCO ₂	partial pressure of carbon dioxide

PCP	<i>Pneumocystis jiroveci</i> pneumonia
PCR	polymerase chain reaction
PCrR	protein:creatinine ratio
PCT	procalcitonin
PCV	packed cell volume
PD	Parkinson's disease
PDW	platelet distribution width
PE	pulmonary embolism/embolus
PEFR	peak expiratory flow rate
PEG	percutaneous endoscopic gastrostomy
PET	positron emission tomography
PFR	peak flow rate
pg	picogram
PICC	peripherally inserted central catheter
PIFT	platelet immunofluorescence test
PIP	proximal interphalangeal
PK	pyruvate kinase
PkS	Parkinson's syndrome
PLA-2	phospholipase A2
PLED	periodic lateralized epileptiform discharge
PLMD	paroxysmal leg movement disorder
PmA	pulmonary artery
PMA	paramethoxymethamphetamine
PMF	progressive massive fibrosis
PMLE	progressive multifocal leukoencephalopathy
PNH	paroxysmal nocturnal haemoglobinuria
PNS	peripheral nervous system
PO	<i>per os</i> (by mouth)
PO ₂	partial pressure of oxygen
PO ₄ ³⁻	phosphate
POTS	postural orthostatic tachycardia syndrome
PP	pancreatic polypeptide
ppb	parts per billion
PPD	purified protein derivative
PR	<i>per rectum</i>
PR3	proteinase 3
PRA	plasma renin activity
PrC	provocation concentration
PRL	prolactin
PRV	polycythaemia rubra vera

PS	protein S
PSA	prostate-specific antigen
PSMA	prostate-specific membrane antigen
PT	prothrombin time
PTA	percutaneous transluminal angioplasty
PTC	percutaneous transhepatic cholangiogram
PTH	parathyroid hormone
PTR	prothrombin ratio
PTTK	partial thromboplastin time with kaolin
PUO	pyrexia of unknown origin
PV	plasma volume
PVA	polyvinyl alcohol
PvC	provocation concentration
PVNS	pigmented villonodular synovitis
PW	pulsed wave
PWI	perfusion-weighted imaging
PxCT	proximal convoluted tubule
qds	<i>quarter die sumendum</i> (four times daily)
RA	refractory anaemia
RAPA	rheumatoid arthritis particle agglutination test
RAS	renal artery stenosis
RAST	radioallergosorbent test
RAt	right atrial/atrium
RBC	red blood cell
RBP	retinol-binding protein
RCC	red cell count
RDT	rapid diagnostic test
RDW	red cell distribution width
REM	rapid eye movement
RES	reticuloendothelial system
Ret-He	reticulocyte haemoglobin content
RF	rheumatoid factor
RFLP	restriction fragment length polymorphism
Rh	rhesus
RhA	rheumatoid arthritis
RhMK	Rhesus monkey kidney
RIA	radioimmunoassay
RiCoF	Ristocetin Cofactor
RID	radial immunodiffusion
RIF	right iliac fossa

RIPA	ristocetin-induced platelet aggregation
RIS	radiology information system
RNP	ribonucleoprotein
RNV	radionuclide ventriculography
RPR	rapid plasma reagins
RSV	respiratory syncytial virus
RTA	renal tubular acidosis
rTMS	repetitive transcranial magnetic stimulation
RUQ	right upper quadrant
RV	right ventricle; residual volume
s	second
SAA	serum amyloid A
SAAG	serum ascites albumin gradient
SAH	subarachnoid haemorrhage
SaO ₂	arterial oxygen saturation
SARS	severe acute respiratory syndrome
SB	Sudan black
SBE	subacute bacterial endocarditis
SBO	small bowel obstruction
SBP	spontaneous bacterial peritonitis
sbt	serum bactericidal test
SC	subcutaneous
SCC	squamous cell carcinoma
SCID	severe combined immunodeficiency
SCLC	small-cell lung cancer
SDH	subdural haemorrhage
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SeHCAT	⁷⁵ selenium homotaurocholate
SG	specific gravity
SGLT2	sodium–glucose cotransporter 2
SHBG	sex hormone-binding globulin
SI	Système International
SIADH	syndrome of inappropriate antidiuretic hormone
SLA	soluble liver antigen
SLE	systemic lupus erythematosus
SMA	smooth muscle antibody
SNAP	sensory nerve action potential
SO ₄ ²⁻	sulfate
SOB	shortness of breath
SOD	sphincter of Oddi dysfunction

SOL	space-occupying lesion
SPECT	single photon emission computed tomography
SpO ₂	oxygen saturation
SSFP	steady-state free precession
SSP	single strand polymorphism
SSPE	subacute sclerosing panencephalitis
SSR	somatostatin receptor
STD	sexually transmitted disease
STEMI	ST-elevation myocardial infarction
sTfR	serum transferrin receptor
STfR	soluble transferrin receptor assay
STIR	short tau inversion recovery
SUV	standardized uptake value
SVC	superior vena cava
SWS	slow wave sleep
SXR	skull X-ray
T	testa
T1W	T1-weighted
T2W	T2-weighted
TA	temporal arteritis
TACO	transfusion-associated circulatory overload
TB	tuberculosis
TBLB	transbronchial lung biopsy
TBNA	transbronchial needle aspiration
TCR	T-cell receptor
TCT	thrombin clotting time
tds	<i>ter die sumendus</i> (three times daily)
TE	echo time
TfR	transferrin receptor
TFT	thyroid function test
Tg	thyroglobulin
TG	triglyceride
THR	total hip replacement
TIA	transient ischaemic attack
TIBC	total iron binding capacity
TIPS	transjugular intrahepatic portosystemic shunt
TLC	thin-layer chromatography; total lung capacity
TLCO	transfer factor for carbon monoxide
TMA	thrombotic microangiopathy; transcription-mediated amplification

TMS	transcranial magnetic stimulation
TN	trigeminal neuralgia
TnI	troponin I
TnT	troponin T
TOE	transoesophageal echocardiography
TPA	tissue plasminogen activator
TPHA	<i>Treponema pallidum</i> haemagglutination assay
TPN	total parenteral nutrition
TPO	thyroid peroxidase
TR	repetition time
TRALI	transfusion-related acute lung injury
TRAP	tartrate-resistant acid phosphatase
TRH	thyrotropin-releasing hormone
tRNA	transfer ribonucleic acid
TRP	tubular reabsorption of phosphate
TSE	transmissible spongiform encephalopathy
TSH	thyroid-stimulating hormone
TTE	transthoracic echocardiogram
TTG	tissue transglutaminase
TTP	thrombotic thrombocytopenic purpura
U	unit
UCTD	undifferentiated connective tissue disease
U&E	urea and electrolytes
UFC	urinary free cortisol
UFE	uterine fibroid embolization
UK	United Kingdom
UMN	upper motor neurone
URTI	upper respiratory tract infection
US	ultrasound
USA	United States
USS	ultrasound scan
UTI	urinary tract infection
UV	ultraviolet
VATS	video-assisted thoracoscopic surgery
VC	vital capacity
vCJD	variant Creutzfeldt–Jakob disease
VDRL	Venereal Disease Research Laboratory
VEP	visual evoked potential
VHF	viral haemorrhagic fever
VHL	von Hippel–Lindau

VIP	vasoactive intestinal peptide
VLCFA	very long-chain fatty acid
VLDL	very low-density lipoprotein
VMA	vanillylmandelic acid
VO ₂	oxygen uptake
VO _{2max}	maximum oxygen uptake
VP	vasoactive peptide
V/Q	ventilation/perfusion
VRE	vancomycin-resistant <i>Enterococcus</i>
VSD	ventricular septal defect
VTE	venous thromboembolism
vWD	von Willebrand disease
vWF Ag	von Willebrand factor antigen
WBC	white blood count/cells
WCE	wireless capsule endoscopy
WHO	World Health Organization
w/v	weight by volume
XDP	cross-linked fibrin degradation product
XDR-TB	extensively drug-resistant tuberculosis
XLA	X-linked agammaglobulinaemia
ZN	Ziehl–Neelsen
ZPP	zinc protoporphyrin

Approach to investigations

Why do tests?

Patients seldom present to their doctors with *diagnoses*—rather, they have symptoms or signs. The major challenge of medicine is being able to talk to the patient and obtain a history, then carry out a physical examination looking for pointers to their likely underlying problem. Our elders and, some would argue, betters in medicine had fewer tests available to them than we have today, and their diagnoses were often made solely from the history and examination. Of course, they would claim that their clinical acumen and skills were greater than ours, and that we rely too heavily on the huge armoury of laboratory and other investigations available today. This, in part, is probably true, but we cannot ignore the fact that advances in science and technology have spawned a bewildering array of very useful and sophisticated tests that help us to confirm our diagnostic suspicions.

By 'test' we mean the measurement of a component of blood, marrow, or other body fluid or physiological parameter to determine whether the patient's value falls within or outside the normal range, either suggesting the diagnosis or, in some cases, actually making the diagnosis for us.

Factors affecting variable parameters in health

Many measurable body constituents vary throughout life. For example, a newborn baby has an extremely high haemoglobin concentration, which falls after delivery. This is completely normal and is *physiological*, rather than *pathological*. A haemoglobin level this high in an adult would be pathological, since it is far outside the normal range for the adult population.

Factors affecting measurable variables

- Age.
- Sex.
- Ethnicity.
- Altitude.
- Build.
- Physiological conditions (e.g. at rest, after exercise, standing, lying).
- Sampling methods (e.g. with or without using a tourniquet).
- Storage and age of sample.
- Container used, e.g. for blood sample, as well as anticoagulant.
- Method of analysis.

Reference ranges (normal values)

These are published for most measurable components of blood and other tissue, and we have included the normal ranges for most blood and cerebrospinal fluid (CSF) analytes at the end of the book.

What makes a test useful?

A really good test, and one that would make us appear to be outstanding doctors, would be one that would *always* be positive in the presence of a disease and would be *totally specific* for that disease alone; such a test would never be positive in patients who did not have the disorder. What we mean is that what we are looking for are sensitive tests that are *specific* for a given disease. Sadly, most tests are neither 100% sensitive nor 100% specific, but some do come very close.

How to use tests and the laboratory

Rather than request tests in a shotgun or knee-jerk fashion where every box on a request form is ticked, it is far better to use the laboratory selectively. Even with the major advances in automation where tests are batched and are cheaper, the hospital budget is finite and sloppy requesting should be discouraged.

Outline your differential diagnoses: what are the likeliest diseases, given the patient's history, examination findings, and population from which the patient come?

Decide which test(s) will help you make the diagnosis: request these and review the diagnosis in the light of the test results. Review the patient and arrange further investigations as necessary.

The downside of tests

It is important to remember that tests may often give 'normal' results, even in the presence of disease. For example, a normal electrocardiogram (ECG) in the presence of chest pain does *not* exclude the occurrence of myocardial infarction with 100% certainty. Conversely, the presence of an abnormality does not necessarily imply that a disease is present. This, of course, is where clinical experience comes into its own—the more experienced clinician will be able to balance the likelihood of disease with the results available, even if some of the test results give unexpected answers.

Sensitivity and specificity

Sensitivity	% of patients with the disease and in whom the test is positive
Specificity	% of people without the disease and in whom the test is negative

Quick-fix clinical experience

This simply does not exist. Talking to patients and examining them for physical signs and assimilating knowledge gained in medical school are absolute requirements for attainment of sound clinical judgement. Those students and doctors who work from books alone do not survive effectively at the coal face! It is a constant source of irritation to medical students and junior doctors, when a senior doctor asks for the results of an investigation on the ward round and you find this test is the one that clinches the diagnosis. How *do* they do it? Like appreciating good wine—they develop a nose for it. You can learn a great deal by watching your registrar or consultant make decisions. This forms the basis of your own clinical experience.

Laboratory errors and how to avoid them

It is a fact of life that the sophisticated automated analysers in current use are not 100% accurate 100% of the time, but they come pretty close. In order to keep errors to a minimum, precautions need to be taken when sampling biological material, e.g. blood.

Minimizing spurious results using blood samples

- Use correct bottle.
- Fill to line (if anticoagulant used). This is less of a worry when vacuum sample bottles are used since these should take in exactly the correct amount of blood, ensuring the correct blood:anticoagulant ratio. This is critical for coagulation tests.
- Try to get the sample to the laboratory as quickly as possible. Blood samples left lying around on warm windowsills, or even overnight at room temperature, will produce bizarre results, e.g. crenated red blood cells (RBCs) and abnormal-looking white blood cells (WBCs) in old EDTA samples.
- Try to avoid rupturing red cells when taking the sample (e.g. using narrow-gauge needle, prolonged time to collect whole sample); otherwise a 'haemolysed' sample will be received by the laboratory. This may cause spurious results for some parameters (e.g. $[K^+]$).
- Remember to mix (not shake) samples containing anticoagulant.

Variations in normal ranges in health

As discussed earlier, most of the normal ranges for blood parameters discussed in this book are for non-pregnant adults. The reason for this is that blood values, e.g. haemoglobin (Hb), red cell count (RCC), are high in the newborn and many full blood count (FBC), coagulation, and other parameters undergo changes in pregnancy.

Part I

The patient

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Symptoms and signs

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Abdominal distension

Patients may describe generalized abdominal swelling or localized fullness in a specific area of the abdomen.

In the history enquire specifically about

- Change in bowel habit.
- Weight loss.
- Associated pain.

Generalized swelling

Consider

- Fat.
- Fluid.
- Faeces.
- Flatus.
- Fetus.
- Full bladder.

Ascites

Fluid in the peritoneal cavity. Look for shifting dullness and fluid thrill on percussion, stigmata of chronic liver disease, lymphadenopathy, and oedema, and assess the jugular venous pressure (JVP).

Causes

- Malignancy.
- Cirrhosis/portal hypertension.
- Hypoproteinaemia.
- Right heart failure.

Investigations

- Urea and electrolytes (U&Es).
- Liver function tests (LFTs).
- Serum albumin.
- Ascitic tap for cytology, and microscopy, culture, and sensitivity (M,C&S).
- Serum-ascites albumin gradient.
- Ultrasound scan (USS) of the abdomen.

(See Fig. 1.1.)

Flatus

Gaseous distension. Need to exclude bowel obstruction. Assess for colicky abdominal pain, bowel habit, flatus, and vomiting. Look for resonant distension on percussion, altered or absent bowel sounds, and focal tenderness with rebound and guarding. Always check for herniae and perform a per rectum (PR) examination in suspected obstruction.

Causes

- *Intraluminal*: faecal impaction, gallstone ileus.
- *Luminal*: inflammatory stricture (e.g. Crohn's), tumour, abscess.
- *Extraluminal*: herniae, adhesions, pelvic mass, lymphadenopathy, volvulus, intussusception.

- *Paralytic ileus*: drug-induced, electrolyte disturbances.
- Age-related causes of obstruction.
- *Neonatal*: congenital atresia, imperforate anus, volvulus, Hirschsprung's disease, meconium ileus.
- *Infants*: intussusception, Hirschsprung's, herniae, Meckel's diverticulum.
- *Young/middle-aged adults*: herniae, adhesions, Crohn's.
- *Elderly*: herniae, carcinoma, diverticulitis, faecal impaction.

Investigations

- Full blood count (FBC).
- U&E.
- Abdominal X-ray (AXR) (erect and supine).
- Consider barium enema, barium follow-through, sigmoidoscopy, surgical intervention for complete acute obstruction.

Localized swelling/masses: common causes according to site

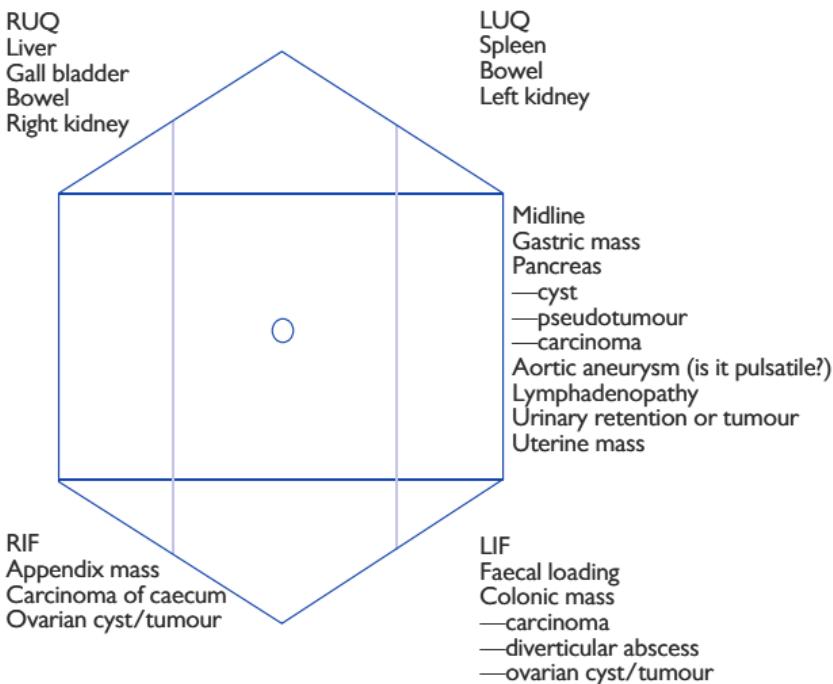


Fig. 1.1 Main causes of abdominal swelling according to site.

Investigate according to site

- Consider USS abdomen and pelvis.
- Computed tomography (CT) scanning.
- Barium studies.
- Intravenous urogram (IVU).

⇒ OHCM 10e, p. 62, p. 604.

Abdominal pain

Abdominal pain may be acute or chronic. Severe acute pain may indicate a surgical emergency, including perforation, peritonitis, or obstruction. Assess nature and radiation of pain, clinical status of the patient, including fever, tachycardia, and hypotension.

Common causes of abdominal pain according to site

Epigastric pain

Peptic ulcer disease, gastritis or duodenal erosions, cholecystitis, pancreatitis.

Perumbilical pain

Pancreatitis, mesenteric artery ischaemia (older patient with vascular disease).

Right upper quadrant (RUQ) pain

Biliary colic, cholecystitis, hepatitis, peptic ulcer.

Left upper quadrant (LUQ) pain

Splenic, peptic ulcer.

Loin pain

Renal colic (colicky radiating loin → groin), pyelonephritis, renal pathology.

Left iliac fossa (LIF) pain

Constipation, diverticular disease, irritable bowel syndrome (IBS), pelvic referred pain, inflammatory bowel disease (IBD).

Right iliac fossa (RIF) pain

Appendicitis, pelvic referred pain, IBD (e.g. Crohn's of terminal ileum).

Suprapubic pain

Urinary tract infection (UTI), cystitis, salpingitis.

Generalized pain

Gastroenteritis, irritable bowel, constipation, generalized peritonitis.

Pitfalls

- Metabolic causes, e.g. diabetic ketoacidosis (DKA), hypercalcaemia, Addison's disease, porphyria, lead poisoning.
- Atypical referred pain, e.g. myocardial infarction (MI), pneumonia.

Investigations

- FBC.
- U&E, e.g. deranged electrolytes following vomiting, diarrhoea, or bowel obstruction.
- Plasma glucose.
- Serum amylase (\uparrow in pancreatitis and bowel obstruction).
- Urinalysis and midstream urine (MSU), e.g. haematuria, proteinuria, glucose.
- LFTs (consider obstructive vs hepatic picture).
- Plain AXR (erect and supine to assess for perforation and bowel obstruction).
- Kidney, ureter, bladder X-ray (KUB) for renal tract calculi.
- USS abdomen, particularly for biliary tract, gall bladder, and renal tract.
- IVU to assess for renal tract calculi/pathology.

Alteration of behaviour

This is usually reported by a relative or friend, rather than by the patient. Often the patient will have little or no insight into the disease and taking a history can be difficult. In addition to a full general and neurological physical examination, a mental state examination is required.

Find out if this is the first episode of altered behaviour or if the episodes are recurrent. Is there a gradual change in behaviour (and personality) over time?

Acute delirium

Causes

- Sepsis (common).
- Acute intracranial event, e.g. haemorrhage.
- Metabolic disturbance, e.g. uraemia, hypercalcaemia (common).
- Intracerebral tumour (including meningioma).
- Drugs—especially interactions in the elderly.
- Alcohol (and withdrawal syndrome).
- Hypoxia (common).
- Hypoglycaemia (iatrogenic in diabetic patients receiving insulin treatment or oral insulin secretagogues, or insulinoma and other causes).

Dementia

- Alzheimer's (common), Pick's (rare).
- Vascular, e.g. multi-infarct.
- Huntington's chorea.
- Vitamin B₁₂ deficiency (severe).
- Hypothyroidism (severe).
- Wilson's disease.
- Alcoholism.
- Normal pressure hydrocephalus.

Note: 'frontal lobe syndrome' from space-occupying lesion (SOL), e.g. meningioma. Presents with disinhibition, impaired social functioning, primitive reflexes, e.g. grasp reflex.

Anxiety states

Usually psychogenic, but consider organic possibilities such as

- Phaeochromocytoma (rare).
- Hyperthyroidism (common).
- Paroxysmal atrial tachycardia (fairly common).
- Alcohol withdrawal (usually history of excessive alcohol intake).

Psychosis

- Schizophrenia.
- Bipolar disorder or pseudo-dementia in:
 - Systemic lupus erythematosus (SLE).
 - Cushing's syndrome.
 - Multiple sclerosis (MS).
 - Thyrotoxicosis ('apathetic' thyrotoxicosis in the elderly).

Temporal lobe epilepsy

- Temporary disturbance of content of consciousness.

Investigations: guided by history and examination

- U&E.
- Glucose (in non-diabetics, take fasting venous plasma in a fluoride oxalate tube with simultaneous serum or plasma for insulin concentration, e.g. suspected insulinoma).
- Chest X-ray (CXR).
- LFTs.
- Thyroid function tests (TFTs).
- FBC.
- Erythrocyte sedimentation rate (ESR).
- Urinalysis (protein, nitrites, glucose).
- Cranial CT scan.
- Serum vitamin B₁₂.
- Arterial blood gases (ABGs) ± carboxyhaemoglobin (COHb).
- Blood cultures.

Consider

- Syphilis serology.
- Human immunodeficiency virus (HIV) test.
- Urine drug screen (⊖ Chapter 11).
- Blood ethanol level (may be low in withdrawal state).
- Electroencephalogram (EEG).
- 24h electrocardiogram (ECG).
- Sleep study.

Alteration in bowel habit

A change in bowel habit in an adult should always alert you to the possibility of bowel cancer. Ask about associated features—PR bleeding, tenesmus, weight loss, mucus, abdominal pain, or bloating.

Has the patient started any new medications, including 'over the counter'? Look for signs of systemic disease.

Consider

- Carcinoma of the colon.
- Diverticular disease.
- IBS.
- Constipation with overflow diarrhoea.
- All of the above may present with alternating diarrhoea and constipation.

Investigations

- Digital rectal examination.
- Proctoscopy.
- Sigmoidoscopy (rigid/flexible).
- Colonoscopy.
- Barium enema.
- CT colonography.

➔ Diarrhoea (pp. 32–3), ➔ Constipation (pp. 29–30), ➔ Incontinence: faecal (p. 60).

Anaemia

Reduced haemoglobin (Hb), no specific cause implied (and *not a diagnosis in itself, so don't be complacent*): ♂ <13.5g/dL, ♀ <11.5g/dL. Often associated with non-specific symptoms such as fatigue, poor concentration, shortness of breath, and dizziness. Older patients may experience palpitations and exacerbation of angina, congestive cardiac failure (CCF), or claudication.

Signs

Pallor of conjunctivae and skin creases, nail pallor and koilonychia (spoon-shaped nails, very rare finding in severe chronic iron deficiency), angular cheilitis, and glossitis. Most of these signs are unreliable and it is difficult to gauge anaemia from skin signs alone.

Causes

(See Table 1.1.)

Two common approaches to assess anaemia are:

1. Red cell dynamics:

- ↑ Red blood cell (RBC) loss/breakdown, e.g. haemolysis (congenital or acquired) or bleeding.
- ↓ RBC production, e.g. vitamin/mineral deficiency, marrow suppression/infiltration, myelodysplasia, Hb disorders (e.g. thalassaemia), chronic disease, renal failure.

2. Red cell indices:

Investigations

FBC and film

Assessment of RBC indices helps direct investigation as above.

Table 1.1 Some causes of anaemia based on the MCV

Microcytic/hypochromic	↓ MCV, ↓ MCHC, e.g. Iron deficiency Thalassaemia Anaemia of chronic disease
Macrocytic	↑ MCV Reticulocytosis (polychromasia on blood film) B_{12} or folate deficiency Chronic liver disease Hypothyroidism Alcohol Myelodysplasia
Normocytic, normochromic	↔ MCV and MCHC Anaemia of chronic disease, e.g. <ul style="list-style-type: none"> • Chronic infection • Inflammation Inflammatory disease or malignancy Acute blood loss Renal failure Myeloma

MCHC, mean corpuscular haemoglobin concentration; MCV, mean cell volume.

Microcytic

- Check iron stores (ferritin or soluble transferrin receptor assay). Note: ferritin is ↑ in acute inflammation and may be misleading. Iron/total iron binding capacity (TIBC) no longer used for assessment of iron deficiency (➡ Assessment of iron status, pp. 244–7).
- Consider thalassaemia screening if not iron-deficient (i.e. ↓ MCV, ↔ ferritin).
- If iron-deficient, assess dietary history (vegetarians) and look for risk factors for blood loss and ↑ demands.
- Premenopausal women: assess menstrual losses.
- Pregnancy/infants/adolescence: consider physiological (↑ requirements).
- All others: look for source of blood loss. The gastrointestinal (GI) tract is the commonest source. Consider oesophagogastroduodenoscopy (OGD) and/or colonoscopy if clinically indicated by symptoms and barium studies.

Macrocytic

- Reticulocyte count.
- Serum B₁₂ and red cell folate levels.
- If folate-deficient: assess dietary history and physiological requirements.
- If B₁₂-deficient: rarely dietary cause alone, usually an associated pathology. Pernicious anaemia (PA) is the commonest cause—check parietal cell antibodies (90% of patients with PA are +ve, but seen in other causes of gastric atrophy, especially in older individuals) and/or intrinsic factor antibodies (+ve in only 50% with PA, but specific). Consider ileal disease and malabsorption.
- LFTs.
- Thyroid function.

Normocytic

- Blood film.
- ESR.
- Renal function.
- Consider myeloma screen in older adults (immunoglobulins (Igs), protein electrophoresis, urine Bence–Jones protein (BJP)). Skeletal survey of value if paraprotein or BJP.
- Autoimmune screen to exclude connective tissue disease.

Haemolysis screen

- FBC, mean cell volume (MCV) (↑ due to reticulocytosis—these are larger than RBCs).
- Blood film (spherocytes, polychromasia, bite cells, and red cell fragmentation).
- Reticulocyte count.
- Serum bilirubin and serum lactate dehydrogenase (LDH).
- Haptoglobins (absent in haemolysis).
- Direct antibody test (DAT) (old term is direct Coombs' test).

Consider

- Congenital haemolytic anaemias: membrane defects, enzyme deficiencies (e.g. glucose-6-phosphate dehydrogenase (G6PD), pyruvate kinase).
- Disseminated intravascular coagulation (DIC)/microangiopathic haemolysis—DIC screen.

Anaphylaxis

Defined as a systemic reaction (local oral angio-oedema is *not* anaphylaxis), with any or all of the following:

- Stridor (laryngeal obstruction).
- Wheeze (bronchospasm).
- Generalized urticaria and/or angio-oedema.
- Hypotension ± loss of consciousness.
- Abdominal pain/cramps, vomiting, and diarrhoea.

Note: not all patients have urticaria or rash—only 50% will do so.

Differentiate IgE-mediated reactions (*anaphylaxis*) from non-IgE-mediated reactions (*anaphylactoid*)—due to direct mast cell degranulation).

Angio-oedema

Angio-oedema is deep tissue swelling which is non-itchy. May be premonitory tingling. May occur with or without urticaria. Caused by bradykinin, not histamine.

Causes

- As for urticaria; also hereditary angioedema (rare).
- Also think of drugs—these are the commonest cause:
 - Angiotensin-converting enzyme (ACE) inhibitors (ACEIs) (elevated bradykinin levels due to inhibition of breakdown).
 - Angiotensin II (AT-II) receptor antagonists.
 - Statins.
 - Proton pump inhibitors.
 - Non-steroidal anti-inflammatory drugs (NSAIDs).

May also be seen in patients with autoimmune disease, such as lupus and rheumatoid arthritis (RhA) (antibodies against C1q), and in older patients in association with paraproteins (myeloma, lymphoma).

Angio-oedema *with* urticaria is *not* due to hereditary angio-oedema.

Investigations

- Check drug history first! If suspect drugs, then stop drugs and wait! If no drugs, then investigate.

Angio-oedema **WITH** urticaria

- Investigate as for urticaria.

Angio-oedema **WITHOUT** urticaria

- Complement C3 and C4.
- If C4 low, check C1 esterase inhibitor (immunochemical and functional).
- Serum IgG and electrophoresis.
- Autoantibody screen.
- FBC and ESR.
- Thyroid function.
- Liver function.

Anorexia

This describes a loss of appetite for food and is associated with a wide range of disorders. In fact, anorexia is a fairly common consequence of underlying disease and represents general undernourishment. Anorexia per se is associated with ↑ morbidity, especially when present in patients undergoing surgery; post-operative infection is commoner, as is prolongation of the hospital stay.

The extent to which it will be investigated depends on the general status of the patient and the presence and duration of any symptoms or signs. Clinical judgement will help!

Causes

- Anorexia nervosa.
- Depressive illness.
- Stress.
- Cancers: any, including carcinoma of the stomach or oesophagus, metastatic, leukaemia, or lymphoma.
- Drugs, including chemotherapy.
- Radiotherapy.
- Renal failure.
- Hypercalcaemia.
- Infections.
- Cigarette smoking.

Investigations

- Full history and examination.
- FBC—looking for anaemia or non-specific changes seen in underlying disease.
- ESR—may be elevated in inflammatory disorders.
- U&E.
- LFTs.
- Serum calcium (Ca^{2+}).
- CXR (e.g. lung cancer, tuberculosis (TB), etc.).
- Cultures of blood, sputum, urine, stool if pyrexial and/or localizing symptoms or signs.

Anuria

Anuria denotes absent urine production. Oliguria (<400mL urine/24h) is commoner than anuria. A catheter must be passed to confirm an empty bladder.

Causes

- Urinary retention—prostatic hypertrophy; pelvic mass; drugs, e.g. tricyclic antidepressants; spinal cord lesions.
- Blocked indwelling urinary catheter.
- Obstruction of the ureters—tumour, stone, sloughed papillae (bilateral).
- Intrinsic renal failure—acute glomerulonephritis, acute interstitial nephritis, acute tubular necrosis (ATN), rhabdomyolysis.
- Pre-renal failure—dehydration, septic shock, cardiogenic shock.

An urgent USS of the renal tract must be performed and any physical obstruction relieved as quickly as possible, either directly (urethral catheter) or indirectly (nephrostomy).

►► Renal function and serum electrolytes must be measured without delay.

Further tests as clinically indicated

- FBC.
- Blood cultures.
- ABGs.
- Uric acid.
- Autoimmune profile.
- ESR.
- Creatine kinase (CK).
- Prostate-specific antigen (PSA) (prostatic carcinoma).
- Serum Ca^{2+} and phosphate (PO_4^{3-}).
- 12-lead ECG.
- CXR.
- Central venous pressure (CVP) measurement via central line (to guide intravenous (IV) fluids).
- MSU (UTI).
- Urine microscopy (for casts).
- Urine osmolality, sodium, creatinine, urea concentrations.
- IVU (Radiology of the urinary tract, pp. 808–11).
- Urinary stone analysis, if available.
- CT pelvis.
- Renal biopsy (if intrinsic renal disease suspected, normal-sized kidneys).

🕒 OHCM 10e, p. 81, p. 293.

Ataxia

Ataxia is an impaired ability to coordinate limb movements. There must be no motor paresis (e.g. monoparesis) or involuntary movements (e.g. the characteristic cogwheel tremor in Parkinson's disease (PD) is not ataxia).

Ataxia may be

- Cerebellar.
- Vestibular.
- Sensory.

Note: many forms of ataxia are hereditary (but are uncommon).

Hereditary causes

- Friedreich's ataxia.
- Ataxia telangiectasia.
- Spinocerebellar ataxia.
- Corticocerebellar atrophy.
- Olivopontocerebellar atrophy.
- Hereditary spastic paraplegia.
- Xeroderma pigmentosa.

Investigations

- Family studies.
- Genetic analysis (discuss with the regional genetics laboratory—counselling may be required).

Vestibular ataxia

- Acute alcohol intoxication.
- Labyrinthitis.

Sensory ataxia

- Loss of proprioception—peripheral neuropathy, dorsal column disease.
- Visual disturbance.

Investigations

- Venous plasma glucose (diabetic neuropathy).
- Serum vitamin B₁₂ (subacute combined degeneration of the cord—rare, but serious).
- LFTs.
- Cryoglobulins.

Cerebellar ataxia

- Demyelinating diseases, e.g. MS.
- Cerebellar infarct or haemorrhage.
- Alcoholic cerebellar degeneration.
- Cerebellar tumour—1° in children, metastases in adults. Note: von Hippel–Lindau (VHL) disease (⌚ OHCM 10e, Chapter 19).
- Nutritional deficiency:
 - Vitamin B₁₂.
 - Thiamine.
- Cerebellar abscess.

- Drugs (supratherapeutic blood levels):
 - Carbamazepine.
 - Phenytoin.
- Tuberculoma.
- Paraneoplastic syndrome.
- Developmental.
- Arnold–Chiari malformation.
- Dandy–Walker syndrome.
- Paget's disease of the skull.
- Wilson's disease (hepatolenticular degeneration).
- Hypothyroidism.
- Creutzfeldt–Jakob disease (CJD) and other chronic infections.
- Miller Fisher syndrome.
- Normal pressure hydrocephalus.

Ataxia should be distinguished from movement disorders, e.g.

- Chorea: Huntington's, Sydenham's, thyrotoxicosis (very rare).
- Athetosis.
- Hemiballismus: characteristic movement disorder, rare.
- Tardive dyskinesia: chronic phenothiazine therapy.

Investigations

- Cranial CT.
- Magnetic resonance imaging (MRI) brain (if demyelination suspected).
- CXR (cerebellar metastases from bronchogenic carcinoma; paraneoplastic syndrome).
- TFTs.
- Triple evoked potentials (demyelination).
- Lumbar puncture (LP) (→ Lumbar puncture, pp. 584–9).
- LFTs.
- Serum drug concentrations, especially anticonvulsants.
- Serum vitamin B₁₂.
- Erythrocyte transketolase (↓ in thiamine deficiency, e.g. alcoholism).
- Isotope bone scan (Paget's, metastases).
- Serum alkaline phosphatase (ALP)—bone isoenzyme (Paget's, metastases).
- Urine hydroxyproline (Paget's disease—reflects bone turnover).
- Caeruloplasmin (Wilson's disease).
- Serum and urine copper (Wilson's disease).

Consider whether the movement disorder is psychogenic (uncommon), rather than due to neuropathology. Uncommon and should not be confidently assumed.

→ OCHM 10e, p. 467.

Bradycardia

Bradycardia is defined as a heart rate of <60 beats per minute. It is a normal physiological response to fitness training but should always be considered a marker of potential cardiac disease until proved otherwise.

Causes

A comprehensive history and thorough examination are important. A transient bradycardia can cause disabling symptoms of dizziness or blackouts in the elderly, whilst persistent bradycardia often heralds systemic disease, e.g.:

- *Iatrogenic*: cardiac drugs, e.g. β-blockers (including eye drops for glaucoma), amiodarone, and calcium channel blockers (e.g. diltiazem and verapamil), cause sinus bradycardia; digoxin (atrioventricular (AV) block). The likelihood of extreme bradycardia or heart block is ↑ with combination therapy.
- *Cardiac causes*: acute MI (often transient in inferior MI); coronary artery disease; sick sinus syndrome; myocardial disease (amyloid, Chagas' disease, sarcoid, myocarditis).
- ↑ vagal tone associated with nausea and vomiting.
- Diminished sympathetic activity.
- *Physiological*: bradycardia is normal in sleep and in athletes.
- *Hypothyroidism*: associated with characteristic symptoms and signs.
- ↑ intracranial pressure (ICP), e.g. cerebral tumour.
- *Hypothermia*, e.g. myxoedema coma.
- *Metabolic*: severe hyperkalaemia, anorexia.
- *Toxic*: severe jaundice.
- *Drug toxicity*: opiates.
- *Infective*: inappropriate bradycardia seen in diphtheria, typhoid.

Investigations

- 12-lead ECG to identify the underlying rhythm.

If there are symptoms of chest pain

- Serum troponin and CK.
- Bedside ECG monitoring.
- Exercise ECG.

If there is a history of intermittent dizziness

- 24h ambulatory ECG monitoring, patient-activated event recorder, or implantable loop recorder, depending on the frequency of symptoms.

If indicated by clinical presentation, consider

- TFTs (hypothyroidism).
- Low reading thermometer (hypothermia—check for J waves on ECG).
- CT brain scan (? intracranial pathology).
- U&E.
- LFTs (especially bilirubin).
- Toxicology screen.

⇒ OHCM 10e, p. 124, p. 808.

Breathlessness

Breathlessness (dyspnoea) is the subjective awareness of difficulty in breathing. Almost universal during exercise, it is a common presenting symptom in a broad spectrum of diseases. A comprehensive history and a thorough examination are therefore essential. Speed of symptom onset, the patient's age and occupation, and local disease prevalence are particularly helpful in devising a differential diagnosis and a guide to investigations.

Causes

- **Acute pulmonary disease:** pneumonia, acute asthma, pulmonary embolus (PE), inhaled foreign body, pneumothorax, acute respiratory distress.
- **Chronic pulmonary disease:** emphysema, chronic bronchitis, ruptured bulla; interstitial disease (sarcoid, fibrosing alveolitis, extrinsic alveolitis, pneumoconiosis).
- **Carcinoma:** bronchogenic carcinoma, lymphangitis carcinomatosis, 2^o carcinoma.
- **Acute cardiac disease:** acute MI (and associated complications of pulmonary oedema, ventricular septal defect (VSD), mitral valve chordal rupture and arrhythmias).
- **Chronic cardiac disease:** left ventricular dysfunction, valvular heart disease (mitral or aortic stenosis and regurgitation), ischaemic heart disease (IHD), pulmonary hypertension, pleural effusion, arrhythmias (especially atrial fibrillation (AF)).
- **Metabolic:** poisoning from salicylates, methanol, and ethylene glycol, DKA, lactic acidosis, hepatic and renal failure.
- **Neuromuscular:** intercostal muscle/diaphragmatic weakness due to Guillain–Barré syndrome (GBS), muscular dystrophy.
- **Haematological:** anaemia.
- **Anxiety and hyperventilation.**
- **Morphological:** kyphoscoliosis, obesity.
- **Laryngeal obstruction:** extrinsic compression (retrosternal goitre), angioedema (often acute drug allergy), laryngeal spasm (hypocalcaemia).

Initial investigations

- FBC.
- U&E.
- Glucose.
- CXR.
- ABGs.
- Peak expiratory flow rate (PEFR).
- 12-lead ECG.

Additional investigations (as indicated)

- Transthoracic echocardiography (TTE).
- 24h ambulatory ECG monitoring.
- Pulmonary function tests.
- CT chest.
- Bronchoscopy.
- Ventilation/perfusion (V/Q) scan/computed tomography pulmonary angiography (CTPA).
- LFTs.
- Ca^{2+} .
- ESR.
- Serum salicylate levels.
- Lactate.
- Lung biopsy.

⇒ OHCM 10e, p. 782.

Bruising

Easy bruising is a common complaint and warrants careful assessment of onset and nature. Recent onset of spontaneous and unusual bruising or bleeding may suggest a serious acquired defect. A lifelong history of bruising and bleeding (e.g. post-tonsillectomy, dental extraction, or surgery) may imply a congenital defect. Family history may be informative.

Examine: skin, mouth, dependent areas, and fundi for mucocutaneous bleeding and purpura (non-blanching haemorrhages into the skin).

Platelet causes

- Thrombocytopenia or platelet dysfunction (e.g. aspirin).
- Marrow failure, infiltration, immune thrombocytopenia (ITP), DIC, hypersplenism, drugs, or alcohol.

Vascular causes

- Congenital, e.g. Osler–Weber–Rendu syndrome.
- Acquired, e.g. senile purpura, vasculitis (Henoch–Schönlein purpura, infection), diabetes, corticosteroid therapy, scurvy, connective tissue diseases.

Coagulopathy

- Congenital—mucocutaneous bruising is suggestive of a platelet-mediated defect (e.g. von Willebrand's disease, Glanzmann's thrombasthenia), rather than a clotting factor deficiency (e.g. haemophilia A and B).
- Acquired, e.g. DIC, vitamin K deficiency.

Hyperviscosity

- Myeloma, Waldenström's macroglobulinaemia (low-grade lymphoma associated with ↑ IgM and ↑ plasma viscosity), ↑↑ white blood cells (WBC) in leukaemia.

Investigations

- FBC and film.
- Coagulation—international normalized ratio (INR) and activated partial thromboplastin time ratio (APTR).
- Bleeding time, measures platelet and vascular phase.
- DIC screen, including fibrinogen, thrombin time, D-dimers or fibrin degradation products (FDPs).

Consider further tests and referral to haematology for

- Factor assays.
- Platelet aggregation studies to assess platelet function.

⇒ OHCM 10e, p. 346.

Calf swelling

Assess whether swelling is bilateral or unilateral, precipitating factors, and duration of onset. Careful examination of the affected leg should be extended to a full examination, particularly of the abdominal and cardiovascular systems.

Causes

Venous and lymphatic

- Deep vein thrombosis (DVT).
- Superficial thrombophlebitis.
- Varicose veins.
- Post-phlebitic limb (post-DVT).

Soft tissue/musculoskeletal

- Calf haematoma or trauma.
- Ruptured Baker's cyst (synovial effusion in the popliteal fossa associated with rheumatoid disease).
- Cellulitis (associated fever, sepsis, tachycardia).

Systemic

- CCF (bilateral limb oedema, ↑ JVP, and signs of left ventricular failure (LVF)).
- Hepatic failure.
- Hypoalbuminaemia.
- Nephrotic syndrome.
- Pregnancy: ↑ dependent oedema, but note also ↑ thrombotic risk, and DVT should be excluded.

Deep vein thrombosis (DVT)

Usually affects the lower limb and can extend proximally into the iliofemoral veins and inferior vena cava (IVC), with a higher risk of associated PE and a higher incidence of post-phlebitic limb. Occasionally seen affecting the upper limb, but this is atypical.

Risk factors for DVT

- Age >60 years.
- Previous DVT or PE.
- Recent major surgery, especially orthopaedic lower limb, abdominal, and pelvic.
- Marked immobility.
- Malignancy.
- Pregnancy and postpartum.
- High-dose oestrogen oral contraceptive pill (OCP).
- Family history of venous thromboembolism (VTE).

Investigations

USS Doppler studies, impedance plethysmography, venography, exclude PE. If any associated symptoms, arrange V/Q scan, multislice CT, and pulmonary angiography. Thrombophilia screening for younger patients (age <55), atypical site and extensive clots, spontaneous onset, and family history.

Chest pain

Acute chest pain is a common symptom. A detailed history and a full physical examination should be performed in order to define the most likely cause and necessary investigation pathway.

History

Be sure to ask the following questions about the pain:

- Site and radiation.
- Character.
- Onset and duration.
- Precipitating and relieving features.
- Associated symptoms.
- Response to pain relief, antacids, or nitrates.

Most types of chest pain fall within one of the categories in Table 1.2.

Table 1.2 Pain sources

Pain source	Description of pain
Myocardial ischaemia	Retrosternal, heavy ache, can radiate → jaw and arms, precipitated by exertion, and relieved by rest or nitrates
Aortic dissection	Severe central tearing pain, radiates to back
Gastro-oesophageal disease	Burning central pain; can radiate to shoulders, throat, or abdomen; exacerbated by meals, eased with antacids/milk
Pleuritic pain	Focal sharp pain, exacerbated by inspiration
Pericardial pain	Sharp pain, radiates to left shoulder tip, worse on lying flat and during inspiration, eased by sitting forwards
Musculoskeletal pain	Sharp focal pain exacerbated by movement and palpation

Investigations

(See Table 1.3.)

➲ OHCM 10e, p. 36, p. 48, p. 94, p. 784.

Table 1.3 Investigations for suspected diagnoses

Cardiovascular causes: all patients should have a 12-lead ECG and CXR

Suspected diagnosis	Investigations
Myocardial ischaemia/infarction	Serial ECGs
Consider:	Cardiac markers of necrosis
• Coronary artery disease	FBC
• Aortic stenosis	TFTs
• Hypertrophic obstructive cardiomyopathy	Echocardiogram Exercise electrocardiogram Stress cardiac imaging Coronary angiography
Thoracic aortic dissection	FBC, U&E, X-match
Note: myocardial ischaemia may also be present if it involves the coronary arteries	Echocardiogram (TTE or TOE)
Syphilitic aortitis	CT, MRI Syphilis serology
Mitral valve prolapse	Echocardiogram (TTE or TOE)
Acute pericarditis	FBC, viral titres, ESR Echocardiogram

Pulmonary causes: all patients should have CXR ± ABGs

Suspected diagnosis	Investigations
Pneumonia/pleurisy	FBC, CRP
Acute bronchitis	Sputum and blood cultures
Pulmonary tuberculosis (TB)	Aspiration if empyema suspected Early morning urine (TB) Mantoux test (TB)
Pneumothorax	CXR
Pulmonary embolus	D-dimers 12-lead ECG V/Q scan CT pulmonary angiography
Lung carcinoma	Sputum cytology
Pleural tumour, e.g. mesothelioma	High-resolution CT
Mediastinal tumour	Bronchoscopy Tissue biopsy
Gastro-oesophageal causes	
Oesophageal	FBC
• Spasm	G&S
• Oesophagitis	<i>Helicobacter pylori</i>
• Candidiasis	Endoscopy
• Reflux disease	Oesophageal manometry
• Mallory–Weiss tear	Oesophageal biopsy

(Continued)

Table 1.3 (Contd.)

Suspected diagnosis	Investigations
<i>Peptic ulcer disease</i>	Endoscopy Gastrografin® swallow Barium swallow, meal, or follow-through Erect CXR (if perforation suspected clinically)
<i>Acute pancreatitis</i>	Amylase Abdominal USS
<i>Cholecystitis/biliary colic</i>	FBC, CRP, LFTs Urinalysis Abdominal USS ERCP
Musculoskeletal and dermatological causes	
Suspected diagnosis	Investigations
<i>Muscular</i>	
<i>Bony structures</i>	
Chest wall bony metastases	CXR
Rib/sternal fractures	Bone scan
Costochondritis (Tietze's syndrome)	Spinal X-rays
Ankylosing spondylitis	CT scan
Cervical/thoracic spine disease	
Thoracic outlet syndrome	
<i>Skin/soft tissue</i>	
Acute shingles	Herpetic serology/smear (rarely)
Post-herpetic neuralgia	

CRP, C-reactive protein; G&S, group and save; TOE, transoesophageal echocardiography; TTE, transthoracic echocardiography.

ACC/AHA 2002 guideline update for the management of patients with chronic stable angina.
 ↗ http://www.onlinejacc.org/content/41/1/159?_ga=2.15239422.956431479.1499776108.8-163922176.1499776108.

Clubbing

Soft tissue hypertrophy under the nail bed distorts finger and toenail growth.

Characteristic features

- ↑ lateral and longitudinal nail curvature.
- The skin at the base of the nail becomes spongy.
- The angle between the nail and skin is obliterated.
- In extreme cases, the terminal phalanx becomes bulbous like a drumstick.

Clubbing can be an important visual indicator of major disease, although it can also be congenital. Rarely, clubbing may accompany swollen wrists and ankles as part of a proliferative periostitis seen in hypertrophic pulmonary osteoarthropathy (HPOA). This is associated with squamous carcinoma of the lung.

Major causes

- *Lung disease:* cystic fibrosis, bronchiectasis, empyema, lung abscess, asbestosis, mesothelioma, pulmonary sarcoid.
- *Carcinoma:* bronchogenic (especially squamous cell), mediastinal, pleural, oesophageal, gastric, colonic, thoracic lymphoma, familial polyposis coli.
- *Infection:* infective endocarditis, colonic amoebiasis.
- *Vascular disease:* cyanotic congenital heart disease, atrial myxoma, arteriovenous malformation (AVM).
- *Liver disease:* primary biliary cirrhosis (PBC), chronic active hepatitis.
- *Ulcerative colitis and Crohn's disease:* malabsorption.
- *Rare causes:* thyrotoxicosis, polycythaemia, SLE.

Investigations

As guided by differential diagnosis and clinical suspicion

- FBC.
- ESR.
- C-reactive protein (CRP).
- LFTs.
- TFTs.
- Serum ACE.
- Autoantibodies.
- Blood cultures (at least three sets if infective endocarditis suspected).
- Faecal occult blood (FOB) (three samples).
- CXR.
- Echocardiography (TTE or transoesophageal echocardiography (TOE)).
- OGD and biopsy.
- Colonoscopy and biopsy.
- Abdominal USS.
- CT chest.
- Bronchoscopy, biopsy, washings.
- Liver biopsy.

➲ OHCM 10e, p. 40, p. 77.

Coma

The Glasgow Coma Scale (GCS) is used to assess the level of consciousness (see Table 1.4). The minimum score is 3; the maximum 15.

Assess the level of consciousness and determine whether this is stable, fluctuating, improving, or deteriorating on serial assessments.

Cerebral causes

- Intracranial haemorrhage (subarachnoid haemorrhage (SAH), subdural haemorrhage (SDH), extradural haemorrhage (EDH), intracerebral bleed).
- Large cerebral infarct.
- Pontine haemorrhage (pinpoint pupils).
- Cerebral venous sinus thrombosis.
- Hypertensive encephalopathy.
- Cerebral tumour (associated local cerebral oedema may respond to dexamethasone).
- Head injury.
- Cerebral infection—encephalitis, meningitis, cerebral malaria, brain abscess.
- Post-ictal state.
- Subclinical status epilepticus. (Note: this is an EEG diagnosis.)
- Cerebral vasculitis, e.g. SLE.
- End-stage MS.
- Leukodystrophy.
- CJD (including variant CJD (vCJD)).

Table 1.4 Glasgow Coma Scale

Eye opening	1	Nil
	2	To pain
	3	To voice
	4	Spontaneously
Motor response	1	Nil
	2	Extension
	3	Flexion
	4	Withdrawal from pain
	5	Localizing to pain
	6	Voluntary
Vocal response	1	Nil
	2	Groans
	3	Inappropriate words
	4	Disorientated speech
	5	Orientated speech

Metabolic causes

- Drugs (usually in deliberate overdose;  Chapter 11).
- Alcohol excess. (Note: remember hypoglycaemia as a cause of coma in alcoholics, as well as extradural haematoma.)
- Hypoglycaemia (iatrogenic, overdose of insulin or sulphonylureas, insulinoma, insulin-like growth factor (IGF)-2-associated hypoglycaemia in certain tumours).
- DKA (coma in ~10% of cases—adverse prognostic sign).
- Hyperosmolar non-ketotic coma (HONK) (may present as severe dehydration ± coma).
- Uraemia.
- Late stages of hepatic encephalopathy.
- Severe hyponatraemia (relatively common—especially inappropriate antidiuretic hormone (ADH) syndrome).
- Hypothyroidism (myxoedema coma—rare).
- Hypercalcaemia.
- Inborn error of metabolism, e.g. porphyria, urea cycle disorders.
- Type 2 respiratory failure (carbon dioxide (CO_2) narcosis).
- Hypothermia (severe).
- Hyperpyrexia (neuroleptic malignant syndrome (NMS), after anaesthesia).
- Severe nutritional deficiency—thiamine, pyridoxine, vitamin B_{12} .

Investigations

- Venous plasma glucose (exclude hypoglycaemia with a fingerstick + reflectance meter; confirm with a venous plasma fluoride-oxalate sample).
- U&E.
- LFTs.
- Serum Ca^{2+} .
- Serum osmolality.
- Urine Na^+ .
- Blood cultures.
- Clotting screen ( p. 288, p. 289, p. 290).
- ABGs.
- Drug screen (serum, urine).
- Cranial CT scan.
- LP.
- CXR (bronchogenic carcinoma with cerebral metastases).
- 12-lead ECG.
- EEG.
- Erythrocyte transketolase (\downarrow in thiamine deficiency).
- Serum ammonia (NH_3) (\uparrow in urea cycle disorders).
- Brain biopsy.

Always assess Airway, Breathing, Circulation before assessment of the cause of \downarrow consciousness. Consider psychogenic unresponsiveness.

 OHCM 10e, p. 220, pp. 786–9, p. 834, p. 836.

Confusion

A reliable witness, family member, or carer may be vital in assessing a patient with confusion, and care must be taken to discriminate between acute and chronic symptoms. Acute confusional states carry a very broad differential diagnosis and require careful initial evaluation (see Table 1.5). Any systemic illness can precipitate a confusional state.

Investigations

- FBC, U&E, LFTs, serum Ca^{2+} , BM stix, and blood glucose.
- ABGs.
- MSU, blood cultures, sputum culture.
- CXR.
- ECG.
- Thyroid function.
- Drug/toxicology screen—blood and urine.
- CT scan.
- LP.

►► Always look for a MedicAlert™ bracelet, necklace, or card.

Table 1.5 Causes of confusion

Hypoxaemia	Acute infection, asthma, COPD, etc.
Head injury	Cerebral trauma
Vascular	CVA, TIA, intracerebral, SDH
Infection	Systemic Meningitis or encephalitis
Endocrine/metabolic	DKA, hypoglycaemia, thyrotoxicosis or myxoedema, uraemia, hypercalcaemia, hyponatraemia
Alcohol and drug abuse	Acute intoxication and withdrawal Also consider overdose
Iatrogenic	Full and recent medication history (especially opiates, analgesia, and sedatives)
Post-ictal state	
Cerebral tumour	
Psychiatric	
Wernicke's encephalopathy	

➔ OCHM 10e, p. 576.

Constipation

Patients may use the term constipation to mean infrequent, hard, small volume, or difficult to pass faeces. Patients vary enormously in their threshold to seek medical advice about bowel habit.

Ask about:

- Associated pain.
- PR bleeding.
- Tenesmus.
- Weight loss.

Causes

- Carcinoma of the colon.
- Diverticular disease.
- Anorectal disease—fissure or haemorrhoid.
- Benign stricture.
- Rectocele.
- Sigmoid volvulus.
- Hernia.
- Drugs, especially analgesics.
- Poor fluid intake.
- Low-fibre diet.
- Change in diet.
- Immobility.
- IBS.
- Megarectum.
- Hirschsprung's disease.
- Spinal cord lesion.
- Stroke.
- Jejunal diverticulosis.
- Hypothyroidism.
- Diabetic neuropathy.
- Hypercalcaemia, hyperparathyroidism, hypokalaemia.
- Uraemia.
- Porphyria.
- Pregnancy.
- MS.
- PD.
- Dermatomyositis.
- Myotonic dystrophy.
- Scleroderma.
- Psychological.

Investigations

- Digital rectal examination.
 - Proctoscopy.
 - Sigmoidoscopy.
 - Colonoscopy.
 - Barium enema.
 - U&E.
 - Ca^{2+} .
 - TFTs.
 - FBC.
 - Bowel transit time studies.
 - Anorectal manometry.
 - Electrophysiological studies.
 - Defecating proctography.
- Elderly patients are more prone to constipation.
 *OHCM 10e*, pp. 260–1, p. 534.

Cyanosis

Cyanosis is a blue/purple dusky discolouration of tissue caused by a rise in blood deoxygenated Hb content ($>5\text{g/dL}$). Rarely it may be caused by ↑ sulphhaemoglobin, methaemoglobin, or COHb. Cyanosis may be peripheral affecting only cutaneous areas, or central when mucous membranes of the mouth and tongue are also discoloured.

Causes of peripheral cyanosis

- Central cyanosis.
- Shock.
- Hypothermia.
- Mitral stenosis.
- Raynaud's syndrome.
- Peripheral arterial disease.
- Patent ductus arteriosus (differential cyanosis, i.e. cyanosed toes, but not fingers, is pathognomonic of this condition).

Causes of central cyanosis

Pulmonary disease with severely impaired oxygen transfer

- Pneumonia.
- Asthma.
- Chronic obstructive pulmonary disease (COPD).
- PE.
- Fibrosing alveolitis.

Right-to-left shunt (Eisenmenger's syndrome)

- Atrial septal defect.
- VSD.
- Patent ductus arteriosus.
- Partial anomalous pulmonary venous drainage (APVD).
- AVM.

Methaemoglobinaemia, sulphaemoglobinaemia, carboxyhaemoglobinaemia

- Congenital.
- Ingestion of oxidizing agents, e.g. phenacetin, inorganic nitrates, local anaesthetic.

Cyanosis arising from pulmonary disease can be reversed by administration of oxygen (O_2) to improve alveolar O_2 uptake. O_2 has no effect where right-to-left shunts are the cause. Central cyanosis may be underestimated with significant anaemia and is more apparent in patients with polycythaemia. In methaemoglobinaemia, the arterial concentration of O_2 is normal. This condition can be treated with IV methylthioninium chloride (methylene blue) (☞ Chapter 11).

Investigations

- FBC.
- ABGs.
- CXR.
- 12-lead ECG.
- TTE (proceeding to TOE if shunt is suspected).
- CT chest (if AVM is suspected).
- Cardiac MRI (if APVD is suspected).

☞ OHCM 10e, p. 34.

Diarrhoea

Patients may use the term diarrhoea to describe loose stools, ↑ frequency of defecation, ↑ volume of stool, steatorrhoea, melaena, or faecal incontinence (☞ Incontinence: faecal, p. 60).

Ask about

- Duration.
- Associated features (abdominal pain, vomiting, mucus, or blood PR).
- Systemic symptoms.
- Recent foreign travel.
- Suspect food.
- Is anyone else in the household affected?

Causes

- Infection (including 'traveller's diarrhoea').
- IBD.
- Diverticular disease.
- Colonic carcinoma.
- Other tumour, especially villous adenoma.
- Coeliac disease.
- Tropical sprue.
- IBS.
- Ischaemic colitis/bowel infarction.
- Laxative use!
- Other drugs, e.g. metformin, orlistat.
- Overindulgence in fruit or vegetables.
- Overflow 2° to constipation.
- Carcinoid syndrome (uncommon).
- Gastrinoma (rare).
- VIPoma (rare).
- Glucagonoma (very rare).
- Hyperthyroidism (common).
- Medullary carcinoma of the thyroid (uncommon).
- Bile salt diarrhoea (previous ileal disease or surgery).
- Dumping syndrome (previous gastric surgery).
- Gut motility disorders.
- Malabsorption (cf. pancreatitis, lymphangiectasia, coeliac).
- Lactose intolerance.
- Scleroderma.
- Amyloidosis.
- Whipple's disease.

Investigations

- Stool culture, hot stool for parasites.
- *Clostridium difficile* toxin in stool.
- High rectal swab for parasites. (Note: giardiasis is diagnosed on duodenal biopsy.)
- Rectal examination, proctoscopy, sigmoidoscopy ± biopsy.
- Colonoscopy.

- AXR.
- Barium enema.
- Small bowel follow-through contrast studies.
- Upper GI endoscopy.
- Small bowel biopsy.
- FBC and blood film.
- ESR.
- CRP.
- Serum ferritin and folate.
- U&E (exclude haemolytic uraemic syndrome (HUS), especially in children).
- Urine screen for laxatives.
- Antigliadin, antiendomysial antibodies and anti-tissue transglutaminase (tTG) (coeliac disease).
- TFTs.
- Serum gut hormone profile (gastrin, vasoactive intestinal peptide (VIP), glucagon—seek expert advice).
- 24h urine for 5-hydroxyindole acetic acid (5HIAA).
- Serum calcitonin (medullary carcinoma of the thyroid).
- Lactose hydrogen breath test (for lactose intolerance).
- ¹⁴C-xylose breath test (bacterial overgrowth in the small bowel).
- CT abdomen.
- Mesenteric angiography (ischaemia).

Investigations must be guided by history and examination findings. If the patient is an inpatient, they should be isolated until infection is excluded. Consider HIV and other immune disorders if an unusual bowel organism is found.

Dizziness and syncope

Dizziness is a term that may be used to describe a variety of symptoms, e.g. spinning (rotatory vertigo), light-headedness, muzzy feeling, or unsteadiness on walking. It is therefore important to establish precisely what the patient means by dizziness.

Loss of consciousness or 'blackout' may not be reported by the patient and an eyewitness account is important. Enquire about any awareness of abnormal heart beat (rhythm-induced syncope), chest pain (ischaemia), neurological symptoms (cerebrovascular disease), preceding micturition, change of posture, or unusual sensations (prodromal epileptic symptoms, e.g. strange taste or smell) prior to the collapse.

Causes of dizziness

- Rotatory sensation lasting >10s and precipitated by movement or position—vestibular cause such as labyrinthitis, Ménière's disease, cerebello-pontine angle tumour (acoustic neuroma).
- Rotatory sensation lasting 2 or 3s and precipitated by movement—cervical spondylosis.
- Non-rotatory sensation lasting 2 or 3s and precipitated by movement, position, or standing up—cervical spondylosis, cerebrovascular disease, postural hypotension, cardiac arrhythmia (usually back to normal in minutes), epilepsy (incontinence is common and return to normal may take hours).

Investigations

Suspected vestibular cause

- Halpike manoeuvre.
- MRI or CT cerebello-pontine angle.
- Audiometry.

Suspected non-vestibular cause

- Blood glucose.
- 12-lead ECG.
- 24h ambulatory ECG monitoring.
- EEG.
- MRI or CT head.
- Tilt table test.

Causes of syncope

- Vasovagal: pain, fear, prolonged standing, excess heat, alcohol, or food.
- Micturition (often elderly men standing up during the night to urinate).
- Defecation (often elderly women with constipation).
- Coughing: chronic airways disease.
- Orthostatic hypotension:
 - Autonomic dysfunction (diabetic neuropathy, Shy–Drager syndrome).
 - Drugs (antihypertensives, diuretics, nitrates, tricyclics; dehydration and sodium depletion).
- Carotid sinus syndrome.
- Epilepsy.

- Drugs: alcohol, illicit drugs.
- Cardiac: arrhythmias, outflow obstruction (aortic stenosis, hypertrophic obstructive cardiomyopathy, myxoma).
- Hyperventilation and anxiety.
- Acute cerebrovascular disease: transient ischaemic attack (TIA), stroke, SAH.
- Acute vascular obstruction: PE, MI.
- Hypoglycaemia: poorly controlled diabetes.

Investigations

- 12-lead ECG.
- 24h Holter monitoring.
- Echocardiography.
- MRI or CT head.
- Tilt table test.
- Blood glucose, HbA_{1c}.
- U&E.
- Cardiac markers.
- ABG.
- Toxicology screen.
- V/Q scan.

► Driving and dizziness/syncope

For guidance on driving in the United Kingdom (UK), see  <http://www.dvla.gov.uk>.

Further reading

Brignole M, Alboni P, Benditt DG, et al.; Task Force on Syncope, European Society of Cardiology. Guidelines on management (diagnosis and treatment) of syncope. *Eur Heart J* 2004; 25: 2054–72.

Dysarthria and dysphasia

Dysarthria is difficulty in articulating words. The patient may complain of ‘slurred speech’. Dysphasia is a difficulty in the formation of speech due to interference with higher mental function. These disturbances often occur together, most commonly in the context of a stroke.

Damage to Wernicke’s area causes a *receptive dysphasia*. Speech may be fluent, but meaning is lost. Damage to Broca’s area causes an *expressive dysphasia*. Speech is non-fluent and the patients are aware they are not using the right words.

Causes of dysphasia include stroke (usually with right hemiparesis, arm more affected than leg) or SOL. Psychosis, especially schizophrenia, may cause a similar picture—the so-called ‘word salad’.

Causes of dysarthria

- Stroke (internal capsule or extensive lesion of the motor cortex—acute).
- Motor neurone disease (MND).
- Midbrain or brainstem tumour.
- PD.
- Cerebellar disease (haemorrhage, infarct, MS, hereditary ataxia, alcoholic or paraneoplastic degeneration).
- Syringobulbia (chronic, progressive).
- Neuromuscular (myasthenia gravis (MG), dermatomyositis, myotonic dystrophy).
- Acute alcohol or drug intoxication.

Dysarthria may be more obvious when the (English-speaking!) patient is invited to say ‘*Baby hippopotamus*’, ‘*British constitution*’, etc.

Investigations

- Cranial CT scan.
- Venous plasma glucose.
- ESR.
- Serum lipids.
- 12-lead ECG.
- Echocardiogram.
- Carotid Doppler studies (especially if bruit).
- CXR.
- LFTs.

Less commonly

- Serum muscle enzymes (polymyositis).
- Autoimmune profile.
- Electromyogram (EMG).
- Skeletal muscle biopsy.

 OHCM 10e, pp. 86–7.

Dysphagia

Dysphagia is difficulty in swallowing. The patient may have associated odynophagia (painful swallowing) or regurgitation of food (immediate or delayed?). Elicit whether the dysphagia is for liquid, solids, or both. Is it intermittent or progressive? Are there associated symptoms?

A careful physical examination is mandatory. Pay special attention to the lower cranial nerves; search for lymph nodes in the supraclavicular fossae. Palpate the thyroid and percuss for retrosternal enlargement.

Causes

- Oesophageal carcinoma.
- Benign oesophageal stricture 2° to chronic acid reflux.
- Barrett's oesophagus.
- Achalasia or diffuse spasm.
- Stroke (bilateral internal capsule cerebrovascular accidents (CVAs)—pseudo-bulbar palsy).
- Oesophageal web (+ iron deficiency anaemia = Plummer–Vinson (Patterson–Kelly–Brown) syndrome).
- Pharyngeal pouch.
- Muscular problem (MG, dermatomyositis, myotonic dystrophy).
- Bulbar palsy (MS, MND, poliomyelitis).
- Scleroderma (including CREST syndrome— OHCM 10e, Chapter 12).
- Infection (usually acute pain on swallowing).
- Mediastinal mass (goitre, carcinoma of the bronchus, enlarged left atrium, aortic aneurysm).

Investigations

- FBC.
- ESR.
- Upper GI endoscopy.
- Barium swallow.
- CXR.
- Oesophageal manometry studies ( Gastrointestinal physiology, pp. 526–7).
- Cranial CT or MRI (if neurological signs).
- Acetylcholine (ACh) receptor antibodies and Tensilon® (edrophonium). test if MG is suspected ( Edrophonium (Tensilon®) test, p. 631).

Note: consider HIV testing if there is oesophageal *Candida*, or herpes simplex or cytomegalovirus (CMV) infection in the oesophagus.

 OHCM 10e, p. 64, pp. 250–1.

Facial pain

Is the pain unilateral or bilateral? Is it constant or intermittent? Precipitating factors or trigger points? A full examination of the head and neck is required in addition to a detailed neurological and systemic examination.

Causes

- Trigeminal neuralgia (TN).
- Temporal arteritis (TA). ►► Risk of visual loss (眼科 OHCM 10e, Chapter 11).
- Herpes zoster (shingles or post-herpetic neuralgia).
- Dental caries, sepsis.
- Sinusitis.
- Temporomandibular joint dysfunction.
- Cluster headache.
- Glaucoma.
- Angina pectoris.
- Tonsillitis.
- Syringobulbia.
- Atypical facial neuralgia.
- Migraine.

Investigations

- ESR—urgent in suspected TA.
 - Temporal artery biopsy if TA strongly suspected. (►► Must be performed rapidly—*within days*—if steroid treatment is commenced. However, do not withhold corticosteroid therapy for this reason!) Because of ‘skip’ lesions, false –ve biopsies may be encountered. Be guided by the full clinical picture, rather than reliance on a single test.
 - Plain radiographs or CT imaging of frontal or maxillary sinuses.
 - MRI to exclude MS, basilar aneurysm, trigeminal schwannoma, neurofibroma as causes of TN.
 - MRI of the cervical spinal cord to exclude syringobulbia if pain is accompanied by brainstem signs.
- 眼科 Headache, pp. 49–50 and 眼科 OHCM 10e, p. 64, pp. 456–7.

Fever of unknown origin (FUO or PUO)

Defined as temperature $>38.3^{\circ}\text{C}$ on several occasions, lasting 3 weeks or more. It is very important to take a full history and consider infectious contacts, recent travel abroad, recent surgery and dental treatment, sexual history, and risk factors for HIV.

Signs

Examine for heart murmurs, splinter haemorrhages, splenomegaly, lymphadenopathy, and rashes/pruritus (see Table 1.6).

Table 1.6 Causes of FUO/PUO

Infection	Abscesses (e.g. subphrenic, pelvic, lung), osteomyelitis, TB, endocarditis, parasites, rheumatic fever, brucellosis, toxoplasmosis, Lyme disease, histoplasmosis, viral (especially Epstein–Barr virus, CMV, hepatitis, and HIV)
Malignancy	Lymphoma, leukaemia, hypernephroma, ovary, lung, hepatoma
Connective tissue	Polyarteritis nodosa, SLE, RA, Still's disease, TA
Other	Sarcoidosis, atrial myxoma, drug fever, IBD, factitious

Investigations

- Re-take the history and re-examine the patient (something might have been missed or new symptoms/signs may have developed).
- FBC, ESR.
- U&E, LFTs, Ca^{2+} .
- CXR.
- MSU, urinalysis.
- Serology for *Brucella* and *Toxoplasma*.
- All biopsy material should be sent for culture, including TB.
- Blood cultures (serial may be necessary).
- Monospot/Paul Bunnell.
- Autoimmune profile (antinuclear antibodies (ANA), rheumatoid factor (RF), ANCA, etc.).
- Bone marrow aspirate/trephine/culture for TB with Ziehl–Neelsen (ZN) stain.
- Abdominal USS (? masses).

Extend investigations as below according to symptoms and signs

- Consult microbiology or infectious disease consultant for advice.
- Stool cultures and fresh stool for ova, cysts, and parasites.
- Repeat serological investigation for changing titres (2–3 weeks).
- Thick and thin blood film for malaria and parasites.
- Mantoux.
- TTE or TOE to exclude endocarditic vegetations.
- CT chest, abdomen, and pelvis.

►► Always re-examine the patient for evolving new signs if the cause remains unknown.

⇒ OHCM 10e, pp. 442–3.

First fit

►► A first fit in an adult requires careful evaluation since the probability of an underlying structural lesion ↑ with age.

Take a careful history, preferably from a witness as well as the patient. Most lay persons will recognize a generalized tonic–clonic fit. However, the occurrence of a few 'epileptiform' movements in patients with syncopal episodes (☞ Dizziness and syncope, pp. 34–5) may cause diagnostic uncertainty.

Be sure to ask about

- Aura preceding the episode. ► Temporal lobe epilepsy—olfactory or gustatory auras (not necessarily followed by convulsions).
- Loss of consciousness—how long? Often overestimated by witnesses!
- Tongue biting.
- Focal or generalized convulsive movements. Note: a clear history of a tonic–clonic fit commencing in a limb and progressing to a more generalized convulsion is highly suggestive of a structural intracerebral lesion; cranial imaging is mandatory.
- Central cyanosis (tonic phase).
- Urinary incontinence.
- Injuries.
- Post-ictal confusion.
- History of trauma.
- Alcohol intake. Remember: alcohol withdrawal fits as well as acute intoxication.
- Drug history—prescribed and recreational.
- History of insulin-treated diabetes or type 2 diabetes treated with oral secretagogues, i.e. sulfonylureas, repaglinide, nateglinide. Note: metformin and thiazolidinediones as monotherapy do not cause significant hypoglycaemia.

A full general and neurological examination is needed, specifically including:

- Fever.
- Meningism, i.e. nuchal rigidity, +ve Kernig's sign (meningoencephalitis).
- Cutaneous rash or ecchymoses (? bleeding diathesis).
- Evidence of head trauma (preceding fit or as a consequence).
- Signs of chronic liver disease.
- Focal neurological deficit. ► Third nerve palsy in an intracranial SOL, including aneurysm of the posterior communicating artery. Sixth nerve lesion may act as a 'false localizing sign' in ↑ ICP.
- MedicAlert™ bracelet (history of epilepsy or diabetes—search personal belongings).

Bilateral extensor plantar reflexes can occur after a generalized fit without a structural brain lesion and there may be transient hemiparesis (Todd's paresis).

Causes

- Epilepsy (➡ OCHM 10e, Chapter 10).
- Hypoglycaemia (acute, severe, history of diabetes?).
- Hyponatraemia (usually <110mmol/L or rapid development).
- Hypocalcaemia (➡ OCHM 10e, Chapter 14).
- Hypomagnesaemia (may accompany hypocalcaemia).
- Hypophosphataemia (rare).
- Alcohol withdrawal. ►► Risk of associated hypoglycaemia.
- Discontinuation of anticonvulsant medication.
- Infection—viral encephalitis or bacterial meningitis. ►► Consider intracerebral abscess, tuberculoma in predisposed patients.
- Encephalopathy—hepatic, uraemic, hypertensive, thyrotoxic (rare—‘thyroid storm’).
- Eclampsia.
- Porphyria.
- Cerebral SLE.
- Head injury.
- Hypoxia.
- Cerebral tumour.
- Stroke—cerebral infarct, haemorrhage.

Investigations

- Venous plasma glucose (fingerprick test at bedside useful as ‘screen’—but can be unreliable).
- U&E.
- Serum Ca^{2+} , magnesium (Mg^{2+}), phosphate (PO_4^{3-}).
- Cranial CT or MRI scan.
- EEG.
- LP (➡ Lumbar puncture, pp. 584–9).
- CXR.
- Serum prolactin (PRL) (may be ↑ after generalized convulsions, but not pseudo-seizures).
- ABGs—remember transient lactic acidosis following generalized tonic-clonic convulsions.
- Blood ethanol (may be undetectable in withdrawal state).
- Serum or urine drug screen.

‘Pseudo-seizures’ may be encountered in patients with atypical recurrent fits (usually long history of epilepsy) and this is unlikely in an adult presenting with a first fit. ► In UK, the DVLA prohibits driving for 12 months following a first fit.

➡ OCHM 10e, pp. 490–2.

Galactorrhoea

Denotes inappropriate breast milk production, i.e. in the absence of pregnancy. The commonest cause is hyperprolactinaemia (\uparrow PRL) due to a pituitary microprolactinoma of <10mm in diameter (→ Precocious puberty, p. 179). Prolactinomas (usually macroadenomas) may cause galactorrhoea in men.

Note: other disease in the pituitary region, certain drugs, and several systemic disorders may be associated with \uparrow PRL (→ OHCM 10e, Chapter 5).

Causes

Normoprolactinaemic galactorrhoea

- This has been described in premenopausal women occurring after the conclusion of:
 - Treatment with the combined contraceptive pill.
 - Breastfeeding (for >6 months afterwards).
- \uparrow sensitivity of lactogenic tissue PRL is postulated, but the mechanism remains uncertain. In part, this may reflect difficulties that can arise in determining whether PRL is persistently elevated. Menstrual disturbances have been described.

Hyperprolactinaemia

- The differential diagnosis and investigation of hyperprolactinaemia are considered in → Galactorrhoea (hyperprolactinaemia), pp. 172–3.

Investigations

- Serum PRL (→ Galactorrhoea (hyperprolactinaemia), pp. 172–3).
- Repeated measurements under controlled conditions may be required since PRL is a 'stress' hormone and may be \uparrow by venepuncture.

Note: if \uparrow PRL is confirmed, further investigations to exclude causes other than a prolactinoma are required.

- Pituitary imaging (CT, or preferably MRI) and visual field testing (Goldmann) may also be indicated if a macroprolactinoma is suspected (PRL concentrations usually very high).

Note: if there is doubt about the nature of the nipple discharge, further specialized investigations may be required on the fluid, including:

- Casein.
- Lactose.
- Microscopy.

Clear fluid may result from benign breast disease.

Note: bloody discharge should prompt urgent specialist investigations to exclude carcinoma of the breast:

- Mammography.
- Biopsy.

→ OHCM 10e, p. 237.

Further reading

Kleinberg DL, Noel GL, Frantz AG. Galactorrhoea: a study of 235 cases, including 48 with pituitary tumors. *N Engl J Med* 1977; **296**: 589–600.

Gout

Gout is a disease of deposition of monosodium urate monohydrate crystals in tissues and relates to hyperuricaemia. Hyperuricaemia is due to an imbalance between purine synthesis and uric acid excretion. Episodes of acute gout may be precipitated by alcohol, trauma, dietary changes, infection, chemotherapy, or surgery. Commoner in men and very rare in premenopausal women.

Clinical features

- Inflammatory arthritis, classically monoarthritis or oligoarthritis affecting the first metatarsophalangeal (MTP) joint of the foot but can affect any joint, including the spine.
- Tenosynovitis.
- Bursitis or cellulitis.
- Tophi—urate deposits in tendons, ear pinnae, and joints.
- Urolithiasis and renal disease.

Investigations

- ESR (may be ↑).
- Urate crystals demonstrated in the synovial fluid or tissues—negatively birefringent on polarized light microscopy.
- Serum urate (not always ↑ in an acute episode, and a normal urate level does not exclude the diagnosis).
- X-ray—soft tissue swelling and punched-out bony erosions.
- Autoimmune profile (AIP) (to exclude rheumatoid).
- Microscopy of synovial fluid (Gram stain and culture).

Treatment

Acute episode

- NSAIDs, colchicine, intra-articular steroids, or oral steroids.
- Avoid precipitating factors and purine-rich foods.
- Urate-lowering therapy indicated for tophi, recurrent attacks, and urine/renal disease, e.g.
 - Allopurinol (xanthine oxidase inhibitor).
 - Probenecid (uricosuric).

Note: asymptomatic hyperuricaemia is commoner than gout, and a high serum urate level with coexistent arthritis is not necessarily due to crystal deposition. Consider important other causes, especially infective arthritis and pseudo-gout.

Pseudo-gout

Calcium pyrophosphate crystal deposition causing acute arthritis or chondrocalcinosis. Crystals are weakly positively birefringent on polarized light microscopy. Associations include old age, dehydration, hyperparathyroidism, hypothyroidism, haemochromatosis, acromegaly, RhA, and osteoarthritis (OA).

➲ OHCM 10e, p. 548.

Gynaecomastia

Gynaecomastia is benign bilateral hyperplasia of glandular and fatty breast tissue in the ♂. The balance between androgens and oestrogens is thought to be of importance in the pathogenesis; many conditions may influence this ratio. Most commonly, it appears transiently during normal puberty (detectable at some stage in ~50% of cases). Gynaecomastia may also be caused by specific endocrine disease or be associated with certain chronic diseases. Treatment with certain drugs is a common cause (~30% of cases) and arises via several mechanisms. Investigations will be guided by the individual circumstances. A careful drug history and thorough physical examination are required, particularly in the post-adolescent period.

When indicated, and after excluding causes such as congenital syndrome and drug therapy, investigations are principally directed at:

- Excluding endocrine carcinoma (rare).
- Identifying associated chronic diseases.

Note:

- Simple obesity is not usually a cause of true gynaecomastia, i.e. the glandular element is not ↑.
- ↑ serum PRL in isolation does not cause gynaecomastia.
- Unilateral, eccentric breast enlargement should prompt exclusion of breast carcinoma (rare).

Causes include

- Physiological states (transient):
 - Newborn.
 - Puberty.
 - Advanced age.
- Klinefelter's syndrome (47,XXY; mosaics).
- 2° hypogonadism, e.g. mumps orchitis.
- Androgen resistance syndromes, e.g. testicular feminization.
- ↑ tissue aromatase activity (converts androgens to oestrogens).
- Oestrogen-producing tumours:
 - Leydig cell tumour.
 - Sertoli cell tumour.
 - Adrenal carcinoma.
- Chronic liver disease.
- Chronic renal failure.
- Panhypopituitarism.
- Tumours producing human chorionic gonadotrophin (hCG).
- Drugs: oestrogens (prostatic carcinoma, transsexuals), spironolactone, cimetidine, digoxin, cytotoxic agents, marijuana.
- Hyperthyroidism (↑ serum sex hormone-binding globulin (SHBG)).
- 1° hypothyroidism.
- Cushing's syndrome.
- Carcinoma of the bronchus.
- Idiopathic.

Investigations

- Testosterone.
- FSH.
- LH.
- LFTs.
- TFTs.
- Oestradiol.
- β -hCG.
- PRL.
- SHBG (affinity of SHBG is higher for testosterone than for oestrogens, therefore ↑ SHBG causes disproportionate ↓ in free testosterone levels).
- Dehydroepiandrosterone sulfate (DHEAS).
- Androstenedione.
- Testicular USS.
- CXR.
- Abdominal CT or MRI imaging (for suspected adrenal tumours).
- Pituitary imaging.
- Karyotype.
- Urinary 17-oxo-steroids.

If carcinoma of the breast is suspected

- Mammogram.
 - Fine-needle aspiration (FNA).
- ⇒ OHCM 10e, p. 230.

Further reading

Braunstein GD. Gynecomastia. *N Engl J Med* 1994; 328: 490–5.

Haematemesis

This literally means vomiting blood and is often associated with melaena (passage of black tarry stools).

Causes

- Chronic peptic ulceration (e.g. duodenal ulcer (DU) or gastric ulcer (GU)) accounts for 50% of cases of bleeding from the upper GI tract.
- Acute GUs or gastric erosions.
- Drugs (e.g. NSAIDs) or alcohol.
- Reflux oesophagitis.
- Mallory–Weiss tear.
- Oesophageal varices.
- Gastric carcinoma (uncommon).

Investigations after admission and stabilization of the patient

- Full history, including drugs, alcohol, past history, indigestion, etc.
- FBC. (Note: Hb will take ~24h to fall; initially may be normal.)
- U&E.
- Cross-match blood.
- Urgent upper GI tract endoscopy.
- Check *Helicobacter pylori* serology ± urea breath test.

⇒ OHCM 10e, p. 30, p. 256.

Haematuria

In health, adults pass between 500,000 and 2,000,000 red cells over a 24h period. Haematuria implies the passage of excess blood that may be detectable using dipsticks (microscopic haematuria) or may be obvious to the naked eye (macroscopic haematuria).

Causes

- Many.
- Glomerular disease, e.g. 1° glomerulonephritis, 2° glomerulonephritis (SLE, vasculitis, infection).
- Vascular or interstitial disease due to hypersensitivity reactions, renal infarction, papillary necrosis, or pyelonephritis.
- Trauma.
- Renal epithelial or vascular tumours.
- Lower renal tract disease, e.g. tumours, stones, infection, drug toxicity (e.g. cyclophosphamide), foreign bodies, or parasites.
- Systemic coagulation abnormalities, e.g. platelet or coagulation factor abnormalities such as profound thrombocytopenia or DIC.

Investigations

- Urinalysis—dipstick, microscopic examination, culture.
- Radiology,* e.g. KUB or IVU.
- Specialist investigation,* e.g. angiography, CT or MRI scanning.
- Cystoscopy.*

Note: ideally these tests (*) should be arranged after discussion with either a nephrologist or a urologist.

 OHCM 10e, p. 80, p. 294.

Haemoptysis

This describes coughing up blood or bloodstained sputum and can vary from faint traces of blood to frank bleeding. Before embarking on investigation, it is essential to ensure that the blood is coughed up from the respiratory tract and is not that of epistaxis or haematemesis (easily confused).

Causes

- Infective, e.g. acute respiratory infection, exacerbation of COPD.
- Pulmonary infarction, e.g. PE.
- Lung cancer.
- TB.
- Pulmonary oedema.
- Bronchiectasis.
- Uncommon causes, e.g. idiopathic pulmonary haemosiderosis, Goodpasture's syndrome, microscopic vasculitis, trauma, haematological disease (e.g. ITP or DIC).

Investigations

- Colour of blood provides clues (pink frothy in pulmonary oedema, rust-coloured in pneumonia).
- Check oxygen (O_2) saturation.
- FBC (? ↓ platelets).
- ESR.
- Coagulation screen.
- Sputum culture.
- CXR.
- Arrange bronchoscopy after discussion with the respiratory team.

⇒ OHCM 10e, pp. 48–9.

Headache

⇒ Facial pain, p. 38.

Headache is an extremely common complaint. Most patients self-medicate and only a small proportion will seek medical advice. Headache may be acute or chronic, constant, recurrent, or gradually progressive. It may arise from structures within the cranial vault or from external causes (⇒ OCHM 10e, Chapter 10).

Causes differ according to age; temporal arteritis is very uncommon in patients under ~55 years, for example. Migraine may be associated with classic features (⇒ OCHM 10e, Chapter 10). Remember to enquire about the combined OCP—may exacerbate migraine. ‘Tension’ headaches predominate.

Causes in adults include

- ‘Tension’ headache (very common; usually recurrent and stereotyped).
- Migraine. Although common, many patients who believe they have ‘migraine’ probably have ‘tension’ headaches. Classic migraine predominantly affects adolescents and young adults.
- Cluster headaches.
- As part of a generalized viral illness, e.g. ‘flu’.
- Causes of ↑ ICP (⇒ OCHM 10e, Chapter 10).
- Acute infective meningitis (bacterial, viral most commonly).
- Encephalitis (most commonly viral, e.g. herpes simplex).
- Intracerebral haemorrhage.
- Post-traumatic (common).
- Intracerebral tumour (1° or 2°, benign or malignant).
- Acute SAH.
- Subdural haematoma.
- Acute glaucoma.
- Acute sinusitis.
- Rubeosis iridis (2° glaucoma in patients with advanced diabetic eye disease).
- TN.
- Referred pain, e.g. from dental caries or sepsis.
- Arterial hypertension; malignant or accelerated phase; essential hypertension is rarely the cause of headache.
- TA. ►► Visual loss preventable with prompt corticosteroid therapy (⇒ OCHM 10e, Chapter 10).
- Venous sinus thrombosis.
- Benign intracranial hypertension (mimics intracerebral tumour).
- Pneumonia caused by *Mycoplasma pneumoniae* may be associated with headache (meningoencephalitis).
- Nocturnal hypoglycaemia (often unrecognized) may cause morning headaches in patients with insulin-treated DM.
- Analgesia-withdrawal headache (⇒ OCHM 10e, Chapter 10).
- Hangover following alcohol excess.
- Otitis media.
- Chronic hypercalcaemia (rare).

Investigations

- ESR (► TA—exclude with urgency).
- CRP.
- FBC.
- U&E.
- Throat swabs.
- Blood cultures (if febrile).
- LP (→ Lumbar puncture, pp. 584–9).
- Skull X-ray (SXR) ± cervical spine X-ray.
- Sinus X-rays (may be local tenderness in sinusitis).
- Cranial CT (→ Computed tomography, pp. 598–600).
- CXR (cerebral metastases from bronchogenic carcinoma).
- Urinalysis.
- Intraocular pressure measurement and refraction.
- Cerebral angiography (if aneurysm or AVM).
- Serum Ca²⁺.

→ OHCM 10e, p. 64, pp. 456–7.

Heart sounds and murmurs

Auscultation of the heart should be conducted over several cardiac cycles. Heart sounds and murmurs are traditionally assessed at the apex, *lower left sternal edge*, *aortic area*, and *pulmonary area*, but they may radiate into other regions such as the axilla or carotid arteries. The carotid pulse should be palpated simultaneously in order to time cardiac events. The following should be identified:

- First (S1) and second (S2) heart sounds.
- Added heart sounds such as third (S3) or fourth (S4) heart sounds, opening snaps, ejection clicks, and prosthetic sounds.
- Murmurs, including location, intensity, and characteristics.

The *first heart sound* is produced by closure of the mitral and tricuspid valves. It is best heard at the apex and is timed just prior to the carotid pulse. The *second heart sound* is caused by closure of the aortic (A2) and pulmonary (P2) valves and is heard just after carotid pulsation. Closure of the pulmonary valve is slightly delayed relative to the aortic valve and so the second heart sound is normally split. This split is exaggerated by inspiration (see Table 1.7).

Normal and abnormal heart sounds are shown in Table 1.7.

The *third heart sound* is heard just after S2 and arises as a consequence of rapid ventricular filling and volume overload. The *fourth heart sound* occurs just before S1 and is caused by atrial contraction against a stiff ventricle or pressure overload. Abnormal valves may cause extra heart sounds on opening, e.g. an opening snap or ejection click. The heart sounds generated by artificial valve closure are referred to as prosthetic heart sounds. These should be crisp, not muffled (see Table 1.8).

Murmurs may be graded according to the following criteria

1. Very soft (just audible under optimal conditions).
2. Soft.
3. Moderate (easily heard with a stethoscope).
4. Loud ± palpable thrill.
5. Very loud/palpable thrill.
6. Heard without a stethoscope/palpable thrill.

Innocent murmurs are generated by turbulent flow such as in high cardiac output states, e.g. pregnancy, fever, anaemia, and thyrotoxicosis. They have the following characteristics:

- No accompanying thrill.
- Never > grade 3.
- Systolic.
- Maximal at the left sternal edge.
- Normal heart sounds.
- Normal pulses, ECG, and CXR.

Systolic murmurs are synchronous with the carotid pulse and caused by

- Abnormal regurgitation through a structure that is normally closed in systole, e.g. AV valve, septum (pansystolic).
- Normal systolic flow through a narrowed or stenosed valve, e.g. aortic valve, pulmonary valve (ejection systolic).

Table 1.7 Normal and abnormal heart sounds

Description	Diagram	Differential diagnosis
Normal		 Normal
Loud S1		 Hyperdynamic circulation—anaemia, fever, thyrotoxicosis Mitral stenosis Left atrial myxoma
Soft S1		 Low cardiac output Heart failure Tachycardia Mitral regurgitation Chronic obstructive pulmonary syndrome Systemic hypertension Dilated aortic root
Loud S2 (A2)		 Aortic stenosis Cardiac failure
Soft S2 (A2)		 Pulmonary stenosis
Soft S2 (P2)		 Pulmonary hypertension
Loud S2 (P2)		 Normal physiological splitting exaggerated in: right bundle branch block, pulmonary stenosis, pulmonary hypertension
Normal split S2		 Atrial septal defect
		
Fixed splitting S2		 Left bundle branch block Systemic hypertension Aortic stenosis
		
Reversed splitting S2		

Table 1.8 Heart murmurs

Pansystolic murmur		Mitral regurgitation Tricuspid regurgitation VSD
Late systolic murmur		Mitral valve prolapse Hypertrophic obstructive cardiomyopathy Aortic coarctation
Ejection systolic murmur		Aortic stenosis Pulmonary stenosis
Early diastolic murmur		Aortic regurgitation Pulmonary regurgitation
Mid-diastolic murmur		Mitral stenosis Tricuspid stenosis
Mixed murmur		Mixed valvular heart disease Patent ductus arteriosus AVMs Collateral circulations

Diastolic murmurs are audible after the carotid pulse and arise from

- Incompetence of the cardiac outflow valves, e.g. aortic or pulmonary valves.
- Narrowing of the cardiac inflow valves, e.g. mitral or tricuspid valves.

Mixed murmurs (systolic and diastolic) arise from

- Mixed valvular disease (stenosis and regurgitation).
- Patent ductus arteriosus.

Murmurs arising from left heart structures are accentuated in expiration, whereas right heart murmurs are augmented in inspiration (see Table 1.9).

Table 1.9 Added heart sounds

S3				Normal (young adult) Left ventricular failure Right ventricular failure Mitral regurgitation Constrictive pericarditis (pericardial 'knock') Normal (elderly) Left ventricular hypertrophy Left ventricular diastolic dysfunction Systemic hypertension Aortic stenosis Acute ischaemia
S4				
Opening snap				Mitral stenosis Tricuspid stenosis
Ejection click				Aortic stenosis Systemic hypertension Pulmonary stenosis Pulmonary hypertension
Mid-systolic click				Mitral valve prolapse
Prosthetic heart sounds				Artificial valve replacement

Hepatomegaly

Measure the liver edge below the (right) costal margin after percussing out the upper and lower borders. Bruits may be heard in hepatoma and a friction rub may occur with malignant deposits. Other signs may suggest the underlying diagnosis (☞ Pitfalls below).

Common causes

- CCF.
- Malignant deposits.
- Hepatitis/cirrhosis (usually alcoholic or infectious, e.g. Epstein–Barr virus (EBV), viral hepatitis).

Foreign residence?

If so, consider amoebic and hydatid cysts, schistosomiasis, and malaria.

Investigations

- FBC, film, LDH (leukaemia, lymphoma).
- ESR.
- Virology (EBV, CMV, and hepatitis A, B, and C antibody serology).
- LFTs—transaminases.
- Serum albumin.
- Prothrombin time (PT) (hepatocellular damage).
- γ -glutamyl transpeptidase (GGT), MCV (alcohol).
- ALP (obstructive causes; malignant deposits if isolated ↑).
- Serum Iggs may be polyclonal ↑ in immunoglobulin G (IgG) (autoimmune hepatitis), immunoglobulin A (IgA) (alcoholic liver disease), or immunoglobulin M (IgM) (PBC).
- Serum protein electrophoresis (myeloma, amyloid).
- Reticulocytes, bilirubin (if ↑, suggests haemolysis).
- Haemoglobinopathy screen (thalassaemia/sickle disorders).
- USS to assess liver texture, splenomegaly, lymphadenopathy.
- CXR and cardiac investigations (cardiomyopathies, sarcoid).
- α -fetoprotein (AFP) (primary hepatocellular carcinoma).
- Serum ferritin, transferrin saturation, DNA analysis (haemochromatosis).
- Mitochondrial antibodies and autoimmune markers, e.g. ANA (autoimmune hepatitis), ANCA (primary sclerosing cholangitis).
- Caeruloplasmin, urinary copper (Wilson's disease).
- α 1-antitrypsin (α 1-antitrypsin deficiency).
- Porphyria screen.

Pitfalls

- Hepatomegaly is a common sign but may not necessarily implicate liver pathology.
- End-stage cirrhosis may commonly present with a small, shrunken liver.

☞ OCHM 10e, p. 63, p. 604.

Herpes zoster

The pattern of the eruption varies from mild to dense with the involvement of several dermatomes. Complications may occur if involvement of the eye, motor nerves, and autonomic nerves (bladder), or when the disease presents as an encephalomyelitis or purpura fulminans.

►► In the immunocompromised host, zoster is more likely both to occur and to disseminate.

Investigations

- Confirm the diagnosis by isolation of the virus from the vesicular fluid.
- Consider underlying disorders if recurrent or severe attacks.
- Look for lymphadenopathy (Hodgkin's or other lymphoma).
- FBC, blood film, LDH (\uparrow in lymphoma).
- Serum protein electrophoresis (myeloma, amyloid).
- Serology for HIV (zoster is common in adult HIV individuals).
- Immunodeficiency work-up.

Pitfalls

The rash is not always unilateral—it may be bilateral.

➲ OHCM 10e, p. 462.

Hyperlipidaemia

Abnormalities of lipid metabolism are common in Western societies. Populations with high levels of cholesterol have high rates of vascular morbidity, especially cardiovascular disease (CVD), and premature death. Vascular risk can be estimated from published risk tables or calculators.

Various classifications of hyperlipidaemia exist, each with a characteristic lipid profile. Many patients with lipid disorders have cutaneous markers, which identify to a certain extent the type of lipid abnormality.

Clinical features

Lipid abnormalities may cause dermatological manifestations

- Grey-yellow plaques or xanthomata in tendons, especially the forearm and Achilles. Usually indicative of elevated low-density lipoprotein (LDL) cholesterol.
- Corneal arcus, a thin white rim around the iris—whilst this is common in the elderly, it is not a sign of ↑ LDL, except in the under 40s.
- Yellow, fatty deposits or xanthelasmata around the eyelids—associated with elevated LDL, these painless, non-tender plaques are common in the elderly.
- Yellow streaks in palmar creases—palmar xanthomata are associated with IDL cholesterol.
- Plaques over tibial tuberosities and elbows—tubero-eruptive xanthomata. Often seen with hepatosplenomegaly with elevated triglycerides.
- Eruptive xanthomata—in severe triglyceridaemia, associated with pancreatitis and hepatomegaly.

Hyperlipidaemia may be 2° to drugs such as corticosteroids, oestrogens, and progestogens, as well as a range of conditions such as hypothyroidism, myeloma, and alcoholism, each of which may be associated with specific clinical signs.

Hypertension

Blood pressure (BP) measurements are graded into a number of categories by the British Hypertension Society (see Box 1.1):

Box 1.1 British Hypertension Society grading of hypertension

Optimal blood pressure	<120/80
Normal blood pressure	<130/85
High-normal blood pressure	130–139/85–89
Grade 1 hypertension (mild)	140–159/90–99
Grade 2 hypertension (moderate)	160–179/100–109
Grade 3 hypertension (severe)	≥180/110

Hypertension should not be diagnosed on the basis of a single BP reading. Unless urgent treatment is required, e.g. malignant hypertension, the BP should be rechecked over a number of weeks to confirm the presence of sustained hypertension.

Causes

Remember that the cause of hypertension in most (95%) cases is unknown ('essential' hypertension). One of the following identifiable causes can be found in the remaining 5%:

- Renal disease, e.g. polycystic kidney disease.
- Renovascular disease, e.g. renal artery stenosis (RAS).
- Endocrine disease, e.g. Cushing's syndrome, Conn's syndrome, phaeochromocytoma, acromegaly.
- Coarctation of the aorta.
- Drugs, e.g. NSAIDs, OCP, steroids, erythropoietin (Epo), sympathomimetics, liquorice.
- Pregnancy, e.g. pre-eclampsia, eclampsia.

Routine investigation

The investigation of hypertensive patients has the following aims

- To confirm the presence and severity of hypertension.
- To assess overall cardiovascular risk.
- To identify target organ damage.
- To identify 2° causes (where present).

Routine investigations should include

- Urinalysis (protein, blood, glucose).
- U&E.
- Plasma glucose (ideally fasted).
- Lipid profile (ideally fasted).
- 12-lead ECG.

CXR, urine microscopy and culture, and echocardiography are not required routinely but should be considered where indicated by your initial assessment and investigation of the patient. The use of 24h ambulatory BP monitoring is often useful where clinic readings are thought to be unreliable because of 'white coat' hypertension.

Further investigation

Where more detailed assessment is required (for instance, to rule out a 2^o cause or to identify end-organ damage), the following investigations may be appropriate:

Renal investigations

- Renal USS (to assess overall renal morphology).
- Renal artery Doppler studies (for RAS).
- Renal artery magnetic resonance (MR) imaging (for RAS).
- Captopril renogram (for RAS).
- Renal angiography (for RAS).
- Renal vein renin measurements (for Conn's syndrome).

Endocrine investigations

- Renin and aldosterone studies for Conn's syndrome (consult your local endocrine laboratory).
- Investigations for Cushing's syndrome.
- Investigations for acromegaly.
- Urinary catecholamine (and metabolite) excretion.

Further reading

Williams B, Poulter NR, Brown MJ, et al. Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004—BHS IV. *J Hum Hypertens* 2004; **18**: 139–85.

Incontinence: faecal

➔ Alteration of bowel habit, p. 9; ➔ Constipation, pp. 29–30; ➔ Diarrhoea, pp. 32–3.

Causes include

- Any cause of diarrhoea (➔ *OHCM 10e*, Chapter 6).
- Overflow diarrhoea from severe constipation.
- IBD (acute or chronic).
- Coeliac disease (diarrhoea is a variable feature).
- Infectious diarrhoea (➔ *OHCM 10e*, Chapter 1).
- Hyperthyroidism (may cause diarrhoea; rare cause of incontinence).
- Carcinoma of the colon (stricture).
- Diverticular disease of the colon (acute attack, chronic stricture).
- Neurological (multiple CVAs, MS, spina bifida, post-childbirth neuropathy) may often be associated with sphincter disturbances.
- Drugs, e.g. laxatives, orlistat (causes fat malabsorption).
- Causes of steatorrhoea (➔ *OHCM 10e*, Chapter 6).
- Intestinal hurry, e.g. post-gastrectomy (➔ *OHCM 10e*, Chapter 6).
- Diabetic diarrhoea (autonomic neuropathy—rare; diagnosis of exclusion but may cause nocturnal faecal incontinence).
- VIPoma (very rare).

Investigations

Non-invasive tests

- Stool cultures (ova cysts, parasites). Note: *Clostridium difficile*—relatively common in patients who have received recent antibiotic therapy.
- FBC (anaemia, especially iron deficiency).
- CRP.
- ESR.
- U&E.
- TFTs.

Imaging

- Pelvic/abdominal X-ray.
- Barium enema.
- CT abdomen.

Procedures

- Colonoscopy.
- Sigmoidoscopy ± biopsy.

➔ *OHCM 10e*, p. 58.

Incontinence: urinary

➔ Anuria, p. 14.

Consider

- Common causes of polyuria (➔ OHCM 10e, Chapter 7); these may present as, or aggravate, urinary incontinence.
- Acute or chronic confusional state (common; loss of voluntary sphincter control).
- UTI (very common—always exclude).
- Drug-induced, e.g. thiazide or loop diuretics; α -adrenergic blockade, e.g. doxazosin (uncommon).
- Psychological, e.g. severe depression.
- Immobility, e.g. PD (Shy–Drager syndrome is uncommon).
- Other causes of autonomic neuropathy (➔ OHCM 10e, Chapter 10).
- Detrusor muscle instability.
- Urethral incompetence.
- Stool impaction.
- Spinal cord compression.
- Tabes dorsalis.

Investigations

- U&E.
- Urinalysis for blood, protein, glucose, nitrates, and nitrites.
- MSU for culture and sensitivity (C&S).
- Plasma glucose (if glycosuria).
- Serum Ca^{2+} .

In selected patients, consider referral to urology or gynaecology services for consideration of:

- Bladder manometry studies.
- Post-voiding USS of the bladder.
- Pelvic imaging, e.g. CT scan.

➔ OHCM 10e, pp. 648–9.

Indigestion

This term is often loosely used by patients to describe a variety of symptoms. These are often regarded as representing relatively minor, and usually intermittent, pathology. However, serious pathology, e.g. carcinoma of the stomach, may present as a vague complaint of 'indigestion'. The symptoms may be retrosternal or abdominal. A detailed history is essential, focusing on features that raise the probability of serious pathology, e.g. dysphagia and weight loss.

Examination

Examination should include a search for the following signs, particularly in the middle-aged and elderly patients:

- Anaemia (especially iron deficiency—common).
- Ascites.
- Troisier's sign (malignant involvement of the left supraclavicular lymph nodes due to carcinoma of the stomach—rare).

Note: the presence of associated pathologies, e.g. pernicious anaemia ( OHCM 10e, Chapter 8)—↑ risk of stomach cancer—will alter the threshold for more detailed expert investigation. Carcinoma of the stomach is commoner in Japanese.

Peptic ulceration may have classic elements that point to the diagnosis. Non-ulcer dyspepsia is very common and is often treated empirically with antacids, H₂ receptor antagonists, or H⁺ pump inhibitor drugs. The clinical challenge is to identify the patient for whom more detailed, and often invasive, investigation is indicated.

Alternative causes, e.g. cardiac ischaemia, should be considered in the differential diagnosis; similarities of the symptoms between cardiac and upper GI disorders are well recognized and sometimes pose considerable diagnostic difficulties.

Causes include

- Oesophageal acid reflux.
- Hiatus hernia.
- Inflammatory disease.
- Peptic ulcer disease of the duodenum or stomach.
- Biliary colic (usually distinctive clinical features).
- Malignancy of the oesophagus, stomach, or rarely small intestine.
- Cardiac symptoms, usually ischaemia.
- IBS.
- Symptoms arising from other structures within the chest or abdomen.

Investigations

- FBC.
- U&E.
- ESR.
- Upper GI endoscopy ± tissue biopsy.
- LFTs.
- CK if MI/acute coronary syndrome (ACS) suspected.
- Troponin (T or I) if MI/ACS suspected.

- Serum amylase (normal in chronic pancreatitis; may be ↑ by a duodenal ulcer eroding the posterior wall).
- Barium swallow and meal (for oesophageal disease).
- CLO test for *Helicobacter pylori*.
- Urea ^{13}C breath test for *H. pylori*.
- USS of the biliary tract (Ultrasound, p. 802).
- Cholecystogram.

If diagnosis remains uncertain, consider

- CT abdomen (discuss with a radiologist).
- Serum gastrin (Zollinger–Ellison syndrome, OHC 10e, p. 716).
- 24h ambulatory oesophageal pH monitoring.
- Oesophageal manometry (oesophageal motility disorders).

Infective endocarditis signs

Infective endocarditis is characterized by infection of the endocardial surface of the heart. The left heart valves are the most commonly affected, but the right heart valves and congenital heart lesions, such as VSDs, may also become infected. Vegetations (composed of the organism, white cells, platelets, and fibrous tissue) are formed at the site of infection. They give rise to periodic septicaemia and may embolize to other parts of the body. There is gradual destruction of the valve with ↑ valvular dysfunction, regurgitation, and heart failure.

Clinical features

- Pyrexia (low-grade or swinging).
- Pale conjunctivae suggestive of anaemia (of chronic disease).
- Clubbing (chronic low-grade infection).
- Cardiac murmur (new or changing).
- Left or right heart failure.
- Splenomegaly (friction rub if splenic infarction is present).
- Microscopic haematuria (on urinalysis).

Embolic phenomena

Embolic phenomena are common and produce clinical signs classically associated with infective endocarditis:

- Splinter haemorrhages (>5, sited in the proximal finger and toenail beds).
- Janeway lesions (palmar macular spots).
- Osler's nodes (painful nodules on the palmar surface of the fingers or toes).
- Roth spots (retinal haemorrhages).
- Conjunctival haemorrhages.
- Microvascular infarction (in the distal limbs).

Further reading

Prendergast BD. Diagnostic criteria and problems in infective endocarditis. *Heart* 2004; **90**: 611–13.
Task Force on Infective Endocarditis of the ESC. Guidelines on prevention, diagnosis and treatment of infective endocarditis. *Eur Heart J* 2004; **25**: 267–76.

Irregular pulse

In health, the pulse is usually regular; although a minor degree of variation in heart rate with respiration (sinus arrhythmia) is common, particularly in children and young adults. In sinus arrhythmia, the heart rate ↑ with inspiration and ↓ with expiration. This is a benign phenomenon.

An irregular pulse can present as a symptom (with the patient complaining of an awareness of irregular or 'missed'/'extra' heartbeats) or as a sign (incidental finding on clinical examination).

Pulse irregularities are traditionally classified into two groups

- Regular irregularities.
- Irregular irregularities.

A regularly irregular pulse

Most commonly the result of ventricular or supraventricular ectopic activity. Ectopic beats often occur after a certain number of sinus beats—thus in ventricular bigeminy, every other beat will be a ventricular ectopic beat and thus occur prematurely with reduced volume. In trigeminy, every third beat will be early.

A regularly irregular pulse

Can also be evident in second-degree AV block (Mobitz type I or II).

An irregularly irregular pulse is most commonly the result of

- Multiple ectopic beats (supraventricular or ventricular).
- AF.
- Atrial flutter with variable AV block.

Investigations

The key to diagnosis is to record an ECG, whilst the pulse irregularity is present. If the paroxysmal irregularity is infrequent, this can prove challenging. A 12-lead ECG is mandatory and may provide an immediate diagnosis. If not, a number of ambulatory ECG monitoring techniques are available:

- 24h ambulatory ECG monitoring.
- Cardiac event monitoring.
- Implantable loop recorder (ILR).

The choice of technique should be guided by how frequently the irregularity is thought to occur.

Additional investigations depend upon the nature of the suspected arrhythmia:

- FBC.
- U&E.
- TFTs.

One may also consider

- CXR (to assess heart size and valvular calcification).
- Echocardiogram (if structural heart disease suspected).
- Exercise treadmill test (if IHD suspected, or to provoke arrhythmias thought to be exercise-related).

Jaundice

This defines the yellow discolouration of the sclerae, mucous membranes, and skin that occurs when bilirubin accumulates. Bilirubin is the major bile pigment in humans and is produced as an end-product of haem catabolism. Jaundice usually only becomes noticeable when the serum bilirubin level is $>30\text{--}60\mu\text{mol/L}$.

Causes

(See Table 1.10.)

- Can be pre-hepatic, hepatic, or post-hepatic.
- Haemolysis.
- Hepatitis (viral, drugs, alcohol).
- Pregnancy.
- Recurrent cholestasis.
- Hepatic infiltration.
- Stones in the common bile duct.
- Carcinoma of the bile duct, head of the pancreas, or ampulla.
- Biliary strictures.
- Sclerosing cholangitis.
- Pancreatitis.

Investigations

(See Fig. 1.2.)

- FBC (? haemolysis).
- Clotting screen (often deranged in liver disease).
- LFTs.
- Viral serology for hepatitis A virus (HAV), hepatitis B virus (HBV), and hepatitis C virus (HCV).
- USS abdomen.
- Consider endoscopic retrograde cholangiopancreatography (ERCP).
- Liver biopsy may be indicated, depending on history, examination, and laboratory findings. Discuss with the gastroenterology team before embarking on this.

⇒ *OHCM 10e*, pp. 272–3.

Table 1.10 Common causes of jaundice

Pre-hepatic	Cholestatic	
	Intra-hepatic	Post-hepatic
Haemolysis	Viral hepatitis	Gallstones
Gilbert's syndrome	Drugs	Carcinoma (biliary tree, head of pancreas, ampulla)
Crigler–Najjar syndrome	Alcoholic hepatitis	Biliary stricture
Dubin–Johnson syndrome	Cirrhosis (any type)	Sclerosing cholangitis
	Pregnancy	Pancreatic pseudocyst
	Recurrent idiopathic cholestasis	
	Infiltration (e.g. amyloidosis, etc.)	

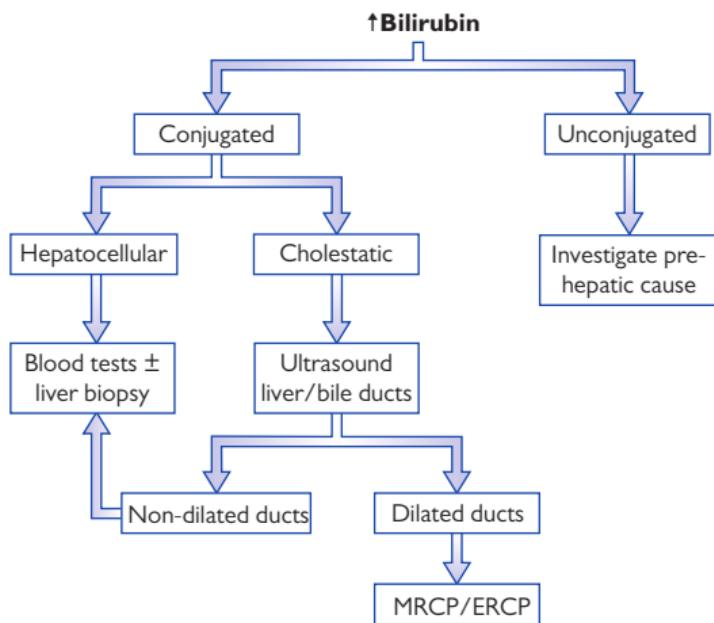


Fig. 1.2 Investigation of jaundice.

Joint pain/swelling

Covers a multitude of disorders including

- OA.
- RA.
- Tendinitis.
- Bursitis.
- Trigger finger.
- Mechanical low back pain.
- Fibromyalgia.
- Other arthropathies.

History and examination

- Ask about affected joints, site of origin, mono- or polyarticular, oligo-articular (e.g. 2–4 joints involved), migratory features, arthralgia (joint pain without swelling).
- Is the pain constant or intermittent?
- Aggravating or precipitating factors?
- Any associated neurological features?
- Is there swelling?
- Associated redness or excessive warmth?
- Drug history (e.g. diuretic-induced).
- Race (e.g. sickle).
- Past history.
- Family history.
- Occupational history.
- Social history.

Investigations

- FBC—a normochromic normocytic anaemia is common in chronic inflammatory disorders. May be microcytic if long-standing inflammation or associated iron deficiency (e.g. induced by NSAIDs).
- ESR—non-specific marker of inflammation.
- CRP—as for ESR.
- Biochemistry screen, especially looking at bone profile and LFTs.
- Consider serum IgG and protein electrophoresis (myeloma).
- Uric acid levels (gout).
- X-ray affected joint(s).
- Consider USS, especially if soft tissue swelling.
- MRI can be useful to help visualize intra-articular structures.
- CT scan.
- Bone scintigraphy (helps identify abnormal bone turnover).
- Dual X-ray absorptiometry (DEXA) scan (useful for diagnosis and monitoring of osteoporosis).
- Arthroscopy may help in selected cases.
- Joint aspiration (allows culture and examination of fluid for crystals).

Jugular venous pulse

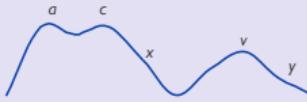
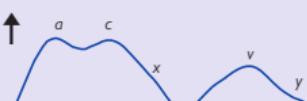
The height and waveform of the internal jugular venous pulse (JVP) reflect right atrial pressure and haemodynamics. The JVP should be inspected with the patient positioned at 45° to the horizontal. The JVP may be distinguished from the carotid pulse by the following features:

- Pulsation is not palpable.
- It may be compressed and obliterated by pressure.
- It rises on compression of the right upper quadrant (hepatojugular reflux).
- It varies with posture.
- Height ↓ with inspiration.

The height of the JVP is measured as the vertical distance between the manubriosternal angle and the top of the venous pulsation. Elevation is defined as >3cm.

There are several components of the jugular venous pulsation waveform. The *a* wave is produced by atrial systole. This is followed by the *x* descent at the end of atrial contraction. The *x* descent is interrupted by a small, barely perceptible deflection called the *c* wave. This deflection is caused by the rapid ↑ in right ventricular pressure just before the tricuspid valve closes. A subsequent *v* wave results from the rise in right atrial pressure as it fills with venous return during ventricular systole and whilst the tricuspid valve remains closed. At the end of ventricular systole, the tricuspid valve opens and the pressure in the right atrium falls, leading to the *y* descent (see Table 1.11).

Table 1.11 Jugular venous pulse waveforms

Description	Diagram	Diagnosis	Comment
Normal		Normal	
↑JVP Normal waveform		Right heart failure Fluid overload Pulmonary embolus Cardiac tamponade	↑ right atrial pressure
Absent a wave		Atrial fibrillation	Poor atrial contraction fails to generate a waves
Large a waves		Tricuspid stenosis Right ventricular hypertrophy	Resistance to right atrial emptying causes ↑ right atrial pressure
Large v waves		Tricuspid regurgitation	Reflux of blood into the great veins with right ventricular contraction
Cannon (a) waves		Complete heart block Ventricular tachycardia	Right atrium contracts against closed tricuspid valves, creating a cannon wave
Rapid y descent		Constrictive pericarditis Cardiac tamponade Tricuspid regurgitation Right heart failure	A steep y descent is caused by right ventricular diastolic collapse (Freidrich's sign)
Absent pulsation ↑JVP		Superior vena caval obstruction	No right atrial pressure can be transmitted to the JVP

Loin pain

Definition

Pain located in the renal angle.

Causes

- Ureteric colic.
- Renal or ureteric obstruction.
- Acute pyelonephritis.
- Renal infarction or papillary necrosis.
- Acute nephritis (uncommon).
- IgA nephropathy—pain caused by extension of the renal capsule.
- Musculoskeletal causes.
- Shingles at T10–12 (obvious if a rash is seen on examination or suspected if pain is in a dermatomal distribution).
- Infection or bleeding into a cyst in polycystic kidneys.
- Vesico-ureteric reflux—pain occurs when the bladder is full; this worsens at the initiation of micturition and then is rapidly relieved on voiding.
- Loin pain-haematuria syndrome—this is recurrent pain which occurs in young women. Angiography reveals tortuous vessels.

Investigations

- U&E.
- Serum creatinine.
- Creatinine clearance (CrC) (if renal impairment).
- FBC.
- ESR.
- Urine dipstick for protein, blood, nitrites, leucocytes.
- Urine microscopy (for casts).
- MSU for C&S testing.
- Blood cultures (if bacteraemia suspected).
- Plain X-ray (KUB view).
- IVU (e.g. if +ve urine dipstick for haematuria).
- Renal USS (useful for rapid, non-invasive exclusion of obstruction).
- CT of urinary tract.
- Angiogram (if suspicion of thrombus, embolus, or loin pain-haematuria syndrome).
- Serum IgA concentration.
- Cystoscopy (specialist procedure).
- Retrograde pyelography.
- Renal biopsy (only after specialist advice).

Lymphadenopathy

Lymph node enlargement may be localized or generalized (see Table 1.12).

Localized cervical lymphadenopathy

- Local causes in the mouth (pharyngitis, dental abscess).
- Scalp (skin malignancies or disease).
- Nose (nasopharyngeal carcinoma).

Enlargement of left supraclavicular nodes

- May suggest carcinoma of the stomach.

Isolated posterior cervical node enlargement

- Is less often due to malignancy.

Other causes

- Sometimes drugs may be associated with lymph node enlargement (phenytoin, antithyroid).

Table 1.12 Causes of lymphadenopathy

Infection	
Viral	Infectious hepatitis, EBV syndromes, HIV, rubella, varicella, herpes zoster
Bacterial	Streptococcal, staphylococcal, salmonella, brucellosis, <i>Listeria</i> , cat-scratch (<i>Bartonella</i>)
Fungal	Histoplasmosis, coccidioidomycosis
Chlamydial	
Mycobacterial	
Parasites	Trypanosomiasis, microfilaria, toxoplasmosis
Spirochaetes	Syphilis, yaws, leptospirosis
Connective tissue	RA, SLE, dermatomyositis, serum sickness
Drugs	e.g. phenytoin
Malignancy	
Haematological	Hodgkin's lymphoma, non-Hodgkin's lymphoma, acute and chronic lymphoid malignancies (chronic lymphocytic/lymphatic leukaemia (CLL), acute lymphoblastic leukaemia (ALL)), acute myeloid leukaemia (AML)
Non-haematological	Metastases from carcinomas (breast, bowel, lung, prostate, kidney, head and neck)
Endocrine	Thyrotoxicosis
Miscellaneous	Sarcoidosis, amyloidosis

Investigations

- FBC, blood film, LDH (leukaemia, lymphoma, Hodgkin's).
- Serology/virology/microbiology/other antigen detection tests:
 - Viral (EBV, hepatitis, CMV, HIV).
 - Bacterial (TB, bacterial endocarditis, syphilis).
 - Fungal (histoplasmosis).
 - Protozoal (toxoplasmosis).
- ANA (collagen disorder, systemic lupus).
- TFTs (hyperthyroidism).
- CXR (sarcoid, TB).
- USS/CT scan (to assess intra-abdominal, mediastinal/hilar lymphadenopathy).
- LFTs/hepatomegaly (\uparrow ALP suggests malignant deposits).
- Lymph node biopsy (groin nodes should usually be avoided because commonly enlarged due to skin and infectious disorders).
- BM (may confirm haematological malignancy).

Note: FNA, although easier to perform, may not be diagnostic and lymph node biopsy should be considered for microbiology and histology.

➔ OHCM 10e, p. 35, p. 594.

Although we have provided a large list of possibilities, common sense should be used in determining the cause. For example, an 80-year-old woman with axillary lymphadenopathy is unlikely to have cat-scratch disease! Common things are common.

Nausea

The so-called vomiting centre is located in the medulla oblongata and is stimulated by the chemoreceptor trigger zone in the fourth ventricle. There are many causes of acute and chronic nausea. These can be divided into GI and non-GI causes.

GI causes of nausea

- Food poisoning (viral, bacterial—common).
- Acute and chronic gastritis (remember *H. pylori*).
- Peptic ulceration.
- Biliary and renal colic.
- IBD.
- Cholecystitis.
- Appendicitis.
- Pancreatitis.
- Gastric outflow obstruction.
- Post-gastrectomy syndrome.
- Acute liver failure.
- Pseudo-obstruction of the bowel.

Investigations

- U&E.
- LFTs.
- ESR.
- CRP.
- Serum or urinary amylase.
- AXR (erect and supine—*beware perforated viscus*).
- Abdominal USS.

Consider:

- OGD.
- Barium swallow and meal.
- Isotopic gastric emptying studies.
- Oesophageal manometry.
- Oesophageal muscle biopsy (rarely indicated).

Non-GI causes

- Acute infections, e.g. UTI.
- Metabolic disorders, including:
 - Hypercalcaemia.
 - Ketoacidosis (diabetic, alcoholic).
 - Uraemia.
- Pregnancy. Note: hyperemesis gravidarum may be associated with ↑ free T4 (FT4), ↓ thyroid-stimulating hormone (TSH).
- Many drugs, notably opiates and digoxin toxicity (check serum levels).
- MI (nausea common; exacerbated by opiates).
- Acute glaucoma.

Investigations

- FBC.
- ESR.
- Venous plasma glucose.
- Urine dipstick (UTI).
- Serum Ca^{2+} .
- Serum drug levels, e.g. digoxin, theophylline.
- 12-lead ECG.
- CK.
- Troponin I.

Neurological causes

- Acute migraine.
- ↑ ICP.
- Acute labyrinthine lesions.
- Ménière's disease.
- Cerebellar lesions (e.g. infarct, haemorrhage, metastases, demyelination).

Investigations

- Cranial CT.
 - MRI if cerebellar lesion suspected.
 - Tilt table test (☞ Tilt table testing, pp. 494–5).
 - Audiometry (specialist technique).
- ☞ OHCM 10e, p. 70. Nystagmus.

Neck stiffness

The main concern in a patient with neck stiffness is that s/he may have meningitis which may result from infection or may reflect infiltration by a disease such as acute leukaemia.

Causes

- Bacterial infection.
- Viral infection.
- Fungal infection.
- TB.
- Infiltration by malignancy (e.g. acute lymphoblastic leukaemia (ALL), high-grade lymphoma, or sometimes acute myeloid leukaemia (AML)).
- Drug-induced.
- Contrast media (myelogram).
- Blood (e.g. post-SAH).
- Mechanical/trauma.
- Connective tissue disease, e.g. RhA.

Investigations

- CT scan of brain ± contrast.
 - LP if no ↑ ICP:
 - Glucose.
 - Protein.
 - M,C&S ± TB culture.
 - Xanthochromia if SAH suspected.
 - If patient immunocompromised, consider:
 - Polymerase chain reaction (PCR) for viruses, e.g. herpes simplex virus (HSV).
 - Toxoplasma serology.
 - India ink stain for *Cryptococcus*.
 - If considering malignancy, send cerebrospinal fluid (CSF) for cytopspin.
- ⇒ OHCM 10e, p. 478.

Nystagmus

An involuntary oscillatory or (more commonly) rapid jerking movement of the eyes that is rhythmic and repetitive. It results from acute or chronic lesions of the eight cranial nerves, brainstem, or cerebellum. The 'slow' phase is pathological, the rapid, rhythmic jerking phase (used arbitrarily to define the direction of nystagmus) being a corrective response. Nystagmus 'to the right' describes the direction of the quick phase. Such 'sawtooth' nystagmus may be evident in the horizontal or vertical plane (including 'downbeat' nystagmus of foramen magnum lesions) or as oscillations around a central point (e.g. in albinism).

Jerk nystagmus

Jerk nystagmus may be graded in severity, depending on whether:

- It occurs only in the direction of directed gaze.
- It occurs when eyes are in the midline, or
- It is present even on looking in a direction contralateral to the rapid movement.

Note: nystagmus (or, more correctly, nystagmoid jerks) may be induced by inappropriate testing, often being present at the extremes of gaze. Do not ask the patient to follow a visual target beyond ~30° of the midline when testing at the bedside.

In unilateral causes

Cerebellar nystagmus

Greatest when gaze directed towards the side of the destructive lesion.

Vestibular nystagmus

Greatest away from the side of the lesion.

Pathological nystagmus

May be due to labyrinthine and vestibular lesions—occurs in one direction only. If visual fixation is removed, nystagmus becomes worse.

Central lesions

Including brainstem lesions caused by, e.g. tumour, MS; cerebellar lesions or medial longitudinal fasciculus lesions leading to internuclear ophthalmoplegia (➡ OCHM 10e, Chapter 10) with ataxic nystagmus.

Investigations

- Positional nystagmus may be investigated by using the Hallpike manoeuvre (➡ OCHM 10e, Chapter 10). Abrupt alteration of the spatial position of the head (from supine, with the head below the bed, rapidly to a sitting position) will induce nystagmus. This will demonstrate benign positional vertigo (common), vestibular disorders, or brainstem lesions.
- Audiometry (specialized investigation).
- Auditory and visual evoked potentials (VEPs) may be pathologically reduced in MS. Examination of CSF may reveal oligoclonal bands (OCBs).

- MRI to include the brainstem. (Upbeat nystagmus will suggest a midbrain lesion and downbeat nystagmus will suggest a foramen magnum lesion.) MRI is superior to CT for demonstrating cerebellopontine angle lesions. Gadolinium enhancement is used to investigate acoustic neuromas.
- Ototoxicity can be caused by some drugs such as gentamicin and phenytoin. Acute poisoning with alcohol or barbiturates may cause transient nystagmus. Chronic alcoholism can lead to permanent cerebellar damage. Excessive doses of anticonvulsant drugs, e.g. phenytoin, are a common cause—measure serum concentrations of the drug.

 OHCM 10e, p. 70.

Obesity

The World Health Organization (WHO) defines obesity as a body mass index (BMI) $>30\text{kg}/\text{m}^2$ (Table 1.13).

Table 1.13 BMI

		BMI (kg/m^2)	
Underweight			<18.5
Normal			18.5–24.9
Overweight			>25.0 –29.9
Obesity	Class	I	30.0–34.9
		II	35.0–39.3
		III	>40

Note: central (abdominal) fat distribution—commoner in men—is associated with greater health risks. The waist-to-hip ratio, or simply the waist girth, can be used to identify levels at which long-term health risks warrant intervention (see Box 1.2):

Box 1.2 WHO BMI grading system

Men	$>102\text{cm}$
Women	$>88\text{cm}$

Aetiology

The great majority of obese subjects have no identifiable metabolic or hormonal defects and detailed investigation is rarely indicated. A chronic imbalance of the equation with energy intake (dietary calories) on the one hand and expenditure (resting metabolic rate + physical activity) on the other is thought to be responsible. Reduced levels of habitual activity allied to an abundance of energy-dense foods appears to account for the current pandemic of obesity and related disorders:

- Impaired glucose regulation.
- Type 2 diabetes mellitus (DM).
- Dyslipidaemia.
- Hypertension.
- CVD.
- OA.
- Impaired physical functioning.
- Gout.
- ↑ surgical risk.
- Depression.
- Certain cancers, e.g. bowel, breast.

Weight gain tends to occur in middle age; ♀ are more at risk than ♂. Socio-economic factors are also important.

Specific causes

Genetic

- For example, Prader–Willi syndrome, Laurence–Moon (Biedl–Bardet) syndrome.

Single gene defects

- For example, mutations of leptin (provides feedback from adipocytes to the hypothalamus about body fat stores) or its hypothalamic receptor (very rare).

Hypothalamic lesions

- Lesions which damage the ventromedial nucleus (the ‘satiety’ area) may lead to obesity.

Lesions include

- Trauma.
- Tumours—craniopharyngiomas and astrocytomas.
- Inflammation—such as TB and meningitis.
- Infiltration—histiocytosis and sarcoidosis.

Cushing's syndrome

- With ‘buffalo’ hump and central obesity.

Hypothyroidism

- Disputed, unless severe myxoedema, but hyperthyroidism is associated with unphysiological weight loss.

Insulinoma

- Often associated with moderate weight gain; rare.

Marked decreased motor inactivity

- For example, severe mental retardation or physical disability.

Investigations

- Weight (calibrated scales).
- Height (stadiometer).
- Waist circumference (maximal).
- BP (large cuff required).
- Venous plasma glucose (or oral glucose tolerance test (OGTT)).
- TFTs.
- LFTs (\uparrow non-alcoholic steatohepatitis in obese subjects).
- Fasting lipid profile (☞ Investigation of hyperlipidaemia, pp. 212–15).
- Serum urate.

Additional investigations

These may occasionally be indicated if clinical features give cause for suspicion of an organic cause:

- Cranial CT or MRI of the pituitary and hypothalamus.
- Investigations for Cushing's syndrome (☞ Obesity/hypercortisolism, pp. 142–6).
- Genetic testing (seek advice of the genetics service).

Further reading

Lean MEJ, Han TS, Seidell JC. Impairment of health and quality of life in men and women with a larger waist. *Lancet* 1998; 351: 853–6.

Oliguria

Causes

Acute renal failure (ARF)—distinguish pre-renal from renal and post-renal causes.

Pre-renal

- Severe sepsis.
- Hypovolaemia, e.g. GI haemorrhage, diuretics.
- Burn injury.
- CCF.
- Addison's disease.
- Acute pancreatitis.

Renal

- ATN (e.g. 2° to nephrotoxins such as aminoglycosides and radiological contrast media).
- Acute cortical necrosis.
- Renal infarction.
- Accelerated hypertension.
- Salicylate overdose.
- Hepatorenal syndrome.

Post-renal

- Renal calculi.
- Retroperitoneal calcinosis.
- Papillary necrosis.
- Bladder, prostate, and cervical tumours.
- Blocked urinary catheter (common!).

Investigations

- U&E.
- Serum creatinine.
- CrC.
- FBC.
- ESR.
- Autoimmune profile.
- LFTs.
- Urinary Na^+ excretion (<20 pre-renal, >40 ATN).
- Urine osmolality ($>500\text{mOsmol/L}$ = pre-renal, $<350\text{mOsmol/L}$ = ATN).
- Urine dipstick for blood, protein, nitrites, and leucocytes.
- Urine microscopy for casts.
- Renal USS (\pm biopsy in selected cases).
- IVU.
- CT pelvis.
- Investigation of renal stones:
 - Serum Ca^{2+} , phosphorus.
 - 24h excretion of oxalate, calcium, creatinine.

Palpitations

Patients generally use the term palpitations to refer to an awareness of an abnormally fast, forceful, or irregular heart rhythm. Palpitations can be physiological, as in the fast and/or forceful heart rhythm felt with exercise or anxiety, or pathological.

Common arrhythmias

Supraventricular arrhythmias

- Sinus tachycardia (⇒ Causes of sinus tachycardia, pp. 110–11).
- AF.
- Atrial flutter.
- Atrial tachycardia.
- AV re-entry tachycardias.
- Supraventricular ectopics.

Ventricular arrhythmias

- Ventricular tachycardia.
- Torsades de pointes.
- Ventricular ectopics.

Investigations

The key to diagnosis is to record a 12-lead ECG, whilst palpitations are present. Although simple in principle, infrequent paroxysmal palpitations can make this very challenging. A 12-lead ECG is mandatory and may provide an immediate diagnosis if the patient is experiencing palpitations as it is performed. As well as assessing the heart rhythm, it is important to inspect the 12-lead ECG for evidence of abnormal AV conduction (short PR interval, pre-excitation) or abnormal repolarization (long QT interval). Check also for evidence of an underlying structural heart disease, e.g. pathological Q waves indicative of a previous MI.

If the patient's palpitations are paroxysmal, a number of ambulatory ECG monitoring techniques are available:

- 24h ambulatory ECG monitoring.
- Cardiac event monitoring.
- ILR.

The choice of technique should be guided by how frequently the palpitations occur.

Additional investigations depend upon the nature of the suspected arrhythmia. It is generally prudent to check:

- FBC.
- U&E.
- TFTs.

One may also consider:

- CXR (to assess heart size and valvular calcification).
- Echocardiogram (if structural heart disease suspected).
- Exercise treadmill test (if IHD suspected, or to provoke arrhythmias thought to be exercise-related).

⇒ OHCM 10e, pp. 36–7, p. 94.

Pancytopenia

Pancytopenia (\downarrow Hb, \downarrow WBC, and \downarrow platelets) may occur because of bone marrow failure (hypoplasia) or inefficient production (myelodysplastic syndrome (MDS)) or peripheral destruction of cells or sequestration (splenomegaly/hypersplenism).

►► Pancytopenia usually means something is seriously wrong.

Bone marrow assessment is necessary to establish whether the marrow is hypocellular or hypercellular in the face of peripheral blood pancytopenia. If hypercellular, the cause may be an infiltrative process (due to leukaemia/carcinoma, granulomatous disease, fibrosis–myelofibrosis, osteosclerotic–osteopetrosis, increased macrophages–haemophagocytic syndromes due to viral infections). Causes of hypoplastic bone marrow failure may be hereditary (e.g. Fanconi's anaemia) or acquired (e.g. drugs). Critically ill patients may develop pancytopenia for multiple reasons (sepsis, haemorrhage, DIC).

Investigations

- FBC, film (aplastic anaemia usually presents with \downarrow lymphocyte count, but minor morphological changes).
- Reticulocytes (\downarrow if production failure).
- Serum vitamin B₁₂, folate (megaloblastic anaemia can be associated with pancytopenia).
- Serology for EBV, hepatitis A, B, and C, HIV (associated with aplastic anaemia).
- Serology for parvovirus infection (if pure red cell aplasia, also consider lymphoma, thymoma).
- ANA (lupus).
- Neutrophil alkaline phosphatase (NAP) score (\uparrow in aplastic anaemia).
- Check for lymphadenopathy, hepatomegaly, and splenomegaly.
- CXR (bronchial carcinoma, sarcoid, TB, lymphoma).
- USS/CT to assess lymphadenopathy/splenomegaly (pancytopenia may be due to hypersplenism and portal hypertension).
- Ham's test for paroxysmal nocturnal haemoglobinuria (PNH) or cell marker analysis of CD55 and CD59.
- Bone marrow (BM) aspirate and cytogenetics (myelodysplasia is a clonal disorder).

⇒ OCHM 10e, p. 364.

Paraesthesiae

This may be described by the patient as an abnormal sensation of aching, pricking, tickling, or tingling commonly in the extremities or face. Often described as feeling like 'pins and needles'.

The selection of investigations will be determined largely by the history (transient? chronic?), the surface anatomical site of the abnormal sensation, and associated symptoms or precipitating factors (e.g. clear history of hyperventilation).

The common causes include the numbness or tingling associated with pressure on the peripheral nerves, such as caused by sleeping awkwardly on an arm ('Saturday night palsy' of the radial nerve), or chronic or recurrent pressure, e.g. on the ulnar nerve at the elbow.

If paraesthesiae is persistent, consider the following conditions, depending on the distribution of the symptoms:

- Carpal tunnel syndrome (with radiation proximally along the forearm; worse at night).
- Peripheral neuropathy (DM, alcohol, drug-induced;  OHC 10e, Chapter 10).
- Sciatica (reduced straight leg raising).
- Meralgia paraesthetica (lateral cutaneous nerve of the thigh).
- Lateral popliteal palsy (common peroneal nerve).

Other less common causes

Peripheral neuropathy due to

- DM.
- Vitamin B₁ or B₁₂ deficiencies.
- Chronic renal failure.
- Chronic hepatic failure.
- Malignancy.
- Neurotoxic drugs:
 - Vinca alkaloids.
 - Metronidazole.
 - Nitrofurantoin.
 - Isoniazid (pyridoxine-dependent).
- Environmental toxins.
- Hypothyroidism.
- GBS (acute).
- Certain porphyrias.
- MS.

Acute hypocalcaemia causes a characteristic perioral paraesthesiae and can be due to many causes, including 1° and 2° hypoparathyroidism and alklosis.

General investigations

- ABGs (acute or chronic acid–base disturbances leading to alterations in ionized Ca^{2+}).
- Serum Ca^{2+} (not all laboratories measure ionized Ca^{2+}).
- Serum parathyroid hormone (PTH) (uncuffed sample).
- Serum Mg^{2+} (see below).
- Venous plasma glucose.
- Vitamin B_{12} (and other investigations in suspected chronic peripheral neuropathy).

If serum calcium or magnesium concentration is low

Identify the cause:

- Chronic GI loss (fistula, excessive diarrhoea, bowel obstruction).
- Chronic renal loss (diuretic drugs, intrinsic renal disease).
- DKA—total body Mg^{2+} may be low, but this very rarely causes symptoms.

Additional investigations

- Urinary Mg^{2+} .

Consider

- USS abdomen/renal tract and subsequent GI investigations.
- U&E.
- Nerve conduction studies (NCS).
- TFTs.
- IGF-1, growth hormone (GH) response during 75g-OGTT (if features of acromegaly present; Acromegaly (growth hormone excess), p. 132).

Peripheral neuropathy

The patient will complain of numbness in hands and feet that progresses proximally in a distribution classically termed 'glove and stocking'. Different aetiologies lead to a motor, sensory, or mixed sensorimotor picture.

Common causes

- Idiopathic (50%, commonest).
- DM.
- Vitamin B₁₂ deficiency (may occur in the absence of anaemia).
- Vitamin B deficiency (e.g. alcoholics).
- Vitamin E deficiency.
- Carcinomatous neuropathy.
- Drugs, e.g. isoniazid, vinca alkaloids, cisplatin, dapsone, gold, metronidazole.
- Paraproteinaemias (e.g. monoclonal gammopathy of undetermined significance (MGUS) or myeloma).

Rarer causes

- Amyloidosis.
- Uraemia.
- Collagen vascular diseases, e.g. rheumatoid, SLE, polyarteritis nodosa (PAN).
- Endocrine disease, e.g. myxoedema, acromegaly.
- GBS.
- Infections, e.g. tetanus, leprosy, diphtheria, botulism.
- Sarcoidosis.
- Hereditary, e.g. Charcot–Marie–Tooth disease.
- Acute intermittent porphyria.
- Toxins, e.g. lead (predominantly motor), arsenic (mixed sensory and motor), mercury (sensory), and thallium (mixed sensory and motor).
- Chronic inflammatory demyelinating polyneuropathy.
- Hereditary motor and sensory neuropathy types I or II.

Investigations

- NCS to confirm the diagnosis.

Further investigations

- In order to determine the underlying cause.
- Discuss with neurology staff.

⇒ OHCM 10e, p. 447.

Peripheral oedema

Swelling of the legs, or peripheral oedema, is a common presenting symptom, which occurs when excess tissue fluid is redistributed by gravity. Severe oedema is usually pathological and swelling of the ankles may progress to ascites and even pleural and pericardial effusion.

Causes of generalized swelling

- Cardiac failure: congestive heart failure, dilated cardiomyopathy, constrictive pericarditis, cor pulmonale.
- Hypoalbuminaemia: liver failure (hepatic cirrhosis), renal failure (nephrotic syndrome), protein-losing enteropathy, malnutrition (malabsorption or starvation).

Causes of localized swelling

- Immobility: common in old age, long-distance travel.
- Infection: cellulitis.
- DVT and/or subsequent venous insufficiency.
- Drugs: calcium channel blockers (nifedipine, amlodipine), NSAIDs.
- Malignancy: compression of deep vein, enlarged lymph nodes or lymphatics.
- Lymphatic obstruction: congenital, infiltrative (filariasis).
- Milroy's disease.
- Pregnancy.
- Wet beriberi.
- Idiopathic.

Patients at special risk

- Pregnancy.
- Prolonged bed rest.
- Following removal of lower limb plaster cast.
- Relative immobility: long-distance travel.
- CCF.

Investigations

These should be guided by the history and examination.

- FBC.
- U&E.
- Albumin.
- ESR.
- Blood cultures.
- ECG.
- Echocardiography.
- Doppler studies of leg veins/contrast venography (according to local availability).
- Abdominal USS.
- Malignancy screen for common cancers.
- Urine dipstick for proteinuria.
- 24h urinary protein excretion or urine protein/creatinine ratio.
- Small bowel biopsy.
- Xylose breath test.

Petechiae and thrombocytopenia

Spontaneous bleeding in the absence of trauma is uncommon with platelet counts $>20 \times 10^9/L$. However, bleeding is much more likely if thrombocytopenia is not immune in origin (e.g. aplastic anaemia, acute leukaemia, drug-induced, chemotherapy, myelodysplasia).

Thrombocytopenia may be inherited or acquired (e.g. DIC). As for pan-cytopenia, these may be classified as due to a failure of production, or ↑ consumption in the peripheries (DIC, ITP), or abnormal tissue distribution (splenomegaly).

ITP may be 1° or 2° (e.g. lymphoma, lupus, HIV).

Drugs (e.g. heparin) and blood transfusion (post-transfusion purpura) may cause severe thrombocytopenia.

Investigations

- FBC, film:
 - Inherited causes may be associated with giant platelets.
 - Morphological abnormalities may suggest MDS.
 - Red cell fragments suggest thrombotic microangiopathies, e.g. TTP.
- LDH (\uparrow in thrombotic thrombocytopenic purpura (TTP) and lymphoproliferative disorders).
- Serum vitamin B₁₂, folate (megaloblastic anaemia can be associated with ↓ platelets).
- ANA, autoimmune screen, IgG (lupus, hyperthyroidism).
- Virology (HIV, EBV, viral hepatitis, CMV).
- Clotting screen (DIC).
- Lupus anticoagulant, cardiolipin antibodies (antiphospholipid antibody syndromes).
- Platelet serology for drug- or transfusion-related causes.
- BM assessment to establish whether thrombocytopenia is due to a BM production problem or due to peripheral consumption (discuss with the haematology team; depending on the degree of thrombocytopenia, other haematological findings, and the age of the patient, a marrow may not be required).

Pitfalls

Thrombocytopenia due to HIV infection must be considered, especially in all younger adults. Not worth checking platelet-associated IgG or IgM since these are elevated in thrombocytopenia caused by immune and non-immune mechanisms, so they add no useful information.

Plethora

A plethoric appearance is typically seen in association with polycythaemia but may also be mistaken for a normal outdoors complexion or cyanosis. Patients with haematocrits above the normal reference range may or may not have an ↑ red cell mass (real or relative polycythaemia, respectively) (see Table 1.14).

Investigations

- FBC, film (repeat FBC as sampling errors can falsely cause elevations of Hb; polycythaemia rubra vera (PRV) may be associated with neutrophilia, basophilia, or ↑ platelets).
- Measurement of red cell mass may be necessary to confirm true polycythaemia.
 - Investigations are then aimed at establishing whether real polycythaemia, if documented, is due to a 1° BM abnormality (PRV) or a 2° disorder (e.g. respiratory disease).
- NAP score (may be raised in PRV). Seldom used now ( Neutrophil alkaline phosphatase, pp. 300–1).
- Vitamin B₁₂ and urate (may be ↑ in PRV).
- ESR/CRP (acute phase reactants may suggest 2° causes).
- Blood gas analysis, O₂ saturation, COHb levels (2° polycythaemia due to respiratory disease, smoking).
- Biochemistry (urea, creatinine; renal disease).
- Epo (↑ in 2° causes).
- USS abdomen (renal cysts, liver disease, uterine fibroids, and other malignancies may 'inappropriately' secrete Epo; also check for splenomegaly in PRV).
- Sleep studies (obstructive sleep apnoea (OSA), supine desaturation).
- O₂ dissociation studies (polycythaemia due to abnormal, high-affinity Hb variant).
- BM aspirate and chromosomal studies/cytogenetics (PRV is a clonal disorder).

Table 1.14 Polycythaemia

↑ red cell count	>6.0 × 10 ¹² /L >5.5 × 10 ¹² /L	♂ ♀
↑ PCV	>50% >45%	♂ ♀
↑ Hb	>18.0 g/dL >16.0 g/dL	♂ ♀

Polyuria

Polyuria (the passage of an excessive volume of urine, which may be associated with frequency of micturition and nocturia) must be differentiated from urinary symptoms associated with prostatic disease and urinary infections. The latter are also characterized by frequency, urgency, and nocturia, but usually small amounts of urine are passed at each void.

Causes include

- DM.
- Cranial diabetes insipidus (DI) (➡ OCHM 10e, Chapter 5):
 - Familial (autosomal dominant).
 - 2° to posterior pituitary or hypothalamic disease, e.g. surgery, tumours, especially metastases, neurosarcoidosis.
- Nephrogenic DI:
 - Familial (X-linked recessive).
 - Chronic intrinsic renal disease, e.g. pyelonephritis.
 - Hypokalaemia.
 - Hypercalcaemia.
 - Sickle-cell crisis.
 - Lithium, colchicine, amphotericin.
 - Post-obstructive uropathy.
- 1° polydipsia (psychogenic).

Investigations

- 24h urinary volume.
- Venous plasma glucose.
- U&E.
- TFTs.
- LH.
- FSH (? panhypopituitarism).
- Serum Ca^{2+} and PTH.
- Sickle-cell test.
- CXR (? mediastinal lymphadenopathy in TB, sarcoidosis).

If no obvious cause found, consider detailed investigations for cranial or nephrogenic DI (➡ Polydipsia and polyuria: diabetes insipidus, pp. 134–6).

➡ OCHM 10e, p. 81, p. 293.

Pruritus

Implies generalized itching and may be associated with many disorders, including:

- Iron deficiency.
- Malignant disease, e.g. lymphoma.
- DM.
- Chronic renal failure.
- Liver disease, e.g. PBC.
- Thyroid disease.
- PRV.
- HIV infection.

Investigations

- Aim to exclude the above diseases.
- FBC.
- Biochemistry screen, including LFTs and renal function.
- Glucose.
- TFTs.

⇒ OHCM 10e, p. 28, p. 535.

Ptosis

Ptosis can be unilateral and bilateral. Bilateral ptosis can be more difficult to recognize. Ptosis must be considered in association with other signs and symptoms. Ptosis may be long-standing, of recent onset, progressive, or intermittent, especially at the end of the day—myasthenia gravis (MG).

Unilateral ptosis

Causes

- Constitutional (congenital).
- Oculomotor (III) nerve palsy—levator palpebrae. ‘Down and out’ pupil with loss of light reflex (e.g. DM, SOL, demyelination).
- Aneurysm (basilar or posterior communicating arteries).
- Cavernous sinus disease.
- Meningitis.
- Horner’s syndrome—superior tarsal muscle (brainstem infarction, syringobulbia, SOL, MS).
- Encephalitis.

If abnormal (reduced) sweating on ipsilateral side face (damage to cervical sympathetic chain)

- Pancoast’s tumour.
- Aortic arch aneurysm.
- Cervical injuries.

No disorder of sweating

- Cluster headache.
- Parasellar tumours.
- Carotid artery aneurysm or dissection.
- Nasopharyngeal tumours.

Investigations

- Venous plasma glucose.
- CXR (Pancoast’s syndrome).
- Cranial CT or MRI.
- Cerebral angiography (aneurysm).

Bilateral ptosis

Causes

- GBS (Miller–Fisher syndrome).
- MD.
- MG.
- Neurosyphilis (bilateral; Argyll Robertson pupils).

Investigations

- Syphilis serology.
- EMG (‘dive-bomber’ in MD).
- Serum anti-acetylcholine receptor antibodies (AChRAb) (MG).
- IV edrophonium (Tensilon®) test (MG;  Edrophonium (Tensilon®) test, p. 631).

 OHCM 10e, p. 73.

Pulmonary embolism

Occurs when a thrombus in systemic veins or the right side of the heart embolizes into the pulmonary arterial system. Impaired gas exchange occurs because of a mismatch between ventilation and perfusion.

Investigations

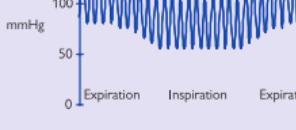
- FBC (may be leucocytosis, neutrophilia most likely).
- ESR (often ↑).
- Plasma D-dimers: ↑ with fresh thrombus.
- ABGs: hypoxia and hypocapnia.
- ECG: look for AF. Usually sinus tachycardia, may be evidence of right ventricular 'strain'. In massive PE, there may be $S_1 Q_3 T_3$.
- CXR: often normal but may show signs of pulmonary infarction or effusion.
- V/Q scan (may be useful for detection of areas of the lungs that are being ventilated but not perfused).
- Multislice CT scan: useful for detection of medium-sized PEs but does not exclude small PEs.

⇒ OCHM 10e, p. 98, pp. 190–1, p. 351, p. 818.

Pulse character

Rate and heart rhythm can be determined from palpation of the radial pulse. The arm should then be elevated to check for a collapsing pulse. Pulse volume and additional characteristics are assessed from palpation of the brachial or carotid pulse (see Table 1.15).

Table 1.15 Pulse character

Description	Diagram	Diagnosis	Comment
Normal	 mmHg	Normal	Normal volume and character
Slow rising	 mmHg	Aortic stenosis	Reduced volume pulse with delayed peak pulsation
Collapsing	 mmHg	Aortic regurgitation High cardiac output: -Thyroid toxicosis -Anaemia -Fever Patent ductus arteriosus	↑volume pulse with rapid rise and fall
Pulsus alternans	 mmHg	Severe heart failure	Pulse is regular, but alternate beats are weak and strong
Pulsus bisferiens	 mmHg	Hypertrophic cardiomyopathy Mixed aortic valve disease	Palpable double pulse
Pulsus bigeminus	 mmHg	Bigeminy	An ectopic beat occurs after every normal sinus beat
Pulsus paradoxus	 mmHg Expiration Inspiration Expiration	Severe asthma Cardiac tamponade Constrictive pericarditis	There is exaggeration of the usual fall in blood pressure during inspiration (>10mmHg)

Purpura

Implies bleeding of varying degrees into the skin. Includes petechial haemorrhages (pinpoint) and ecchymoses (bruises). There are many causes, including disorders of platelets and blood vessels.

Causes

- Congenital, e.g. Osler–Weber–Rendu syndrome (= HHT), connective tissue (Ehlers–Danlos), osteogenesis imperfecta, Marfan's.
- Severe infection (septic, meningococcal, measles, typhoid).
- Allergic, e.g. Henoch–Schönlein purpura.
- Drugs, e.g. steroids.
- Miscellaneous, e.g. senile purpura, scurvy, factitious.
- Thrombocytopenia—any cause (immune, marrow infiltration, deficiency of vitamin B₁₂ or folate, myelofibrosis, DIC, TTP/HUS).

Investigations

- FBC (looking for platelet abnormalities and presence of leukaemic cells or other signs of infiltration).
- Coagulation screen (looking for clotting factor deficiencies, DIC, etc.).
- Bleeding time using template device (previously used as a test of platelet function, but largely abandoned now because of poor reproducibility).

⇒ OHCM 10e, p. 311, p. 315, p. 556, p. 702.

Recurrent thrombosis

The pathogenesis (and hence causes) of thrombosis reflect abnormalities in the dynamics of the circulation, the blood vessel walls, or the blood constituents (Virchow's triad). A hypercoagulable or thrombophilic risk factor is an inherited or acquired disorder of the haemostatic mechanisms, which may be associated with an ↑ likelihood of a thrombotic event (venous or arterial) or recurrent thrombosis. This concept of risk factors for thrombosis is analogous to that for heart disease, and similarly for most patients multiple causal factors operate (see Table 1.16).

Heredity thrombotic disease may be suggested by a positive family history but should be tested for if the venous thrombotic events occur in the absence of acquired causes, at a younger age, at unusual sites (e.g. mesenteric), or as recurrent thromboses.

Investigations in recurrent thrombosis

Inherited thrombophilia screening

- Deficiency of factors, e.g. protein C, protein S, or antithrombin.
- Abnormal protein (FVL).
- ↑ procoagulant (PT, VIII); others (homocysteineuria).
- Consider occult malignancy (PSA in ♂, pelvic USS in ♀).
- FBC (myeloproliferative disorder, PNH).
- Biochemistry (cardiac disease, liver disease, nephrotic syndrome).
- ESR/CRP (ulcerative colitis).
- ANA/lupus anticoagulant/cardiolipin antibodies (antiphospholipid antibody syndromes, lupus).

Pitfalls

Thrombophilia testing may be complicated if the patient is on warfarin/heparin; discuss with the lab before sending samples.

➲ OHCM 10e, p. 375.

Table 1.16 Thromboembolic risk factors

Acquired

Cardiac disease	MI, AF, cardiomyopathy, CCF
Post-op	Especially abdominal, pelvic, or orthopaedic surgery
Pregnancy	
Malignancy	Any
Polycythaemia	
Immobilization	Prolonged
Fractures	Especially hip and pelvis
Obesity	
Varicose veins	
Drugs	e.g. oestrogen-containing oral contraceptive
Inherited	Activated protein C resistance, e.g. FVL mutation Protein C or S deficiency Dysfibrinogenaemias

Retinal haemorrhage

May be

- Flame-shaped (e.g. hypertension).
- Dot and blot (e.g. DM, vein occlusion, or haematological disease).
- Pre-retinal haemorrhage; suggests new vessel formation, e.g. DM or post-retinal vascular occlusion.
- Hyperviscosity syndromes.
- Severe anaemia.
- Severe thrombocytopenia.
- Haemoglobinopathy, e.g. HbSC.

Investigations

- Check BP.
- Renal function.
- FBC ($\uparrow\uparrow$ Hb or platelets).
- ESR or plasma viscosity (hyperviscosity syndromes such as myeloma or Waldenström's macroglobulinaemia).
- Serum Iggs and protein electrophoresis.
- Hb electrophoresis.

Rigors

Fever is due to a resetting of the anterior hypothalamic thermostat, is mediated by prostaglandins (hence aspirin is beneficial), and is most commonly caused by infection. Large variations in temperature may be accompanied by sweats, chills, and rigors. An undulant fever may suggest Hodgkin's disease or brucellosis. 'B' symptoms define fever ($>38^{\circ}\text{C}$), night sweats (drenching), and weight loss ($>10\%$) and suggest a diagnosis of lymphoma. (Fever is unusual in chronic lymphocytic/lymphatic leukaemia (CLL) in the absence of infection.)

Investigations

- FBC, film (Hodgkin's disease is associated with anaemia, neutrophilia, eosinophilia, and lymphopenia).
- LDH (\uparrow in lymphoma, non-specific test).
- Microbiological tests, blood/urine cultures (also consider pyogenic infection and abscesses in more unusual sites, e.g. renal).
- Antigen detection tests for specific pathogens.
- CXR (TB, lymphoma).
- ANA (connective tissue disease).
- BM aspirate/trephine may be necessary as part of leukaemia and lymphoma work-up.

Pitfalls

Not all fever is caused by infection.



OHC 10e, p. 29.

Short stature

The assessment of short stature can be a long and difficult process. Constitutional short stature is the commonest cause. Psychosocial disease must be considered, but extensive investigation is required to rule out organic disease. If no cause is found, a period of observation may make the underlying cause apparent. Specialist evaluation should be undertaken in all cases.

Causes

Endocrine

- GH deficiency.
- GH resistance (very rare).
- Hypothyroidism (readily treatable).
- Cushing's syndrome (rare in children). (Note: corticosteroid treatment for chronic asthma.)
- Rickets.
- Pseudohypoparathyroidism.
- Type 1 DM—Mauriac's syndrome, now rare.

Non-endocrine

- Constitutional short stature (short parents).
- Emotional deprivation.
- Intrauterine growth retardation.
- Achondroplasia.
- Mucopolysaccharidoses (rare).
- Turner's syndrome (46 XO and variants).
- Noonan's syndrome (46 XY, but features of Turner's in a ♂).
- Congenital cardiac disease, e.g. left-to-right shunt, cardiac failure.
- Cystic fibrosis.
- Other causes of malabsorption, e.g. coeliac disease, Crohn's colitis.
- Chronic liver disease.
- Haematological disease, e.g. sickle-cell disease.
- Chronic renal disease.

Investigations

- Current height + weight (compare to any previous data available; plot on growth charts).
- Growth velocity—normal if prior problem, e.g. intrauterine growth retardation.
- Physical stigmata of physical disease. Note: central nervous system (CNS) examination mandatory.
- FBC.
- ESR.
- U&E.
- LFTs.
- TFTs.
- Serum albumin (? nutritional status).
- Venous plasma glucose.
- Serum Ca^{2+} .

- Serum ALP (bone isoenzyme).
- Serum PO₄³⁻ (reduced in rickets).
- X-ray pelvis (Looser's zones), epiphyses (wide, irregular in rickets), ribs (multiple fractures).
- Serum antigliadin and antiendomysial antibodies (coeliac).
- Testosterone or oestradiol, LH, FSH, PRL (if puberty delayed—panhypopituitarism?).
- X-ray of the wrist for bone age. If delayed, measure serum IGF-1 (if IGF-1 normal, then GH deficiency unlikely; if IGF-1 low, consider nutritional and general health status before diagnosing GH deficiency—stimulation tests required;  Endocrinology and metabolism, Short stature, p. 178). If normal—constitutional short stature.
- Karyotype (Turner's and Noonan's syndromes).
- 24h urinary free cortisol (as screen for Cushing's syndrome;  Obesity/hypercortisolism, pp. 142–6).
- CT or MRI of the pituitary (if GH deficiency or panhypopituitarism).

Skin pigmentation

Skin pigmentation can be due to ↑ melanin deposition, e.g. racial differences in skin pigmentation, or due to ↑ melanin deposition seen in sun exposure. Lentigines and freckles are common. Haemosiderin and other substances can ↑ skin pigmentation. ↑ pigmentation can be seen in various dermatological conditions; chronic inflammation and fungal infection can result in ↑ skin pigmentation. Lichen planus and fixed drug eruptions are associated with ↑ pigmentation.

Increased pigmentation may also be found in association with chronic systemic disease

- Addison's disease (palmar creases, buccal pigmentation, recent scars).
- Porphyria cutanea tarda (especially exposed areas—dorsum of the hands).
- Chronic malabsorption syndromes.
- Drugs, e.g. amiodarone, psoralens, mepacrine, minocycline, chloroquine.
- Chronic uraemia.
- Haemochromatosis (so-called 'bronzed diabetes').
- PBC (deep green-yellow jaundice, chronic pruritus).
- Ectopic ACTH syndrome, e.g. bronchial carcinoma.
- Nelson's syndrome (excessive adrenocorticotrophic hormone (ACTH) secretion from pituitary basophil adenoma in Cushing's disease treated by bilateral adrenalectomy).
- Carotenaemia (orange discolouration does not involve the sclerae;  Jaundice, p. 66).
- Chloasma (pregnancy, oestrogen-containing OCP).
- Acanthosis nigricans—most often a marker of insulin resistance in obese patients with type 2 DM. Rarely in association with underlying carcinoma.
- Peutz–Jeghers syndrome (fingers, lips, in association with small intestine polyposis).

Contrast with hypopigmentation

- Localized acquired depigmentation (vitiligo) is a marker of autoimmune disease.
- Oculocutaneous albinism (autosomal recessive).
- Chronic hypopituitarism ( Hypothalamus/pituitary function, pp. 128–30).
- Phenylketonuria.

Investigations

- FBC.
- U&E.
- Venous plasma glucose.
- Antigliadin and antiendomysial antibodies.
- Short tetracosactide (Synacthen[®]) test (if 1° hypoadrenalism suspected;
 ➡ Short Synacthen[®] test, p. 225).
- Urinary porphyrins.
- LFTs, serum albumin, and PT (INR).
- Fe/TIBC/ferritin + genetic markers for haemochromatosis + liver biopsy.
- ESR and/or CRP.
- Autoimmune profile (➡ Chapter 4).
- Testosterone (or oestradiol) + LH, FSH.
- Antimitochondrial antibodies, liver biopsy (PBC).
- Investigations for Cushing's syndrome (➡ Obesity/hypercortisolism,
 pp. 142–6).
- Investigations for causes of chronic renal failure.

Splenomegaly

A palpable spleen is at least twice its normal size, when its length is >14cm. Enlargement may represent changes in the white pulp (lymphoid tissue expansion, inflammation), red pulp (blood congestion, extramedullary haemopoiesis), or occasionally supporting structures (cysts).

Causes in Western societies

- Leukaemias.
- Lymphomas.
- Myeloproliferative disorders.
- Haemolytic anaemias.
- Portal hypertension.
- Infections, e.g. infective endocarditis, typhoid, TB, brucellosis, viral (EBV, viral hepatitis).

Less common causes

- Storage disorders (e.g. Gaucher's).
- Collagen diseases.
- Sarcoid.
- Amyloid.

If foreign residence, consider infectious causes (malaria, leishmaniasis, schistosomiasis) and haemoglobinopathies (HbC, HbE, thalassaemia).

Massive splenomegaly (>8cm palpable below LCM)

- Myelofibrosis.
- Chronic myeloid leukaemia (CML).
- Gaucher's.
- Malaria.
- Leishmaniasis.

Investigations

- Thorough history and physical examination.
- FBC, blood film, LDH (leukaemia, lymphoma, pernicious anaemia).
- Reticulocytes, bilirubin (if ↑, suggests haemolysis).
- Virology/microbiology (sepsis, bacterial endocarditis, EBV, CMV).
- Serum protein electrophoresis (myeloma, amyloid).
- Autoantibody screen, ANA (collagen disease, lupus, RhA).
- Haemoglobinopathy screen.
- LFTs (splenomegaly may be associated with hepatomegaly, or due to portal hypertension).
- Peripheral blood cell markers (immunophenotype—may show leukaemia or lymphoma).
- BM aspirate/trephine/cell markers/cytogenetics.
- Leucocyte glucocerebrosidase activity (Gaucher's disease).
- USS to assess liver texture, splenomegaly, and lymphadenopathy.

⇒ OCHM 10e, p. 63, p. 373, p. 604.

Steatorrhoea

Implies that the patient is passing pale, bulky stools that are offensive (contain fat and tend to float) and are difficult to flush away.

Causes

- Any disorder that prevents absorption of micellar fat from the small bowel.
- Ileal disease.
- Ileal resection.
- Parenchymal liver disease.
- Obstructive jaundice.
- Pancreatic disease, including cystic fibrosis.
- ↓ bile salt concentration.
- Bile salt deconjugation by bacteria.
- Cholestyramine.
- β-lipoprotein deficiency.
- Lymphatic obstruction.

Investigations

Blood tests

- LFTs.
- Bone profile.
- Vitamin B₁₂ and serum (or red cell) folate.
- Autoantibody profile.
- Serum amylase.

Pancreatic investigations

- Pancreatic function tests.
- CT scan.

Small bowel

- Small bowel follow-through.
- Jejunal biopsy (? villus atrophy).
- Bacterial overgrowth (¹⁴C glycocholate breath test).

Parasites

- Stool culture (e.g. *Giardia*).

Ileal disease

- Consider Crohn's.

⇒ OHCM 10e, p. 59.

Stridor

Stridor denotes a harsh respiratory added sound during inspiration. It may be a high-pitched musical sound similar to wheeze but arising from constriction of the larynx or trachea. Stridor may be aggravated by coughing.

►► Progressive breathlessness is accompanied by indrawing of intercostal spaces and cyanosis indicates severe laryngeal obstruction with risk of sudden death.

In young children

Because of the smaller size of the larynx and trachea in children, stridor may occur in a variety of conditions:

- Postural stridor (laryngomalacia).
- Allergy, e.g. nut allergy, insect stings—common. Note: emergency treatment with IM or subcutaneous (SC) adrenaline (epinephrine)—self-administered or by parent, and parenteral hydrocortisone.
- Vocal cord palsy.
- Croup (acute laryngitis—often coryza).
- Acute epiglottitis.
- Inhaled foreign body, e.g. peanut (common—inhalaion further down the respiratory tract, usually into the right main bronchus, may produce localized wheeze or distal collapse; ↗ Patterns of lobar collapse, p. 780).

Investigations

- Pulse oximetry (non-invasive measurement of partial pressure of oxygen (PO_2)).
- Plain lateral X-ray of the neck (for radio-opaque foreign body).
- Endoscopic nasalaryngoscopy.

Adults

- Infection, especially *Haemophilus influenzae*.
- Inflammatory or allergic laryngeal oedema, e.g. penicillin allergy (see above); may be accompanied by anaphylactic shock.
- Pharyngeal pouch (may be recurrent lower respiratory tract infection).
- Inhaled vomitus or blood in an unconscious patient.
- Tetany (due to low serum Ca^{2+} or alkalosis; ↗ OHCM 10e, Chapter 14).
- Large multinodular goitre, carcinoma, or lymphoma of the thyroid (uncommon).
- Laryngeal tumours.
- Bronchogenic tumour with bilateral cord paralysis (subcarinal and paratracheal gland involvement. Note: 'bovine' cough of right recurrent laryngeal nerve palsy).
- Shy–Drager syndrome (of autonomic neuropathy).

Investigations

- CXR.
- Lateral X-ray of the neck.
- USS of the thyroid.
- Endoscopic nasalaryngoscopy.
- Bronchoscopy.
- Barium swallow (pharyngeal pouch).
- CT neck and mediastinum.

↗ OHCM 10e, p. 48.

Suspected bleeding disorder

Bleeding problems present a considerable challenge. Patients may present with simple easy bruising—a common problem—or catastrophic post-traumatic bleeding. The best predictors of bleeding risk are found in taking an accurate history, focusing on past haemostatic challenges (e.g. tonsillectomy, teeth extraction, menses—especially at time of menarche) and current drug history (e.g. aspirin). The history may also help delineate the type of defect. Platelet bleeding (e.g. thrombocytopenia) starts at the time of the (even minor) haemostatic insult but, if controlled by local pressure, tends not to recur. Bleeding due to coagulation factor deficiency tends to be associated with internal/deep muscle haematomas as the bleeding typically occurs in a delayed fashion after initial trauma and then persists.

Inappropriate bleeding or bruising may be due to a local factor or an underlying systemic haemostatic abnormality.

► Acquired causes of bleeding are much commoner than inherited causes.

Causes of bleeding include

- Surgical.
- Trauma.
- Non-accidental injury.
- Coagulation disorders.
- Platelet dysfunction.
- Vascular disorders.

Clinical features

History and presenting complaint. Is this an isolated symptom? What type of bleeding does the patient have, e.g. mucocutaneous, easy bruising, spontaneous, post-traumatic. Duration and time of onset—? recent or present in childhood. Menstrual and obstetrical history are important.

Systemic enquiry

Do the patient's symptoms suggest a systemic disorder, bone marrow failure, infection, liver disease, or renal disease?

Past medical history

Previous episodes of bleeding, recurrent—? ITP, congenital disorder. Exposure to trauma, surgery, dental extraction, or pregnancies.

Family history

First-degree relatives. Pattern of inheritance (e.g. autosomal, sex-linked). If family history is negative, this could be a new mutation (one-third of new haemophilia is due to new mutations).

Drugs

All drugs cause some side effects in some patients. Bleeding may result from thrombocytopenia and platelet dysfunction. Do not forget to ask about aspirin and warfarin.

Physical examination

Signs of systemic disease

Is there any evidence of septicaemia, anaemia, lymphadenopathy ± hepatosplenomegaly?

Assess bleeding site

Check the palate and fundi. Could this be self-inflicted? Check size—petechiae (pinhead); purpura (larger $\leq 1\text{cm}$); bruises (ecchymoses; $\geq 1\text{cm}$).

Joints

Swelling or other signs of chronic arthritis.

Vascular lesions

Purpura—allergic, Henoch–Schönlein, senile, steroid-related, hypergammaglobulinaemic, HHT—capillary dilatations (blanches on pressure), vasculitic lesions, autoimmune disorders, hypersensitivity reactions.

Investigations

- FBC, film, platelet count, biochemistry screen, ESR, coagulation screen.
- Special tests, e.g. BM for 1° haematological disorders; radiology, USS.
- Family studies.

Suspected stroke

A stroke denotes an acute neurological deficit. Strokes may vary in presentation, e.g. rapidly resolving neurological deficit to a severe permanent or progressive neurological defect (e.g. multi-infarct disease). Neurological deficits persisting >24h are termed 'completed stroke' (cf. TIA). With suspected stroke, a full history and general physical examination are mandatory. Risk factors for cerebrovascular disease should be sought, including a history of hypertension (common—major risk factor), DM (common—major risk factor), and dyslipidaemia. Ask about recent falls or trauma. Hemiparesis can occur as a post-ictal phenomenon or a result of migraine or hypoglycaemia (see below). Hysterical or functional paralysis is also seen but should not be confidently assumed at presentation. Neuroanatomical localization of the deficit and the nature of the lesion(s) require appropriate imaging. Note: the post-ictal state may be associated with temporary (<24h) limb paresis (Todd's paralysis) in focal epilepsy (suggests structural lesion—cranial imaging is mandatory).

General investigations

- FBC (polycythaemia, anaemia).
- U&E.
- ESR.
- Protein electrophoresis (if hyperviscosity syndrome suspected, e.g. ↑↑ ESR).
- ECG (AF, IHD—statins reduce the risk of stroke in patients with previous MI).
- CXR (cerebral metastases from bronchogenic carcinoma?).

Specific risk factors

- Venous plasma glucose. Note: severe hypoglycaemia, e.g. insulin-induced or 2° to sulfonylureas, may mimic acute stroke. Always check the capillary fingerprick glucose concentration to exclude this possibility—even if there is no history of DM. Take a venous sample in a fluoride-oxalate tube (+ serum for insulin concentration) if hypoglycaemia confirmed. (Diabetes mellitus, pp. 194–9 for further details of investigation and treatment.) Hyperosmolar non-ketotic diabetic coma may also be misdiagnosed as stroke (plasma glucose usually >50mmol/L with pre-renal uraemia).
- Thrombophilia screen (if indicated by clinical or haematological features).
- Lipid profile (not an immediate investigation; 2° prevention—see above).
- Blood cultures (if subacute bacterial endocarditis (SBE) or other sepsis suspected. Note: cerebral abscess).

Imaging

- Cranial CT scan (\pm IV contrast).
- Echocardiogram (if mural thrombus, endocarditis suspected).
- Carotid Doppler studies—may not be indicated if surgical intervention (endarterectomy) is unlikely because of poor prognosis, e.g. dense hemiplegia or coma.

Consider alternative diagnoses including

- 1° or 2° brain tumour (may present as acute stroke—search for 1°).
- Cerebral abscess (usually clear evidence of sepsis).
- Cerebral lupus (ESR, autoantibodies).

⌚ OHCM 10e, p. 159, pp. 470–5, p. 746.

Sweating

Fairly non-specific symptom, but one which may indicate serious underlying disease.

Causes

- Excess heat (physiological).
- Exercise (physiological).
- Fever—any cause.
- Anxiety.
- Thyrotoxicosis.
- Acromegaly.
- DM.
- Lymphoproliferative disease, e.g. lymphomas.
- Cancer (any).
- Hypoglycaemia.
- Alcohol.
- Nausea.
- Gustatory.
- Neurological disease, e.g. lesions of the sympathetic nervous system, cortex, basal ganglia, or spinal cord.

Investigations

- FBC.
- ESR.
- Biochemistry screen, including LFTs.
- Glucose.
- TFTs.
- Urinalysis and culture.
- Blood cultures.
- CXR.
- Further investigations, depending on results of above.

Tachycardia

Tachycardia is arbitrarily defined as a heart rate above 100 beats per minute. It is a normal physiological response to exercise and to emotional stress but can also herald a cardiac rhythm disorder. One should always begin by assessing the nature of the tachycardia and identifying any underlying cause or contributing factor.

Assessment begins with a 12-lead ECG, performed whilst the patient is tachycardic. This will enable the immediate identification of the heart rhythm. One must then differentiate between sinus tachycardia (which may or may not have a pathological cause) and tachycardias due to other (abnormal) cardiac rhythms.

Causes of sinus tachycardia

- Sympathetic stimulation, e.g. anxiety, pain, fear, fever, exercise.
- Drugs, e.g. adrenaline, atropine, salbutamol.
- Stimulants, e.g. caffeine, alcohol, amphetamines.
- Thyrotoxicosis.
- Heart failure.
- PE.
- IHD, acute MI.
- Anaemia.
- Blood or fluid loss, e.g. post-operative.
- Inappropriate sinus tachycardia (a persistent resting sinus tachycardia, diagnosed when all other possible causes have been excluded).

In assessing abnormal heart rhythms causing tachycardia, it is helpful to divide them into narrow-complex tachycardia (QRS duration <120ms) and broad-complex tachycardia (QRS duration >120ms).

Narrow-complex tachycardias

- Sinus tachycardia (see above).
- Atrial tachycardia.
- Atrial flutter.
- AF.
- AV re-entry tachycardias.

Broad-complex tachycardias

- Narrow-complex tachycardia with aberrant conduction.
- Ventricular tachycardia.
- Accelerated idioventricular rhythm.
- Torsades de pointes.

Investigations

- 12-lead ECG to identify the underlying rhythm.
- Consider bedside monitoring on the Coronary Care Unit (CCU), particularly if the patient is compromised or ventricular arrhythmias are suspected.
- Other investigations depend upon the underlying cause but may include:
 - FBC.
 - U&E.
 - TFTs.
 - Cardiac markers.
 - CXR.
 - ABGs.
 - V/Q scan/CTPA.
 - Echocardiogram.
 - Exercise treadmill test.
 - Cardiac catheter.
 - Electrophysiological studies.

It can be useful to perform carotid sinus massage (► exclude carotid bruits first) or to give IV adenosine (► do not use in asthma/COPD), whilst the patient is on a bedside ECG monitor. Supraventricular tachycardias will usually slow transiently, allowing clearer identification of the underlying atrial activity, and re-entry tachycardias may terminate altogether. Ventricular tachycardias will be unaffected.

© OHCM 10e, Chapter 3.

Tinnitus

Tinnitus is a common symptom in which the patient perceives a sound, often chronic and distressing, in the absence of aural stimulation. It usually manifests as a 'ringing' or 'buzzing' in the ears. Tinnitus may occur as a symptom of nearly all disorders of the auditory apparatus. Psychological stresses may be relevant in some cases.

Causes include

- Acoustic trauma (prolonged exposure to loud noise, e.g. gunshots, amplified music).
- Barotrauma (blast injury, perforated tympanic membrane).
- Obstruction of the external auditory meatus (wax, foreign body, infection).
- Otosclerosis.
- Ménière's disease.
- Drug-induced ototoxicity.
- Gentamicin—may be irreversible.
- Acute salicylate toxicity.
- Quinine toxicity.
- Acute alcohol poisoning.
- Hypothyroidism.
- Hypertension (rare symptom).
- Intra- or extracranial aneurysm (typically causes 'pulsatile' tinnitus).
- Glomus jugulare tumours.

Note: consider acoustic neuroma in unilateral tinnitus (⇒ OHCM 10e, Chapter 10).

Investigations

- FBC.
- Serum concentrations of, e.g. salicylates, gentamicin (► mandatory during systemic therapy).
- TFTs.
- BP.

Audiological assessment

Specialist investigations include

- Assessing air and bone conduction thresholds.
- Tympanometry and acoustic reflex testing.
- Speech perception thresholds.

Consider

- CT temporal bone (acoustic neuroma).
- Cranial MRI (following specialist advice).

⇒ OHCM 10e, p. 464.

Tiredness

Tiredness is a common presenting complaint in the endocrine clinic. Important diagnoses to exclude are hypo-/hyperthyroidism, hypoadrenalinism, hypercalcaemia, and DM. A U&E is useful to exclude hyponatraemia or hypokalaemia (muscle weakness), as well as renal failure.

Recommended investigations for tiredness in the absence of an obvious cause from history and examination

- TSH.
- FT4.
- Synacthen® test.
- Serum Ca²⁺.
- Glucose.
- U&E, creatinine.
- FBC.
- ESR (or CRP).
- LFTs.

Urgency of micturition

Urgency of micturition denotes a strong desire to void and the patient often has to rush to the toilet because of an acute call to micturate. Urinary incontinence may result, especially if physical mobility is impaired. Urgency forms part of a cluster of symptoms which include frequency of micturition (☞ Polyuria, p. 90), nocturia, and hesitancy of micturition.

Men

- Prostatic disease.
- UTI.
- Bladder irritability.
- Urethritis.
- States of polyuria (☞ Polyuria, p. 90); may lead to urinary incontinence (☞ Incontinence: urinary, p. 61).

Investigations to consider

- Urinalysis—stick test for glucose, protein, blood, and nitrites.
- MSU for microscopy and culture.
- FBC.
- U&E.
- Venous plasma glucose.
- ESR.
- Serum PSA.
- PSA is ↑ in 30–50% of patients with benign prostatic hyperplasia, and in 25–92% of those with prostate cancer (depending on tumour volume), i.e. a normal PSA does not exclude prostatic disease. Check the reference range with the local laboratory.
- Transrectal USS of the prostate.
- Prostatic biopsy (specialist procedure).

Women

- UTI.
- Gynaecological disease, e.g. pelvic floor instability, uterine prolapse.
- Bladder irritability.
- Urethritis.
- States of polyuria; may lead to urinary incontinence (☞ Incontinence: urinary, p. 61).

Investigations to consider

- FBC.
 - U&E.
 - MSU for microscopy and culture.
 - Urodynamic studies.
- ☞ OCHM 10e, p. 80, p. 648.

Urticaria

Itchy superficial wheals; may be giant. Distinguish acute from chronic—chronic is rarely allergic in origin. If persists for >24h and fades with brown staining, consider urticarial vasculitis (rare).

Causes

- Allergic: drugs, foods, additives, acute infection, e.g. HBV, *Mycoplasma*.
- Physical: sunlight, heat, cold, pressure, vibration.
- Stress.
- Thyroid disease: hypo- or hyperthyroidism.
- Occult infection: gall bladder, dental, sinus.
- Vitamin deficiency: B₁₂, folic acid, iron.
- Autoimmune: antibodies against IgE receptor on mast cells—very rare.

Investigations

Based on history but should include as baseline

- FBC (with eosinophil count).
- Thyroid function.
- Infection marker (CRP).
- Liver function.

Further tests may include

- B₁₂ and red cell folate.
- Serum ferritin.

Allergy tests are of little value, unless there is a clearly identified trigger.

Vasculitis

Definition

Disease caused by inflammatory destructive changes of blood vessel walls (see Table 1.17).

Table 1.17 Causes of 1° vasculitis

	Granulomatous	Non-granulomatous
Large vessel	Giant cell arteritis (GCA)	Takayasu's arteritis
Medium vessel	Churg–Strauss disease	Polyarteritis nodosa
Small vessel	Wegener's arteritis	Microscopic arteritis

Presentation

Wide variety of clinical presentations affecting one or more organ systems:

- **Skin:** splinter haemorrhages, nailfold infarcts, petechiae, purpura, livedo reticularis.
- **Respiratory:** cough, haemoptysis, breathlessness, pulmonary infiltration, sinusitis.
- **Renal:** haematuria, proteinuria, hypertension, ARF.
- **Neurological:** mononeuritis multiplex, sensorimotor polyneuropathy, confusion, fits, hemiplegia, meningoencephalitis.
- **Musculoskeletal:** arthralgia, arthritis, myalgia.
- **Generalized:** PUO, weight loss, malaise.

Causes of secondary vasculitis

- Infective endocarditis.
- Meningococcal septicaemia.
- Malignancy.
- RhA.
- Henoch–Schönlein purpura.
- SLE.
- Cryoglobulinaemia.
- Drug reaction.

Investigations

- FBC.
- U&E.
- LFTs.
- ESR.
- CRP.
- Protein electrophoresis.
- ANA.
- RF.
- ANCA.
- CXR.
- Biopsy of artery and/or skin lesions.
- Urine dipstick and microscopy.

Visual loss

Total loss of vision may be bilateral or unilateral. Unilateral blindness is due to a lesion either of the eye itself or between the eye and the optic chiasm. Determine whether the visual loss is gradual or sudden. Gradual loss of vision occurs in conditions such as optic atrophy or glaucoma. In the elderly, cataract and macular degeneration are common. Remember tobacco amblyopia and methanol toxicity. Trachoma is a common cause worldwide.

Causes of sudden blindness include

- Optic neuritis, e.g. MS.
- Central retinal artery occlusion.
- Central retinal vein occlusion.
- Vitreous haemorrhage. (Note: proliferative diabetic retinopathy.)
- Acute glaucoma.
- Retinal detachment.
- Temporal (giant cell) cell arteritis (TA). Note: visual loss is potentially preventable with early high-dose corticosteroid therapy (OHC M 10e, Chapter 10).
- Migraine (scotomata).
- Occipital cortex infarction.
- Acute severe quinine poisoning (consider stellate ganglion block).
- Hysteria (rare), e.g. is blindness:
 - Complete? No pupil response or optokinetic nystagmus.
 - Cortical? Normal pupillary light reflex, no optokinetic nystagmus.
 - Hysterical? Normal pupillary light reflex, normal optokinetic nystagmus.
- HELLP syndrome complicating pre-eclampsia—rare.

Investigations will be determined by the history and examination findings; a specialist opinion should be sought without delay.

If TA suspected

- ESR/CRP.
- Autoimmune profile, including cytoplasmic ANCA (cANCA)/perinuclear ANCA (pANCA).
- Temporal artery biopsy (within days; ►► do not withhold steroid therapy).

Investigations in sudden onset of visual loss

- Visual acuity (Snellen chart).
- Goldmann perimetry.
- Intraocular pressure measurement (tonometry).
- Fluorescein angiography (specialist investigation—may delineate diabetic retinopathy in more detail. ►► Risk of anaphylaxis).
- Cranial CT scan.
- Cranial MRI scan.
- LP (CSF protein and OCBs if MS suspected).

Screen for risk factors and causes of cerebrovascular thromboembolic disease:

- Venous plasma glucose.
- Serum lipid profile.
- Carotid Doppler studies.
- 12-lead ECG.
- Echocardiogram.

Wasting of the small hand muscles

Wasting of the small muscles of the hand may be found in isolation or may be associated with other neurological signs. If found in isolation, this suggests a spinal lesion at the level of C8/T1 or distally in the brachial plexus or upper limb motor nerves.

Unilateral wasting of the small muscles of the hand may occur in association with

- Cervical rib.
- Brachial plexus trauma (Klumpke's palsy).
- Pancoast's tumour (may be associated with Horner's syndrome).
- Cervical cord tumour.
- Malignant infiltration of the brachial plexus.

Bilateral wasting of the small muscles of the hand occurs in

- Carpal tunnel syndrome (common).
- RA (common).
- Cervical spondylosis (common).
- Bilateral cervical ribs.
- MND.
- Syringomyelia.
- Charcot–Marie–Tooth disease.
- GBS.
- Combined median and ulnar nerve lesions.
- Cachexia.
- Advanced age.
- Peripheral neuropathies.

Investigations

- ESR.
- CRP.
- RF.
- CXR.
- X-ray cervical spine.
- NCS (⌚) Nerve conduction studies, pp. 606–8).
- EMG (⌚) Electromyogram, pp. 610–11).
- LP, CSF protein, etc. (⌚) Lumbar puncture, pp. 584–9).
- CT thorax.
- MRI of the cervical cord/brachial plexus.

Weight loss

Causes

- Diet.
- Anorexia.
- DM.
- Malnutrition.
- Small intestinal disease (coeliac, bacterial overgrowth).
- Malignant disease (carcinoma and haematological malignancies).
- HIV/AIDS.
- Chronic pancreatitis.
- Chronic respiratory failure.
- Cirrhosis.
- Diuretic therapy.
- Hyperthyroidism.
- Addison's disease.

Investigations

May well need extensive investigation before determining the cause, but start with:

- FBC.
- ESR or CRP.
- Biochemistry screen.
- TFTs.
- MSU, including C&S.
- CXR.
- Stool culture (if appropriate).
- Blood culture.
- Other endocrine tests as appropriate.
- Consider HIV testing.

⊕ OCHM 10e, p. 35, p. 245.

Wheeze

Wheezes (rhonchi) are continuous high-, medium-, or low-pitched added sounds audible during respiration. Typically they are loudest on expiration in asthma and may on occasion be heard without a stethoscope. The implication is reversible or irreversible airway obstruction. If wheeze is audible only during inspiration, this is termed stridor, implying an upper respiratory obstruction. An important distinction must be made between monophonic and polyphonic wheezes and whether wheeze is localized to a single area or is heard throughout the thorax.

Polyphonic wheeze

Wheezes with multiple tones and pitch. The commonest causes of wheeze (usually recurrent) are:

- Asthma.
- COPD (often audible during both phases of respiration).

Fixed monophonic wheeze

A wheeze with a single constant pitch. Implies local bronchial obstruction, usually due to:

- Bronchogenic carcinoma.
- Foreign body.

Note: stridor is a harsh form of monophonic wheeze arising from an upper airway obstruction (☞ Stridor, p. 104).

Investigations

- ABGs. (Note: inspired O₂ concentration should be recorded.)
- Pulse oximetry at the bedside (does not provide information about the partial pressure of carbon dioxide (PCO₂)).
- Spirometry (peak flow rate (PFR), pre- and post-bronchodilator therapy).
- Pulmonary function tests (forced expiratory volume in 1s (FEV₁), forced vital capacity (FVC), total lung capacity; ☞ Flow volume loops/maximum expiratory flow–volume curve, p. 554).
- CXR (posteroanterior (P-A) and lateral).
- Sputum cytology (if tumour suspected).
- CT thorax.
- Bronchoscopy and biopsy (specialist procedure—especially if foreign body or suspected tumour).

☞ OHCM 10e, p. 52.

Part II

Investigations

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Endocrinology and metabolism

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Guiding principles of endocrine investigation

Investigations for endocrine disease have caused a lot of confusion in the minds of clinicians (many still do!). Tests have come and gone over the years and have been adopted with varying degrees of enthusiasm by specialist centres. In particular, there is often confusion over which tests to do, what procedures to follow, and how to interpret the results. In some areas (e.g. Cushing's syndrome), controversy persists among the experts. In others, a clear consensus approach exists.

Some useful general principles

1. Use *dynamic* tests, rather than random (untimed) sampling where the hormone under investigation is secreted in infrequent pulses (e.g. GH) or levels are easily influenced by other factors (e.g. cortisol varies markedly with stress levels and has a marked circadian rhythm; see Table 2.1).

In general

- If you are suspecting a LOW level—do a *STIMULATION* test (to see if it stays *LOW*)
 - If you are suspecting a HIGH level—do a *SUPPRESSION* test (to see if it stays *HIGH*)
2. Use the correct collection method, e.g. ACTH or insulin levels require rapid separation of the sample and prompt freezing (-20°C). Timing of sampling may also be critical. Label samples carefully, including time of collection! Check procedures with the local laboratory. Many units will have protocols for endocrine investigations.
 3. Do tests in the correct sequence, e.g. ACTH levels can only be interpreted once the cortisol status is known. In many cases, simultaneous samples are required for interpretation, e.g. PTH with Ca^{2+} for hypo-/hyperparathyroidism, glucose with insulin for insulinoma.
 4. ‘Normal’ results may be ‘abnormal’, depending on the activity of the hormone axis under investigation. Interpretation of the absolute levels of hormones in isolation may be highly misleading. For example, a serum PTH within the normal range in the presence of hypocalcaemia suggests hypoparathyroidism; ‘normal’ LH and FSH levels in the presence of a very low serum testosterone concentration suggest pituitary failure. In both instances, the regulatory hormone concentration is inappropriately low. Thus, the level of the regulatory hormone (or releasing factor) must be considered in the light of the simultaneous level of the ‘target’ hormone or metabolite.

5. *Results may vary according to the laboratory assay.* Reference ranges vary between laboratories—it is especially important with endocrine tests to interpret your results according to your laboratory's 'normal range'. Also, interfering factors may differ between assays, e.g. different PRL assays cross-react very differently with macroprolactin
 Galactorrhoea (hyperprolactinaemia), pp. 172–3). Some individuals have a heterophile interfering antibody that affects the results of many radioimmunoassays. Resist 'discarding clinical evidence in favour of a numerical value'.
6. *Beware of interfering medication,* e.g. inhaled beclomethasone can suppress serum cortisol levels; administered hydrocortisone (cortisol) is detected by the cortisol assay; synthetic androgens and oestrogens can appear to cause low serum testosterone/oestrogen (as they are not detected in the testosterone/oestrogen assay); some anti-emetics and antipsychotics can raise circulating PRL levels; carbenoxolone or liquorice may cause hypokalaemia. Always ask patients for a full medication list (including herbal remedies and other self-medications).
7. *Take a family history.* Familial forms of many common endocrine problems exist that require important changes in the management approach, e.g. familial hypercalcaemia may suggest MEN-1 or MEN-2 requiring a different form of parathyroid surgery and a risk of phaeochromocytoma (MEN-2).

Endocrine tests are generally expensive and should not be performed unnecessarily or outside standard protocols. Dynamic tests may have cautions and contraindications and can be hazardous if used inappropriately (see Table 2.1). A high degree of organization and close liaison with the laboratory are required to perform these tests in a way that can be clearly interpreted. Dynamic tests should ideally be performed in an endocrine investigation unit. Chemical pathologists (clinical biochemists) and other laboratory staff generally have great experience with performing and interpreting endocrine tests—seek their advice, wherever possible, before embarking on tests with which you are unfamiliar. Tests in children should be performed and interpreted under expert paediatric guidance.

Table 2.1 Random sampling vs dynamic testing

Hormone	Random or dynamic sampling?
GH	<i>Dynamic:</i> glucose tolerance test for excess; insulin stress test or GHRH arginine stimulation test for deficiency
IGF-1	Random
LH, FSH	Random in ♂, post-menopausal Timed with menstrual cycle in premenopausal ♂ Random or stimulated in pre-pubertal children
Testosterone	Random
Oestrogen (oestradiol)	Random in ♂, post-menopausal ♂ Timed with menstrual cycle in premenopausal
ACTH	Random
Cortisol	<i>Dynamic:</i> dexamethasone suppression test for excess; Synacthen® stimulation test if suspect deficiency
TSH	Random
T4 and T3	Random
PRL	Random
ADH/vasopressin	Do not normally measure directly
Osmolality	<i>Dynamic:</i> water deprivation test if suspect DI
PTH	Random, but need simultaneous Ca ²⁺ value
Insulin	Fasting, plus simultaneous glucose value
Calcitonin	Random
Renin/aldosterone	Upright usually, off medication
Metanephhrines	Measure in urine, 24h sample or seated random plasma sample
5HIAAs	Measure in urine, 24h sample

Further reading

- American Association of Clinical Endocrinologists Clinical Guidelines. AACE/ACE clinical practice guidelines. <https://www.aace.com/publications/guidelines>.
- Endocrine Society. Endocrine Society Guidelines. <https://www.endocrine.org/guidelines-and-clinical-practice>.
- Ismail AA, Barth JH. Wrong biochemistry results. *BMJ* 2001; 232: 705–6.

Hypothalamus/pituitary function

Hypothalamic dysfunction

Causes

- Familial syndromes (Laurence–Moon–Biedl, Prader–Willi).
- Tumours (especially craniopharyngiomas, dysgerminomas, optic gliomas, meningioma—rarely pituitary tumours).
- Pituitary surgery.
- Infiltration (histiocytosis X, sarcoidosis).
- Trauma.
- Meningitis.
- Encephalitis.
- TB.

Symptoms and signs

- Obesity/hyperphagia.
- Somnolence.
- Thermoregulation.
- DI.
- Hypogonadism.
- Precocious puberty.

Investigations

- MRI.
- Water deprivation test for DI (➡ Polydipsia and polyuria: diabetes insipidus, pp. 134–6), tests of pituitary function.

Hypopituitarism

Definition

Failure of one or more pituitary hormones (usually multiple).

Causes

- Congenital.
- Pituitary tumour (including infarction of tumour ‘apoplexy’).
- Craniopharyngioma.
- Post-cranial irradiation.
- Following pituitary irradiation.
- Metastases to the pituitary (especially the breast).
- Post-surgery.
- Empty sella syndrome (occasionally).
- Sheehan’s syndrome (infarction with postpartum haemorrhage).
- CTLA-4 Ig immunotherapy for malignancy.

Symptoms and signs

Often very vague, e.g. tiredness, normocytic anaemia (easily missed). Combined with impotence or amenorrhoea—very suggestive. Other clues include loss of body hair (especially axillary), reduced shaving, hyponatraemia, and growth failure in children. DI is not a feature (unless there is also hypothalamic damage), as ADH can be secreted directly from the hypothalamus. There may also be signs of an SOL—bitemporal hemianopia (rarely

optic nerve compression, homonymous hemianopia); headache (especially following apoplexy); III, IV, V₁, V₂, or VI cranial nerve lesions; and CSF rhinorrhoea. Occasionally, galactorrhoea following pituitary stalk compression by a tumour ('disconnection'). Note: generally GH is lost first, then LH/FSH, and ACTH/TSH last. If multiple pituitary hormones are deficient, GH deficiency can be assumed.

Investigations

(See Fig. 2.1.) Basal-free T₄ (not TSH, which can be misleadingly normal), LH, FSH, PRL, and oestrogen/testosterone are usually sufficient to test the thyroid and gonadal axes. Adrenal and growth hormone testing requires dynamic tests (e.g. insulin tolerance test (ITT), but see below). Severe GH deficiency = peak stimulated GH <12.3mU/L (4.1ng/mL) using GHRH arginine test; <15.3mU/L (5.1ng/mL) using ITT testing. Note that the short Synacthen® test (➔ Short Synacthen® test, p. 225) is only suitable for testing the hypothalamopituitary–adrenal axis if pituitary failure is long-standing (>6 weeks), allowing time for adrenal atrophy to occur.

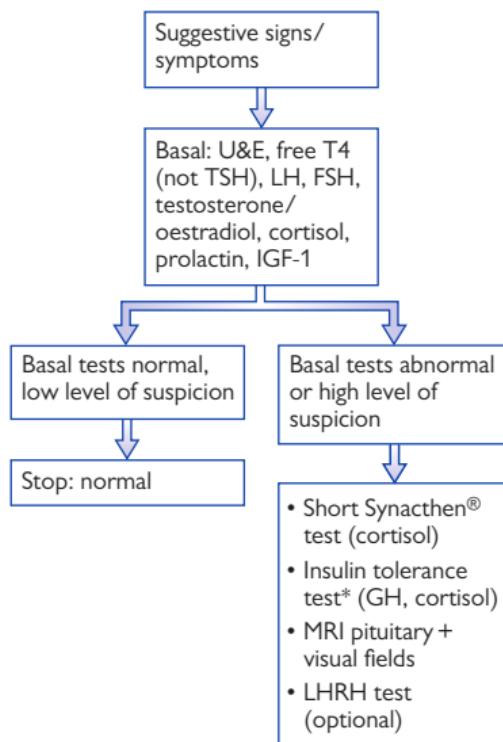


Fig. 2.1 Investigation of suspected hypopituitarism. Note: specialist paediatric advice should be taken in children.

* ➔ See also note under Alternative investigations, p. 130.

Alternative investigations

- GHRH arginine stimulation test. Although the ITT (Test protocols, pp. 216–17) is the traditional gold standard test for GH and 2° adrenal insufficiency, the GHRH arginine test has equal sensitivity and specificity for GH deficiency. It is also safer and better tolerated and is gradually replacing the ITT.¹
- Combined anterior pituitary testing—giving LHRH, thyrotropin-releasing hormone (TRH), ACTH, and GHRH (Combined anterior pituitary function testing, p. 218)—no clear advantage of this approach has been demonstrated and the results of the LHRH test, in particular, are difficult to interpret in pre-pubertal children.
- Long (depot) Synacthen® test—rarely required (Adrenal failure, pp. 162–4; Long (depot) ACTH test, p. 225).

Further reading

Molitch ME, Clemons DR, Malozowski S, Merriam GR, Vance ML; Endocrine Society. Evaluation and treatment of adult growth hormone deficiency. *J Clin Endocrinol Metab* 2011; **96**: 1587–609.

¹ National Institute for Health and Care Excellence. UK NICE guidelines for GH replacement 2003.  <https://www.nice.org.uk/guidance/ta64>.

Acromegaly (growth hormone excess)

For GH deficiency, see  Hypothalamus/pituitary function, pp. 128–30.

Clinical features

- Often insidious over many years.
- Enlarging hands and feet with rings having to be resized.
- Increase in shoe size.
- Coarsening of facial features, especially enlargement and broadening of the nose.
- Sweating.
- Headache.
- Malocclusion (protuberance of the lower jaw) and splaying of the teeth.
- Skin tags.
- Hypertension.
- Cardiac failure.
- Renal stones.
- Arthritis.
- Colonic polyps.
- Sleep apnoea.
- Carpal tunnel syndrome.
- DM.
- May be local symptoms from the pituitary tumour and symptoms/signs of loss of other pituitary hormones ( Hypothalamus/pituitary function, pp. 128–30).
- GH excess commencing before puberty results in gigantism (tall stature).

Investigations

- A random GH level is not helpful—may be high in normal people.
- A random IGF-1 level should be measured and compared with laboratory normal ranges corrected for age. This can be used as a screening test, but IGF-1 assays vary in reliability. IGF-1 levels should be raised in all cases of acromegaly, but levels can be affected (reduced) by fasting and systemic illness.
- Where IGF-1 results suggest GH excess, this should be confirmed in a standard 75g OGTT with glucose and GH measurements at 0, 30, 60, 90, and 120min.
- If no GH values are <2mU/L, then a diagnosis of acromegaly is confirmed.
- The vast majority (99%) of cases of acromegaly are due to pituitary tumours. If a pituitary tumour is not seen on MRI scanning, yet acromegaly is confirmed, a GHRH level should be requested to exclude ectopic production of this polypeptide by non-pituitary tumours stimulating the release of GH from the pituitary.
- For follow-up of treated cases of acromegaly, IGF-1 levels (more sensitive) and/or random GH levels can be used.
- Life expectancy appears to return to normality when the nadir of GH values is <3mU/L or IGF-1 levels return to the reference range.

Further reading

Katznelson L, Laws ER Jr, Melmed S, et al.; Endocrine Society. Acromegaly: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2014; **99**: 3933–51.

Polydipsia and polyuria: diabetes insipidus

'First-line tests'

It is relatively common for patients to report excess thirst or ↑ need to pass urine. Figure 2.2 and Box 2.1 summarize the causes. Prostatism and urge incontinence resulting in urinary frequency should be distinguished by history-taking as the patients do not have thirst. Then the first step is to identify straightforward causes, such as drugs (diuretics), DM, hypercalcæmia, hypokalaemia, and chronic renal failure with U&E, creatinine, glucose, and Ca^{2+} . Measuring 24h urine volume is also useful as volumes over 3L are likely to be pathological and volumes under 2L do not require further investigation. A GTT should not be required to diagnose DM as the renal threshold for glucose needs to be exceeded (~10mmol/L) to cause polyuria and there should be glucose in the urine.

Box 2.1 Causes of polyuria/polydipsia

Diabetes mellitus

- DI (cranial or nephrogenic)
- High Ca^{2+}
- Low K^+
- Chronic renal failure
- 1° polydipsia (including dry mouth, e.g. Sjögren's)

'Second-line tests'

Once other diagnoses have been excluded, subsequent tests aim to distinguish DI from 1° polydipsia (compulsive water drinking). A carefully supervised water deprivation test should be performed (Water deprivation test, pp. 220–1). However, it is not always easy to arrive at a conclusive diagnosis. Serum Na^+ levels are helpful, as DI is unlikely if $\text{Na}^+ < 140\text{mmol/L}$. Morning spot urine osmolality after overnight water restriction (not shown on chart) is occasionally useful—values $> 600\text{mOsmol/L}$ make significant degrees of DI unlikely. Measuring 24h urine volume is also useful, as volumes over 3L are likely to be pathological. However, obligate urine volumes as low as 2L could still cause the patient to complain of polyuria. In such borderline cases, the distinction between partial DI, normality, and 1° polydipsia can be very difficult. It has been proposed that in an adult or child over 2 years of age, a 24h urine volume of $> 40\text{mL/kg}$ body weight, a plasma osmolarity of $< 300\text{mOsmol/L}$, and a –ve test for glucose is diagnostic of DI, but this requires confirmation.

Guidance on interpretation of second-line tests, including the water deprivation test, is given in Table 2.2. Note that 1° polydipsia may be a psychiatric condition but can also occur in patients with a dry mouth (e.g. Sjögren's syndrome, anticholinergic drugs) or who have been previously encouraged to drink regularly 'to help their kidneys'.

Distinction between partial cranial DI and habitual (psychogenic) water drinking is complicated by the fact that drinking very high volumes over time may 'wash out' the renal medullary concentrating gradient. In this situation, a plasma vasopressin level at the end of the water deprivation test may be very helpful to distinguish a lack of vasopressin from a lack of vasopressin action. A 24h urine volume is also helpful as volumes of <3L/day are unlikely to cause renal 'washout'. Clues to 1° polydipsia include an initial plasma osmolality (and serum Na^+) that is low, plasma osmolality rising to >295mOsmol/L, and thirst not abolished by desmopressin, despite a rise in urine osmolality. Note that 'full-blown' cranial DI results in urine volumes of around 500mL/h (12L/day).

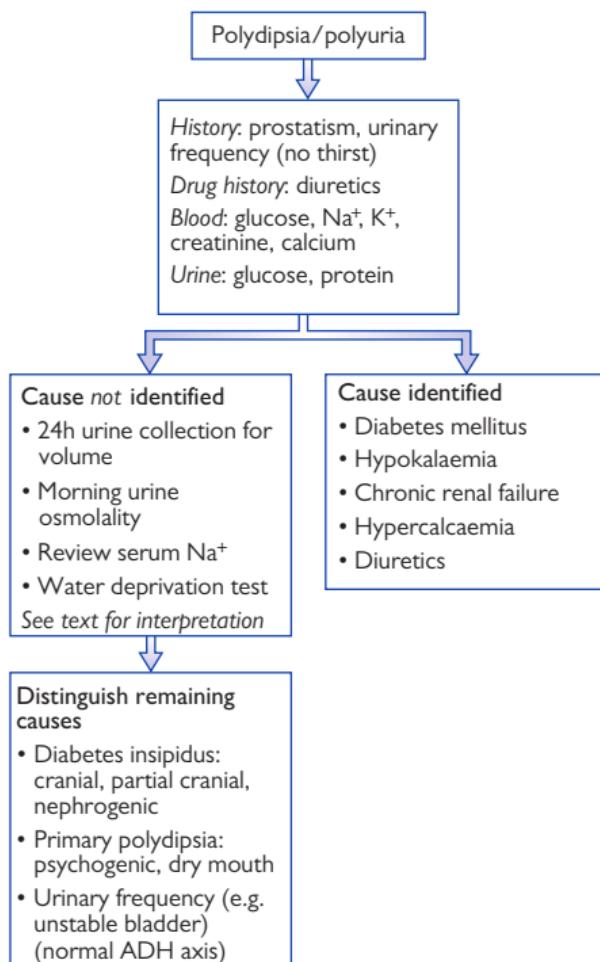


Fig. 2.2 Investigation of polydipsia/polyuria. Note: once cranial DI is diagnosed, further investigations into the underlying cause are required (➔ Hypothalamic dysfunction under Hypothalamus/pituitary function, p. 128).

Interpretation of second-line tests for polyuria/polydipsia

Table 2.2 Interpretation of second-line tests for polyuria/polydipsia

	Normal	Partial DI: cranial (C) or nephrogenic (N)	Primary polydipsia
Random serum Na ⁺	Normal	>140mmol/L	<140mmol/L
Random serum osmolality**	Variable	>290	<290
Morning urine mOsm ^{**}	Variable	Unlikely if >600 (C) Excluded if >600 (N)	
End of water deprivation test before desmopressin**	Urine >600 Plasma 280–295	Urine <600 Plasma >295	Urine >600* Plasma 280–295
Urine osmolality after desmopressin SC**	>600	Rises to >600 or >50% ↑ (C); rises to <600 or <50% ↑ (N)	Rises to >600* or >50% ↑
Plasma vasopressin at end of water deprivation test	Normal for plasma osmolality	Low for plasma osmolality (C); normal for plasma osmolality (N)	Normal for plasma osmolality

* With long-standing large-volume polyuria (>3L/day), these values may not be achieved due to washout of the renal medullary concentrating gradient—if results equivocal, see text.

** Osmolalities are all expressed in mOsmol/L.

'If all else fails'

In cases of doubt, a carefully supervised therapeutic trial of desmopressin can be useful to distinguish DI from 1° polydipsia (Diagnostic trial of desmopressin, p. 222). This should be done as an inpatient, as there is a risk of significant hyponatraemia in habitual water drinkers. The principle is that patients able to regulate water intake according to their thirst (DI) should not develop a hypo-osmolar plasma. In 1° polydipsia, the urine volume will fall and the urine concentrating gradient will gradually recover. However, if the patient continues to drink due to their psychological drive, rather than their thirst, they will become water-overloaded and hypo-osmolar.

An additional valuable test to distinguish partial DI from 1° polydipsia is hypertonic saline infusion testing, which usually requires access to a plasma vasopressin assay but has been used with urinary vasopressin levels.^{2–3} MRI scanning typically shows an ↑ signal in the posterior pituitary, which is lost in cranial DI. However, this sign is not helpful in distinguishing more subtle degrees of DI from other causes.

2 Robertson GL. Diabetes insipidus: diagnosis and management. *Best Pract Res Clin Endocrinol Metab* 2016; 30: 205–18.

3 Diederich S, Eckmanns T, Exner P, Al-Saadi N, Bähr V, Oelkers W. Differential diagnosis of polyuric/polydipsic syndromes with the aid of urinary vasopressin measurements in adults. *Clin Endocrinol (Oxf)* 2001; 54: 665–71.

Hyponatraemia (including syndrome of inappropriate antidiuretic hormone)

Hyponatraemia is a very common clinical problem. Figure 2.3 shows a flow chart for investigation. If patients are on diuretics, further evaluation is usually not possible. The diuretic will need to be discontinued. If this is not possible, hyponatraemia is likely to be attributable to an underlying condition (cardiac, renal, or liver failure). Pseudo- or dilutional hyponatraemia is important to exclude at an early stage (see Box 2.2). A careful clinical assessment should be made of the volume status, including identification of oedema, fluid loss (e.g. diarrhoea, fistula leakage), and signs of dehydration, including postural drop in BP. A urine Na^+ and TSH estimation is useful at this stage (see Fig. 2.3). Note that the most important diagnosis not to miss is hypoadrenalinism, as this can be fatal if untreated. Clinicians should have a low threshold for performing a short Synacthen® test (➡ Short Synacthen® test, p. 225). Hypoadrenalinism due to pituitary failure may not be accompanied by hyperkalaemia, hypotension, or hyperpigmentation and can easily be missed. Cerebral salt wasting occurs within days of brain injury (e.g. SAH, neurosurgery, or stroke) and is probably due to release of brain natriuretic peptides.

Box 2.2 Causes of pseudohyponatraemia

With normal serum osmolality

- Hyperproteinaemia (e.g. myeloma)
- Hyperlipidaemia (hypertriglyceridaemia)
- Glycine or sorbitol (from bladder irrigant)

With raised osmolality

- Hyperglycaemia
- Mannitol
- Glycerol

The syndrome of inappropriate antidiuretic hormone (SIADH) is a diagnosis of exclusion (see Box 2.3).

Box 2.3 Criteria for diagnosing SIADH

- Hyponatraemia present
- No diuretics
- No oedema
- Normal renal function
- Normal adrenal function
- Normal thyroid function
- Urine $\text{Na}^+ > 20 \text{ mmol/L}$
- Euvolaemic

Table 2.3 Causes of SIADH

Cause	Examples
Drugs	Carbamazepine, chlorpropamide, opiates, psychotropics, cytotoxics
CNS disorders	Head trauma, post-pituitary surgery (transient), stroke, cerebral haemorrhage, GBS, meningitis, encephalitis, fits
Malignancy	Small-cell lung cancer, pancreas, prostate
Chest disease	Pneumonia, TB, abscess, aspergillosis
General stimuli	Nausea, pain, smoking
Other	Acute intermittent porphyria

All the criteria in Box 2.3 should be met. A specific cause for SIADH is frequently not found or there may be a combination of precipitating factors (see Table 2.3). In the elderly, a state of chronic SIADH is relatively common and usually explains hyponatraemia persisting for many years without any other apparent cause. Affected individuals should be encouraged to drink less than a litre a day ('5 cups or less'), to only drink if they are thirsty, and to avoid exacerbating factors (see Table 2.3).

Features of SIADH/hyponatraemia that are often underappreciated

1. Other than a CXR, there is no requirement to search for an underlying malignant cause. If there is underlying malignancy, it is usually extensive, very apparent, and incurable (e.g. extensive small-cell carcinoma of the lung).
2. The urine osmolality does not have to be high. In individuals drinking large volumes of fluid, a urine osmolality as low as 250mOsmol/L (i.e. less than plasma) may be inappropriately concentrated, reflecting true SIADH.
3. Conditions previously diagnosed as 'sick cell syndrome' are now thought to represent SIADH in ill patients.
4. 'Water intoxication' is usually the combination of SIADH and excessive fluid intake. Healthy patients drinking to excess can rarely exceed the renal capacity to excrete a water load (~12L/day) and hence do not become hyponatraemic. A degree of SIADH is required for potomaniacs (excess water drinkers) to become hyponatraemic.
5. The post-operative state contains many precipitants to SIADH (nausea, pain, opiates, pneumonia) and ADH secretion is promoted by hypovolaemia from blood loss. The administration of '3L of IV fluid a day' post-operatively frequently results in hyponatraemia.

6. Symptoms of hyponatraemia, such as drowsiness, coma, or fits, are dependent on the rate of fall of serum Na^+ , not the absolute value. Patients who are alert with $\text{Na}^+ < 125 \text{ mmol/L}$ have clearly been chronically hyponatraemic and their serum Na^+ requires only gentle correction. However, a very rapid fall in serum Na^+ to $< 130 \text{ mmol/L}$ (typically due to massive infusion of hypotonic fluid into the bladder) may cause coma and needs to be corrected as a medical emergency with hypertonic saline.

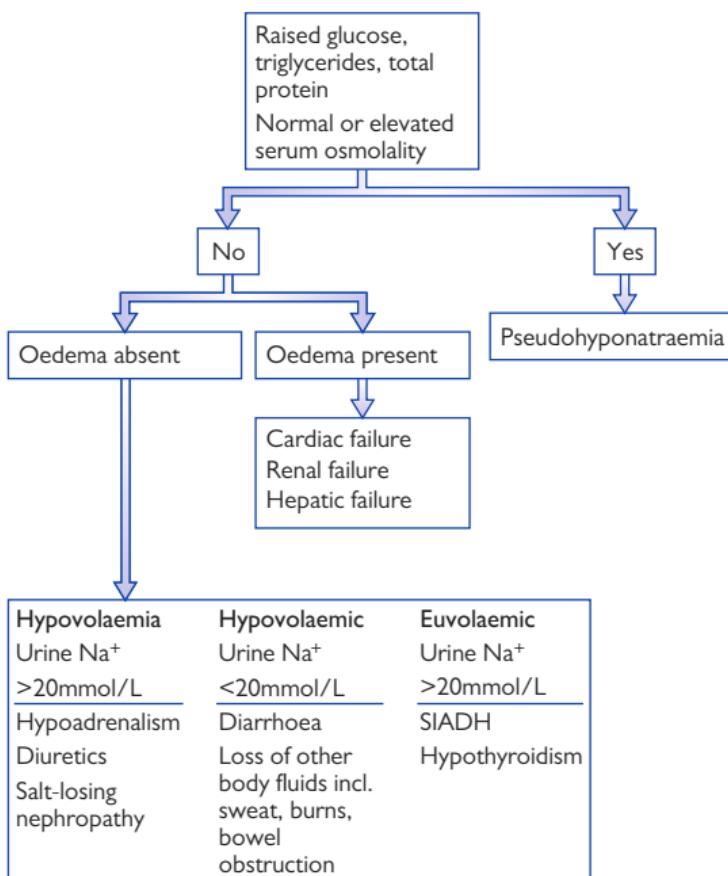


Fig. 2.3 Investigation of hyponatraemia.

Further reading

Spasovski G, Vanholder R, Allolio B, et al.; Hyponatraemia Guideline Development Group. Clinical practice guideline on diagnosis and treatment of hyponatraemia. *Eur J Endocrinol* 2014; **170**: G1–47.

Obesity/hypercortisolism

Endocrinologists are frequently asked to determine whether there is an underlying endocrine cause in patients who are obese. 2° causes of obesity are listed in Box 2.4. A long history of obesity, typically going back to childhood, is characteristic of constitutional obesity and further investigation, other than thyroid function, is rarely necessary. However, simple obesity may result in effects suggestive of hypercortisolism, e.g. striae, bruising, central obesity, rounded facial features, mild hyperandrogenism in women, buffalo hump, hypertension, and hyperglycaemia. Rapidly progressive obesity, marked hypertension, hypokalaemia, proximal muscle weakness, poor sleep, osteoporosis/vertebral collapse, and marked hirsutism or acne are more suggestive of hypercortisolism and require further investigation. Hypothalamic damage is usually apparent from the history.

Box 2.4 Secondary causes of obesity

- Constitutional
- Hypothyroidism
- Cushing's syndrome
- Hypothalamic damage (extreme hyperphagia)
- Genetic, e.g. Prader–Willi
- GH deficiency
- Drugs, e.g. antidepressants

The optimal approach to the diagnosis of hypercortisolism (Cushing's syndrome) is probably the most controversial subject in endocrinology. Endocrinologists who have seen many cases of Cushing's syndrome have seen exceptions to every rule, and the episodic nature of ACTH and cortisol secretion means that low values can occur even in disease. True cyclical Cushing's disease also occurs but is rare.

Diagnosis consists of two phases

1. Does the patient have hypercortisolism or not?
2. What is the cause of the hypercortisolism? Phase 1 must be completed first, as phase 2 tests can only be interpreted if hypercortisolism is present.

Investigation of hypercortisolism phase 1

Does the patient have hypercortisolism?

Patients being investigated for hypercortisolism should look Cushingoid. One exception is malignant ectopic ACTH secretion (e.g. from small-cell lung cancer) which can cause profound hypokalaemia before Cushingoid appearance is apparent. Depression and alcoholism may cause abnormal tests for hypercortisolism without representing a true hypercortisolae-mic state and hence are termed 'pseudo-Cushing's syndrome'. Such depressed patients often do not appear Cushingoid and alcoholism should

be identifiable clinically and biochemically. If there is a high degree of suspicion of hypercortisolism in a depressed patient, midnight cortisol levels <140nmol/L or a -ve result on dexamethasone–corticotropin-releasing hormone (CRH) testing (→ Low-dose dexamethasone suppression test, p. 223) may be helpful in excluding the diagnosis. Note that iatrogenic or factitious Cushing's syndrome is usually due to a steroid other than hydrocortisone (not detected in the cortisol assay) and characteristically results in an apparent suppression of the hypothalamopituitary–adrenal axis on testing.

Four tests are used to determine whether a patient does have hypercortisolism

1. *24h urinary-free cortisol (UFC) collections.* Three collections with simultaneous creatinine excretion estimation are ideal. If the creatinine excretion varies by >10% between collections, the samples are not true 24h collections and should be repeated. If two or more collections have a value >3 times the laboratory upper limit of normal (e.g. >800nmol/24h), then the diagnosis of hypercortisolism is secure. Patients with intermediate values should have repeat sampling after several weeks or additional tests. Steroids, adrenal enzyme inhibitors, statins, and carbamazepine must be discontinued prior to testing. False +ves can be caused by pregnancy, anorexia, exercise, psychoses, alcohol, and alcohol withdrawal.
2. *Low-dose dexamethasone suppression test (LDST).* This can be performed overnight or over 2 days (→ Low-dose dexamethasone suppression test, p. 223), the latter having less false +ves. Some authorities believe it adds little to UFCs as when cortisol secretion is high, the UFC is clearly raised, but in times when it is intermediate, the LDST may be normal. It is a useful outpatient screening test (→ Overnight dexamethasone suppression test under Low-dose dexamethasone suppression test, p. 223) in individuals who cannot reliably collect 24h urine samples.
3. *Late-night salivary cortisol test.* High salivary cortisol levels (>4nmol/L) measured between 11 p.m. and 12 midnight indicate loss of diurnal rhythm and are one of the best tests of hypercortisolism. Salivary samples can be collected as an outpatient by drooling into a collection tube or use of a cotton pledgelet. The tests should be repeated on two occasions. An alternative is a venous sample taken via an indwelling cannula in as relaxed a state as possible, preferably during sleep, but this requires an inpatient admission. Values <140nmol/L make hypercortisolism very unlikely.
4. *Dexamethasone-suppressed CRH test* (→ Low-dose dexamethasone suppression test, p. 223). This is a modification of the LDST, which was initially claimed to have a specificity of 100% for hypercortisolism, but subsequent series suggest <80%.

Summary

In patients who appear Cushingoid, three UFCs should be performed (note causes of false +ves). If these give equivocal results, additional tests are required, including further UFCs, late-night salivary cortisols, and a formal 2-day LDST followed by CRH.

Investigation of hypercortisolism phase 2

What is the cause of hypercortisolism?

The common and rare causes of hypercortisolism are summarized in Tables 2.4 and 2.5, along with useful clinical features. ~65% of cases are due to a pituitary adenoma (Cushing's disease), 20% due to an adrenal adenoma or carcinoma, and 10% due to ectopic ACTH production.

These are the three main causes to be distinguished using a combination of the tests shown under Investigations below. Distinction between a pituitary adenoma (which may not be visualizable on MRI) and a small indolent tumour (typically lung carcinoid) represents the greatest challenge. Despite extensive investigation, the cause will remain uncertain in some of these cases.

Investigations

1. *Plasma ACTH level* (separate and freeze immediately). Undetectable plasma ACTH levels are strongly suggestive of an adrenal tumour. However, ACTH secretion is intermittent and two suppressed values with simultaneous high cortisol levels ($>400\text{nmol/L}$) are preferable and should prompt adrenal CT scanning.
2. *High-dose dexamethasone suppression test* (➡ High-dose dexamethasone suppression test, p. 224). Greater than 90% suppression of basal UFC levels is strongly suggestive of a pituitary adenoma. Lesser degrees of suppression are seen with ectopic ACTH.
3. *Inferior petrosal sinus sampling (IPSS)*. This is an excellent diagnostic tool but requires expert radiological support and should only be performed in tertiary referral centres. A total of 100mg IV of CRH is also given via a peripheral vein, while sampling, to ensure active secretion of ACTH during the test. ACTH levels are compared between the

Table 2.4 Common causes of hypercortisolism (Cushing's syndrome)

Cause	Pathology	Characteristic features
ACTH-secreting pituitary adenoma (Cushing's disease)—65%	Pituitary adenoma	Typical features of hypercortisolism with little virilization
Ectopic ACTH secretion—10%	Malignant: small-cell lung cancer, thymic carcinoid, medullary thyroid cancer Indolent/benign: bronchial/pancreatic carcinoids, phaeochromocytoma	Malignant: rapid progression, marked hypokalaemia, proximal muscle weakness, ↑ BP, tumour clinically apparent, few Cushingoid signs Indolent: indistinguishable from Cushing's disease, tumour not easily detected
Adrenal tumour—20%	Adrenal adenoma Adrenal carcinoma	Adenomas: typical Cushingoid signs, sometimes virilization Carcinomas: rapid progression (months) with virilization, poor prognosis

inferior petrosal sinus on both sides and a peripheral vein. Sampling is performed at -15 , 0 , $+15$, and $+30$ min after CRH injection. Ratios >2 (ideally >3) post-CRH are strongly suggestive of pituitary-dependent disease. Risks include failure to enter the sinus and sinus thrombosis.

4. *Imaging.* Pituitary and adrenal imaging should not be performed without biochemical testing, as non-functioning tumours of the pituitary and adrenal are common (false +ve), and conversely functioning pituitary tumours are often too small to be visualized by MRI (false -ve). However, if the findings are consistent with the biochemical tests, this is useful supportive evidence. Patients with findings suggestive of ectopic ACTH production should have thin-slice CT of the chest looking for a bronchial adenoma, and MRI scanning of the pancreas for an islet tumour. ^{111}In -labelled octreotide scanning may also be useful in locating small tumours.
5. *Plasma CRH levels.* Very rarely, 'ectopic ACTH' syndrome is actually due to ectopic CRH production stimulating ACTH from the pituitary (see Table 2.5). Raised plasma CRH levels may be diagnostic in this condition.

Table 2.5 Rare causes of hypercortisolism (Cushing's syndrome)

Cause	Pathology	Characteristic features
Ectopic CRH secretion	Variety of tumours, mostly carcinoids	Clinical features indistinguishable from Cushing's disease, but no pituitary tumour, ↑ serum CRH and may fail to suppress with high-dose dexamethasone
Ectopic gastrin-releasing peptide secretion	Medullary thyroid cancer	Very rare, resembles ectopic CRH
Factitious ACTH administration	Injections of ACTH	Very difficult to distinguish from ectopic CRH secretion or Cushing's disease but, if isolated from their ACTH source, become adrenally insufficient in days
Cyclical Cushing's disease	Cyclical secretion from pituitary adenoma	Cushing's disease with intermittently -ve tests
Pseudo-Cushing's syndromes	Depression or alcoholism	Clinical evidence of Cushing's disease may be limited; evidence of depression or alcoholism
Bilateral micronodular adrenal hyperplasia	Often associated with Carney complex	Investigation suggestive of adrenal tumour (ACTH suppressed), but adrenals normal or slightly enlarged and contain pigmented nodules
Bilateral macronodular adrenal hyperplasia	Sporadic or familial	Investigation suggestive of adrenal tumour (ACTH suppressed), but marked or very marked bilateral nodular enlargement of adrenals on CT scanning

Additional tests include

- Metyrapone test: here the adrenal enzyme blocker metyrapone is used to lower cortisol levels. Pituitary adenomas respond by ↑ ACTH production, but ectopic sources of ACTH do not. The test can also be used to confirm that ACTH levels are truly suppressed in adrenal tumours (rarely necessary).
- Peripheral CRH test:* ACTH levels are measured before (-30, -15min) and +15 and +30min after injection of 100mg IV of CRH into a peripheral vein. A rise in ACTH levels of >34% is suggestive of a pituitary adenoma. The addition of 5µg IV of desmopressin improves the response rate and reduces false –ves.

Summary

(See Fig. 2.4.)

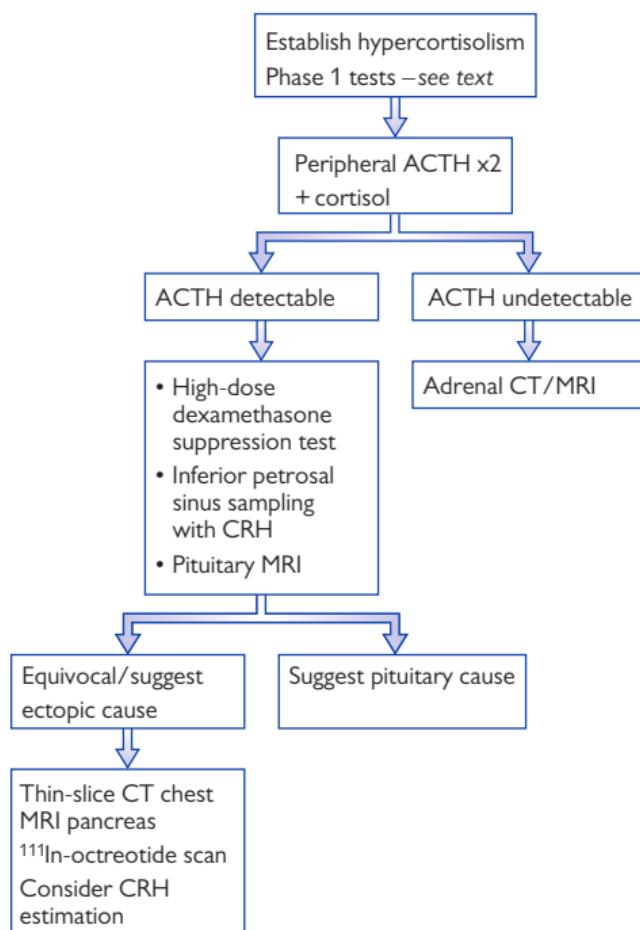


Fig. 2.4 Hypercortisolism. Flow chart for diagnosing the cause once hypercortisolism is established.

Further reading

Nieman LK, Biller BM, Findling JW, et al. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2008; **93**: 1526–40.

Endocrine hypertension

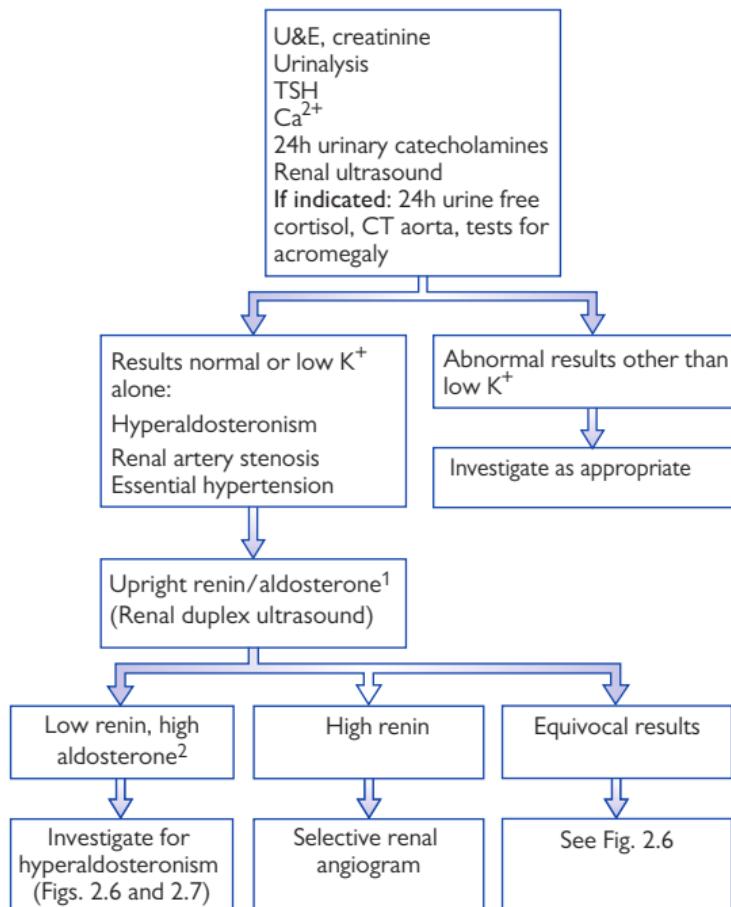
Ninety-five per cent of cases of hypertension are ‘essential hypertension’ with no specific underlying cause. If hypertension is very marked, occurs in younger patients, is difficult to control with drugs, is episodic/fluctuating, is of recent onset, is familial, is associated with recurrent hypokalaemia, or has associated features (see Table 2.6), then an underlying cause should be excluded.

History and examination should include features of conditions in Table 2.6, with particular attention to paroxysmal attacks, drugs (e.g. liquorice), and family history.

Table 2.6 Secondary causes of hypertension

Physical features	Notes
Absent	
Phaeochromocytoma	May be familial, e.g. in MEN-2 (may have mucosal neuromas), von Hippel–Lindau syndrome, neurofibromatosis; paroxysmal ↑ BP in only 60% of cases with headache, sweating, and palpitations
Hyperaldosteronism	Multiple syndromes, including Conn’s syndrome (see Table 2.7)
Renal artery stenosis	Congenital or acquired (atheroma)
Renal disease	Any cause, including polycystic kidneys
Hyper-/hypothyroidism	Diastolic hypertension with hypothyroidism, systolic hypertension with hyperthyroidism
Hyperparathyroidism	Does not usually improve after surgical cure
Drugs	Epo, ciclosporin, cocaine, amphetamines, steroids, liquorice, oestrogens, and androgens
<i>Physical features present</i>	
Coarctation of the aorta	
Cushing’s syndrome	
Acromegaly	
Pregnancy-induced	

Figure 2.5 provides a flow chart for further investigations. At least three separate BP readings should be obtained—24h BP monitoring may be useful where ‘white coat hypertension’ is suspected.



Notes:

1. Ideally, this test should be performed off all antihypertensive drugs for 2 weeks (6 weeks for spironolactone) except alpha blockers.
2. Low renin and very low aldosterone should prompt investigations for 'apparent mineralocorticoid excess'.

Fig. 2.5 Investigation of causes of hypertension.

The majority of 2° causes of hypertension can be rapidly excluded by the investigations shown in the first box of Fig. 2.5. If the results are normal or the only abnormality is a low K⁺ level, then the possibilities of hyperaldosteronism or RAS remain to be distinguished from essential hypertension. Further investigation should be driven by the severity of the hypertension, the (young) age of the patient, and the difficulty in obtaining control with drugs.

Investigation of renal artery stenosis/high renin levels

Selective renal angiography remains the gold standard for diagnosing RAS—other imaging methods can miss the diagnosis. Three-dimensional (3D) MR angiography is now considered a non-invasive alternative. High renin levels associated with hypertension (off drugs) in the absence of RAS should prompt a search for a juxtaglomerular cell tumour of one kidney. Note that the presence of hypertension is essential, as many conditions associated with a low or normal BP can result in 'appropriate' hyper-reninaemia (e.g. diuretics; cardiac, renal, or liver failure; hypocortisolism; hypovolaemia). High renin levels can also occur in essential hypertension.

Investigation of hyperaldosteronism

Hypertension with persistent hypokalaemia raises the possibility of hyperaldosteronism which may be due to a variety of causes (see Table 2.7). Note that investigation for hyperaldosteronism is also appropriate with K⁺ levels in the normal range if other investigations are –ve and hypertension is marked, difficult to control, or in a younger patient. The optimal approach to investigation remains controversial and equivocal cases frequently occur. If there is marked hypokalaemia of recent onset, a 24h UFC (and review of medication) is indicated to exclude recent-onset hypercortisolism (usually due to ectopic ACTH production) in which Cushingoid features have not yet become apparent. True hyperaldosteronism is never due to a malignant lesion, so that if hypertension can be medically controlled, it is not always necessary to establish a definitive diagnosis of aetiology. A detailed scheme is provided in Fig. 2.6.

Establishing hyperaldosteronism

The initial investigation is an upright aldosterone/renin ratio, performed when the patient has been upright or sitting (not lying) for at least 2h. The sample needs to be taken to the laboratory, separated, and frozen immediately. Ideally, the patient should be on no antihypertensives other than α-blockers (e.g. doxazosin), as most drugs can affect interpretation of the test results (see Table 2.8). This is difficult to achieve in subjects with very marked hypertension. Combination antihypertensive therapy and spironolactone cause the most confusion. An undetectable renin with an unequivocally high aldosterone level makes the diagnosis very likely. A normal or raised upright renin excludes hyperaldosteronism. Borderline results should be repeated off interfering medication and after K⁺ replacement (hypokalaemia can inappropriately lower aldosterone). A low renin with a normal aldosterone can be seen in essential ('low renin') hypertension. Refer to the laboratory for normal and diagnostic ranges. Additional tests (e.g. renin after Na⁺ restriction/furosemide, aldosterone after captopril, Na⁺ loading, or IV saline) are used in specialist centres, but their exact role in testing remains unresolved.

Table 2.7 Investigating established primary hyperaldosteronism

	Change in aldosterone with posture	CT findings	Adrenal venous sampling (ratio of aldosterone between sides)	Response to glucocorticoids*	Treatment of choice	Notes
Adenoma (Conn's)	None/fall	Unilateral nodule	>10:1	Absent	Surgery	
Renin-responsive adenoma	Rise	Unilateral	>10:1	Absent	Surgery	
Unilateral hyperplasia	None/fall	'Normal'	>10:1	Absent	Surgery	
Bilateral hyperplasia	Rise	'Normal'	No difference	Absent	Medical	
Glucocorticoid remediable aldosteronism (GRA)	None/fall	Normal	No difference	Present	Steroids	Very raised 18-oxo cortisol. Positive genetic screening**

* Dexamethasone 0.5mg 6-h for 2–4 days resulting in suppression of aldosterone levels to nearly undetectable levels (usually associated fall in BP also).

** Positive for chimeric CYP11B1/CYP11B2 gene.

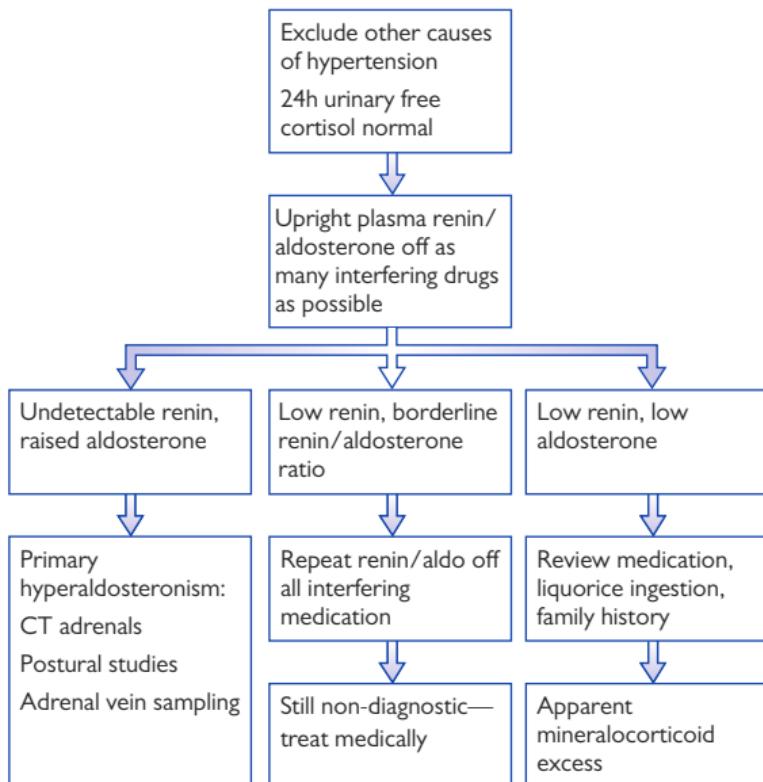


Fig. 2.6 Investigation of hyperaldosteronism/mineralocorticoid excess in patients with hypertension.

Table 2.8 Renin/aldosterone testing and drugs

Drug	Effect on PRA	Effect on aldosterone
Drugs that ↑ PRA		
Spironolactone	↑	Variable
Ca ²⁺ channel blockers	May ↑	↓
ACE inhibitors*	↑	↓
Diuretics	↑	↑
Vasodilators	↑	↑
Drugs that ↓ PRA		
α-blockers	↓	↓
NSAIDs	↓	↓

PRA, plasma renin activity.

* Angiotensin II receptor antagonists are likely to have same effects.

Investigating the cause of established primary hyperaldosteronism

There are five causes of established 1° hyperaldosteronism with suppressed renin and high aldosterone (see Box 2.5). Surgery (unilateral adrenalectomy) is indicated for adenoma (65% of cases), the unusual renin-responsive adenoma, and the rare cases of unilateral hyperplasia, but not for bilateral hyperplasia (idiopathic hyperaldosteronism, 30% of cases) or the rare familial glucocorticoid-remediable aldosteronism (GRA). Tests to distinguish these are summarized in Box 2.5 and Fig. 2.7.

➔ OCHM 10e, pp. 228–9.

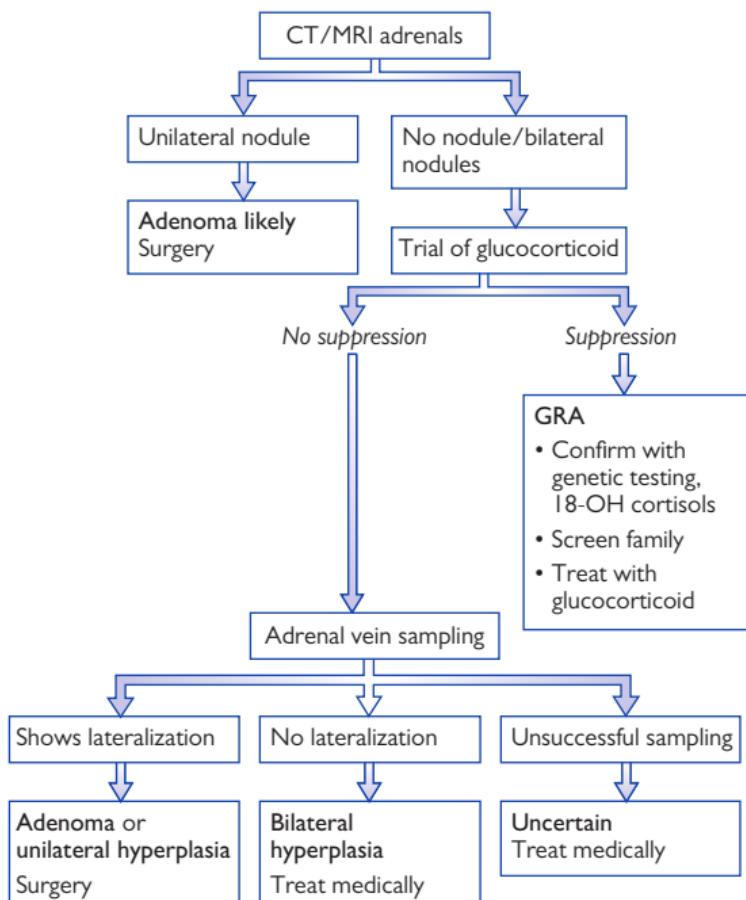


Fig. 2.7 Identifying the cause of established primary hyperaldosteronism.

Box 2.5 Causes of hyperaldosteronism/apparent mineralocorticoid excess

Primary hyperaldosteronism (decreased renin, increased aldosterone)

- Aldosterone-producing adenoma (Conn's syndrome)
- Renin-responsive adenoma
- Idiopathic unilateral hyperplasia
- Idiopathic bilateral hyperplasia
- Glucocorticoid-remediable hyperaldosteronism

Apparent mineralocorticoid excess (decreased renin, increased aldosterone)

- Liquorice ingestion, carbenoxolone, fludrocortisone
- Congenital 11 β -hydroxysteroid dehydrogenase deficiency
- Liddle's syndrome
- Congenital adrenal hyperplasia (11 β -hydroxylase or 17 α -hydroxylase def.)
- Hypercortisolism

If hyperaldosteronism is established and a nodule is visible on CT/MRI imaging, it is reasonable to proceed to unilateral adrenalectomy/excision of the nodule. If no nodule or bilateral nodules are seen, then adrenal vein sampling is the most useful test to determine whether surgery should be performed. Aldosterone levels after glucocorticoid administration or genetic testing for the chimeric CYP11B1/CYP11B2 gene should be performed beforehand to exclude GRA (see Table 2.7, p. 151—family members may be only mildly hypertensive, making family histories unreliable). Unfortunately, the right adrenal vein cannot be catheterized in up to 25% of cases and there is a risk of precipitating adrenal haemorrhage. Postural studies identifying a >50% rise in aldosterone comparing recumbent and 2–4h of standing/walking suggest idiopathic hyperplasia, but a small renin-responsive adenoma not visible on CT could give similar results.

Investigating the cause of apparent mineralocorticoid excess

Rarely, investigation reveals low renin and low aldosterone levels in the presence of hypertension, hypokalaemia, and alkalosis. There are five causes of this (see Box 2.5). A 24h UFC estimation will rapidly exclude recent-onset, aggressive hypercortisolism. Repeated enquiry should be made for drug and liquorice product ingestion. The remaining causes may be diagnosed by urinary cortisol/cortisone ratio (11 β -OH steroid dehydrogenase deficiency—often referred to alone as ‘apparent mineralocorticoid excess’) or other appropriate changes in urinary and plasma cortisol metabolites (e.g. raised DOC levels—11 β -hydroxylase or 17 α -hydroxylase deficiency) or responsiveness to amiloride (Liddle's syndrome).

Phaeochromocytoma

1. *Clinical features.* Phaeochromocytoma is rare, but an important diagnosis not to miss—can result in fatal hypertensive crisis, especially during surgery or after inadvertent β -adrenoreceptor blockade without α blockade. It can be sporadic (90%) or be the first clue to a familial syndrome (see Table 2.6). ~10% of cases are extra-adrenal, 10% multiple, and 10% malignant ('tumour of 10%). Ninety per cent of cases have sustained or paroxysmal hypertension, but paroxysmal attacks of some nature are a feature of only 55% of cases. Pure adrenaline-secreting lesions can occasionally cause hypotension. They are always intra-adrenal. Phaeochromocytoma needs to be excluded in cases of incidentally found adrenal masses. Paragangliomas are non-secreting phaeos.
2. *Diagnostic tests.* Plasma free or 24h urinary fractionated metanephhrines have replaced catecholamine estimations or catecholamine metabolites (vanillylmandelic acids (VMA)), as they are more sensitive and specific and are released continuously. A single clearly positive estimation in the presence of hypertension is usually sufficient. If non-diagnostic, sampling in the recumbent position may help confirm normal levels (metanephhrines are 2-fold higher in the seated position). Mild ↑ can be seen in anxiety states and with very small lesions detected in the follow-up of familial, recurrent disease. Causes of false +ve results include methyldopa, levodopa, labetalol, sotalol, tricyclic and monoamine oxidase inhibitor (MAOI) antidepressants, paracetamol, sulfasalazine, sympathomimetics, cocaine, clonidine withdrawal, intracranial events (e.g. SAH, posterior fossa tumour), or metabolic stress (e.g. hypoglycaemia, MI).
3. *Finding the tumour.* Once the diagnosis is established, blockade (typically with increasing twice-daily (bd) doses of phenoxybenzamine) should be established before invasive investigation. The tumours are usually large (>2cm) and bright on T2-weighted (T2W) images. CT/MRI scanning therefore identifies virtually all adrenal lesions. Radionuclide scanning with ^{131}I -MIBG (iodine-131-meta-iodobenzylguanide) is useful to confirm activity if >1 adrenal nodule is present and to identify extra-adrenal lesions where no adrenal lesion is seen. Note that extra-adrenal phaeochromocytomas (paragangliomas) are usually in the chest or abdomen but can occur in the neck (including chemodectomas of the carotid body), pelvis, and bladder. Biopsy of suspected phaeochromocytoma lesions is contraindicated.
4. *Malignant phaeos.* The only reliable indicator of malignancy in phaeos is the presence of distant metastases or local invasion on histology. The histological appearance of the tumour cells themselves surprisingly has no significance.
5. *Genetic testing.* Up to 30% of phaeos, especially if familial, will have a genetic basis (see Table 2.9). Genetic testing is now recommended to be considered in all confirmed cases, especially in familial, syndromic, or recurrent cases. Fourteen gene loci have been associated with phaeochromocytoma—the commonest are shown in Table 2.9. Genetic testing can be targeted to limited loci according to the clinical presentation, the location of the tumour, and whether it is metastatic or not (☞ Further reading, p. 156).

☞ OCHM 10e, pp. 228–9, p. 738, p. 837.

Table 2.9 Phaeochromocytomas: genetic mutations associated with phaeochromocytomas or paragangliomas

Gene	Syndrome	Associated features	Frequency
RET	MEN-2	Medullary carcinoma of the thyroid, hyperparathyroidism	5%
VHL	von Hippel–Lindau syndrome	Haemangioblastomata, renal, pancreatic tumours	8%
SDH B		Large, malignant, extra-adrenal paragangliomas	6%
SDH D		Often head and neck paragangliomas	4%
SDH C		Rare	0%
Neurofibromatosis	von Recklinghausen's/disease	Skin neurofibromata, café-au-lait spots	4%

SDH, succinate dehydrogenase.

Further reading

Lenders JW, Duh QY, Eisenhofer G, et al.; Endocrine Society. Pheochromocytoma and paraganglioma: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* 2014; **99**: 1915–42.

Hypokalaemia

Persistent hypokalaemia ($<2.5\text{ mmol/L}$) can cause muscle weakness, cramps, tetany, and polyuria, and exacerbate digoxin toxicity and predispose to cardiac arrhythmias. The majority of cases are due to the common causes (see Box 2.6) and are relatively easy to diagnose. However, puzzling cases where none of these features are present occur and prompt further investigation. A flow chart is shown in Fig. 2.8.

Box 2.6 Common causes of hypokalaemia*

- Diuretics
- Vomiting/diarrhoea
- Intestinal fistula
- Laxative or diuretic abuse
- Steroids (including fludrocortisone), liquorice, ACTH therapy

* See text for investigation of rare causes.

Note in Fig. 2.8 the importance of identifying the presence of acidosis and hypertension. Occult diuretic and purgative use should always be borne in mind. The commonest cause of persistent hypokalaemia with no other cause presenting in adulthood is Gitelman's syndrome (NCCT-Na-Cl cotransporter defect), an asymptomatic congenital disorder, which can usually be separated from the rare, more severe Bartter's syndrome (which usually presents neonatally or in early childhood and represents gene defects in the renal tubular proteins NKCC2, ROMK, or CLCNKB) by low serum Mg^{2+} levels.

➲ OHCM 10e, p. 98, p. 674.

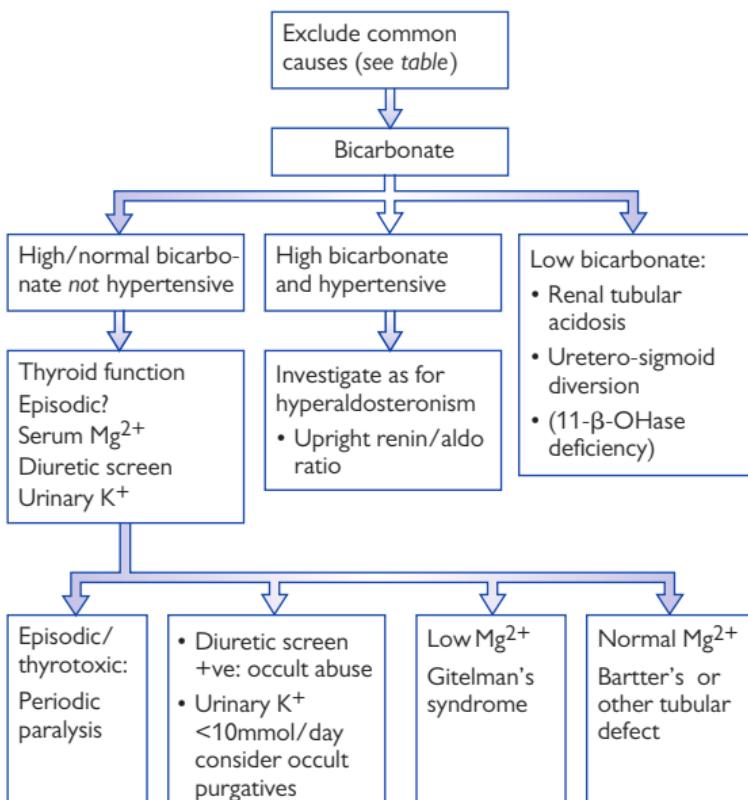


Fig. 2.8 Investigation of hypokalaemia.

Further reading

Shaer AJ. Inherited primary tubular hypokalemic alkalosis: a review of Gitelman and Bartter syndromes. *Am J Med Sci* 2001; 322: 316–22.

Hyperkalaemia

Artefactual and common causes need to be excluded, of which renal failure is the most important (see Table 2.10). If these fail to reveal a cause, then hypoadrenalinism (which can be life-threatening), isolated mineralocorticoid deficiency, and type IV renal tubular acidosis (RTA) need to be excluded.

Table 2.10 Causes of hyperkalaemia

Artefactual	Other	Rare, but important
Sample left unseparated overnight	Excess K ⁺ replacement	Hypoadrenalinism
Sample haemolysed	K ⁺ -sparing diuretics, ACE inhibitors	Type IV RTA
Myeloproliferative disease (leakage of K ⁺ from high cell counts)	Renal impairment, especially acute and after trauma or surgery Metabolic acidosis (especially DKA), rhabdomyolysis, burns, massive blood transfusion	Isolated mineralocorticoid deficiency

Hypoadrenalinism is suggested by concomitant hyponatraemia, hypotension (including postural), malaise, and skin pigmentation. Diagnosis is by short Synacthen® testing (➔ Adrenal failure, pp. 162–4). Note that hyperkalaemia is not a feature of 2° (pituitary) hypoadrenalinism since aldosterone production is maintained by the renin–angiotensin system. Type IV RTA is common in patients with diabetes. It is associated with renal tubular dysfunction, as well as mildly impaired glomerular function. Serum creatinine is usually at, or above, the upper limit of normal. It is a state of hyporeninaemic hypoaldosteronism. Renin/aldosterone testing is suggestive, but there is no definitive test. Isolated mineralocorticoid deficiency is usually congenital (e.g. due to aldosterone synthase deficiency) but can be acquired (e.g. HIV disease). High renin and low aldosterone levels would be expected. Aldosterone resistance (pseudohypoaldosteronism with high aldosterone levels, but biochemical mineralocorticoid deficiency) has been described.

➔ OCHM 10e, p. 93, p. 301, p. 674.

Adrenal failure

For causes of 1° adrenal failure, see Table 2.11.

Hypoadrenalinism is often insidious in clinical onset. However, it is an important diagnosis to make, as it can be life-threatening, especially at times of stress. The key is to have a high index of suspicion. 1° adrenal failure is suggested by hyperkalaemia, hyponatraemia, hypotension (including postural), malaise, weight loss, nausea, abdominal pain, and skin pigmentation. In pituitary (2° adrenal failure, hyperkalaemia, hypotension, and pigmentation are absent, and malaise may be the only feature. Signs/symptoms of gonadal failure (e.g. loss of libido, reduced shaving, or amenorrhoea), if present, mandate exclusion of pituitary failure. Random cortisol levels can be misleading, as they may be high in the morning and low in the evening. Nonetheless, a random cortisol level $>550\text{nmol/L}$ excludes the diagnosis and is a useful test in patients undergoing severe stress/illness (e.g. in intensive therapy unit (ITU)).

► *Do not delay treatment.* Where there is a strong suspicion of adrenal failure, treatment must not be delayed pending investigation. A short Synacthen® test or random cortisol should be performed immediately and treatment commenced with steroids, awaiting results. Alternatively, treatment with dexamethasone 0.5mg daily (which does not cross-react in the cortisol assay) can be used and then discontinued for the day of testing. Patients on other forms of glucocorticoid therapy should discontinue treatment on the morning of the test and ideally 24h beforehand (12h for hydrocortisone or cortisone acetate). Mineralocorticoid replacement need not be discontinued.

Short ACTH (Synacthen®) test

The standard test for adrenal failure is the short ACTH test. A low dose of synthetic ACTH (0.5 or 1.0 μg) test was previously in vogue but has not been confirmed to be useful.

For 2° (pituitary) adrenal failure, alternative tests include the insulin tolerance test (→ Insulin tolerance test, pp. 216–17) and the metyrapone test. However, these tests involve applying a stress and carry a risk in patients who are profoundly hypoadrenal. They are only indicated in patients within 6 weeks of pituitary surgery or with a pituitary insult where hypotrophy of the adrenal cortices has yet to develop.

Test to distinguish primary vs secondary adrenal failure

In the context of known pituitary disease and with failure of other pituitary hormones, adrenal failure can be assumed to be 2° (pituitary) in origin. Where isolated adrenal failure is identified, 1° adrenal failure is most likely and suggested by ↑ skin pigmentation and hyperkalaemia.

Table 2.11 Causes of 1° adrenal failure

Cause	Associated features	Diagnostic tests/notes
Autoimmune adrenalitis (>90% of cases in developed countries)	Autoimmune damage may be associated with polyglandular failure types 1 and 2	Anti-adrenal (21-hydroxylase) antibodies
Drugs	Ketoconazole, mitotane, etomidate, rifampicin, phenytoin	Exacerbate pre-existing adrenal impairment
TB	Extra-adrenal TB	Calcified or enlarged adrenals, extra-adrenal TB, but may only show shrunken glands
Other infections, e.g. histoplasmosis, syphilis	Seen in North and South America	Adrenal glands enlarged
Metastatic malignancy	Common with breast, lung, melanoma, or GI cancer, although does not always cause adrenal failure	Enlargement/deposits in adrenal glands on CT
Bilateral adrenal haemorrhage	Anticoagulation, adrenal vein sampling	Signs of haemorrhage on CT
AIDS	CMV/TB, <i>Cryptococcus</i> adrenitis	
Adrenoleukodystrophy	Especially in ♂ <15 years, dementia, quadriplegia	
Adrenomyeloneuropathy	Neuropathy, blindness—may appear after adrenal failure	
Familial glucocorticoid deficiency	Defective melanocortin 2 receptors, including Allgrove's syndrome, hypoadrenalinism associated with seizures, achalasia, and alacrima from childhood	
Defective cholesterol metabolism		
Congenital adrenal hypoplasia	Mutation in <i>DAX1</i> or related genes, causing failure of adrenals to develop. Adrenal insufficiency from birth	

Three additional tests can be used to confirm the level of adrenal failure

1. *Basal plasma ACTH.* This is usually the only additional test required.

High levels are seen in 1° adrenal failure; ‘normal’ or low levels are seen in 2° adrenal insufficiency. Note that the sample must be taken and separated immediately at least 24h after the last dose of a short-acting glucocorticoid (e.g. hydrocortisone) to avoid pharmacological suppression. Patients on a longer-acting steroid may have to have the test repeated >24h after cessation of the steroid if the result is equivocal.

2. *Anti-adrenal antibodies* (anti-21 hydroxylase antibodies). These antibodies are present in around 70% of patients with autoimmune adrenalitis (Addison’s disease), the commonest cause of 1° adrenal insufficiency. However, they can also be present without adrenal failure in patients with other autoimmune conditions.
3. *Long (depot) ACTH test.* Chronic stimulation with ACTH can recover function in adrenal glands that have failed because of lack of pituitary ACTH, but not in 1° adrenal failure. This is given in the form of ACTH in oil on 2 consecutive days (→ Long (depot) ACTH test, p. 225) or as an infusion over 48h. With the advent of reliable ACTH assays, this test is rarely indicated.

Additional diagnostic tests—exclude adrenoleukodystrophy in males

While the majority of cases of 1° hypoadrenalism are due to autoimmune disease in developed countries, there are multiple other rare causes. These should particularly be considered where adrenal failure occurs in childhood and/or is associated with neurological disease or hypogonadism (see Table 2.11). In particular, adrenoleukodystrophy (ALD) should be excluded. All ♂ diagnosed with 1° adrenal failure should have serum sent for very long-chain fatty acids (VLCFAs—raised in ALD), as early bone marrow transplantation (and, to a limited extent, treatment with ‘Lorenzo’s oil’) may prevent irreversible progressive neurological disease from developing (e.g. spastic paraparesis).

Further reading

Bornstein SR, Allolio B, Arlt W, et al. Diagnosis and treatment of primary adrenal insufficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2016; 101: 364–89.

Amenorrhoea

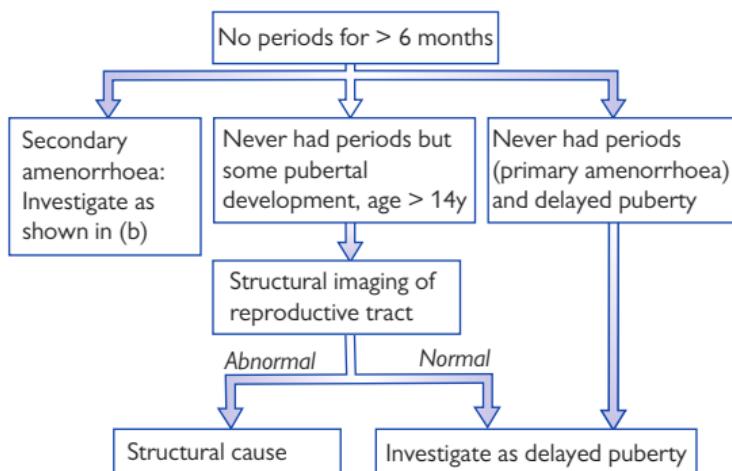
Amenorrhoea is often separated into 1° (never menstruated) and 2° (cessation of periods after menarche) amenorrhoea, but many causes are shared between the two categories. Structural assessment of the genital tract should be performed earlier in investigation of 1° amenorrhoea. Investigation of oligomenorrhoea is similar to 2° amenorrhoea. Menorrhagia and intermenstrual bleeding are due to different causes, often gynaecological in origin. 'Irregular periods' can fall into either category, depending on whether it actually refers to intermenstrual bleeding or variably spaced (anovulatory) periods. A plan of investigation is shown in Fig. 2.9.

In 2° amenorrhoea, it is helpful early on to identify 1° ovarian failure (e.g. due to Turner's syndrome, premature ovarian failure, radiation, mumps orchitis, radiation, chemotherapy, or non-45XO gonadal dysgenesis) characterized by high gonadotrophins (LH, FSH). Where the gonadotrophins are equivocal or low, amenorrhoea due to hyperprolactinaemia or thyrotoxicosis should be excluded, but the commonest diagnosis is chronic anovulation due to polycystic ovarian syndrome. In this condition, the ovaries still produce oestrogen, resulting in a +ve progesterone withdrawal test; 10mg of medroxyprogesterone is given daily for 5 days and the test is +ve if any menstrual bleeding occurs in the following week. If the test is -ve, a pituitary (e.g. pituitary tumour) or hypothalamic (e.g. stress, anorexia nervosa, systemic illness, or weight loss) cause resulting in profound oestrogen deficiency must be considered.

Further reading

Azziz R. The evaluation and management of hirsutism. *Obstet Gynecol* 2003; 101: 995–1007.

(a)



(b)

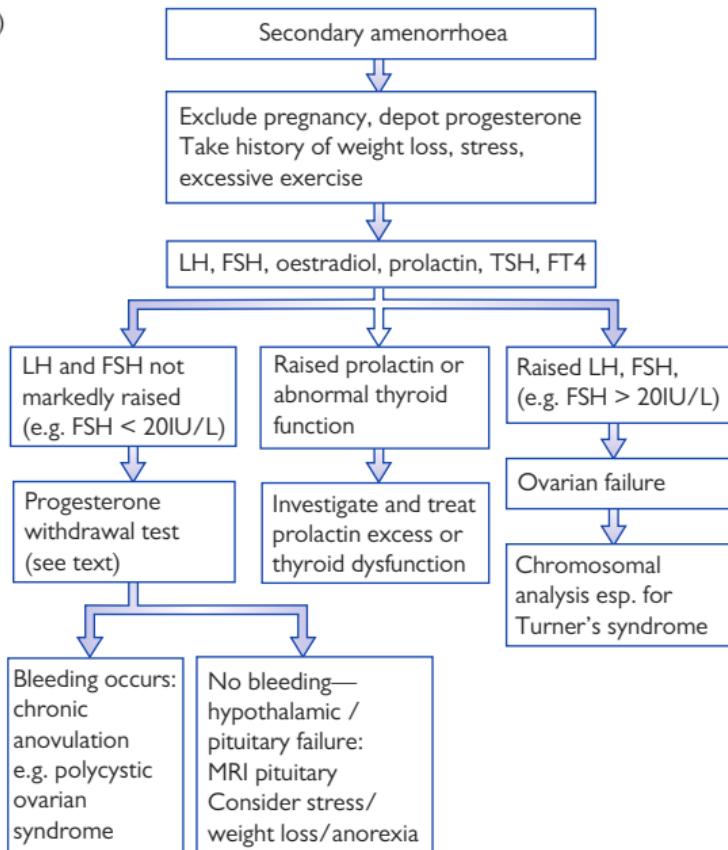


Fig. 2.9 Investigation of amenorrhoea: (a) 1° and (b) 2°. See Delayed puberty, p. 176 for investigation of delayed puberty.

Infertility

Detailed assessment of infertility is beyond the scope of this text and is best referred to a specialist in this area. However, the general physician can take the following basic steps, always remembering that the couple should be assessed together as the problem may lie with the man, the woman, or a combination of both:

1. Semen analysis of the ♂ and, where possible, a post-coital test to confirm that live semen are delivered to the vaginal tract.
2. If amenorrhoea is present in the ♀, investigate as in Fig. 2.9.
3. If the ♀ is menstruating, determine if the cycles are ovulatory, e.g. by day 21, progesterone levels, or home measurement urinary dipstick of the LH surge.

If live semen are delivered and ovulation is occurring, then structural damage or chlamydial infection in the female genital tract is likely, and will require gynaecological assessment.

Hirsutism/virilization (raised testosterone)

Hirsutism refers to an ↑ in androgen-dependent terminal hairs in the ♀, typically over the face/chin, lower abdomen, arms and legs, and around the areola of the breast. Virilization reflects much higher androgen levels and comprises the features shown in Box 2.7. Over 20% of women have more androgen-dependent hair than they consider to be normal. In >95% of cases, this is associated with androgen levels in the ♀ normal range or slightly elevated in association with polycystic ovarian syndrome. Some drugs, such as ciclosporin, diazoxide, minoxidil, and androgenic steroids can also cause hirsutism. A history of recent onset (<6 months) and rapidly progressive hirsutism, particularly when associated with features of virilization and a testosterone level of >5nmol/L, should prompt a search for alternative adrenal or ovarian causes (see Fig. 2.10).

➲ OHCM 10e, p. 230.

Box 2.7 Features of female virilization

- Clitoral enlargement
- Temporal hair loss
- Breast atrophy
- Deepening of voice

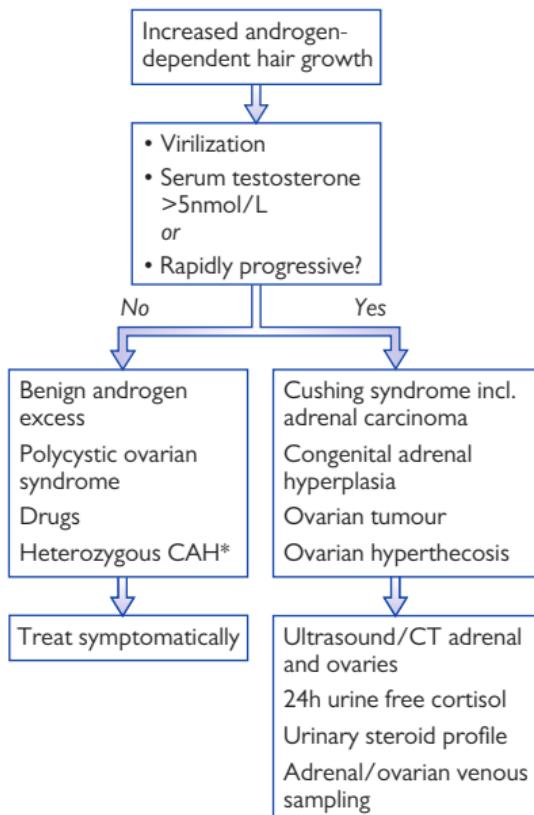


Fig. 2.10 Investigation of hirsutism. * CAH, congenital adrenal hyperplasia.

Further reading

- Martin KA, Chang RJ, Ehrmann DA, et al. Evaluation and treatment of hirsutism in premenopausal women: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2008; **93**: 1105–20.
- Speiser PW, Azziz R, Baskin LS, et al.; Endocrine Society. Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010; **95**: 4133–60.

Galactorrhoea (hyperprolactinaemia)

Galactorrhoea is always due to PRL and should be confirmed by asking the woman to demonstrate a secretion of milky appearance from the nipple. Rarely, galactorrhoea can occur with PRL levels in the normal range and regular menses, but usually it is associated with mildly raised levels and amenorrhoea in ♀ or very elevated levels in ♂. There is no link with breast size—gynaecomastia in ♂ is associated with excess oestrogen. Once dopamine-blocking drugs (major tranquillizers and anti-emetics, but not antidepressants), depot progesterone administration, and hypothyroidism have been excluded, all patients should have pituitary imaging to exclude a large tumour pressing on the pituitary stalk, especially if there are modest PRL levels ($<10,000\text{IU/L}$) associated with a large tumour ($>2\text{cm}$) (see Fig. 2.11). If the PRL levels are disproportionately low for the tumour size, serial dilution of the sample and re-estimation should be considered to exclude an artefactually low level due to the 'hook effect'. Very high PRL levels ($>10,000\text{IU/L}$) are invariably associated with true prolactinomas. Nipple manipulation (e.g. to check if galactorrhoea has ceased) and chest wall trauma (including shingles) can also stimulate PRL levels.

Asymptomatic raised prolactin (macroprolactin)

If PRL is found (accidentally) to be persistently raised ($>1000\text{IU/L}$), but menstruation is normal and there is no galactorrhoea, consider the possibility of macroprolactin. This is a circulating complex of PRL multimers, and sometimes PRL autoantibodies of no biological importance, but gives a high reading in the PRL assay and the result often varies widely between assays. If the laboratory is alerted to a mismatch between PRL levels and the clinical picture, they can easily screen for this with polyethylene glycol precipitation. Stress and epileptic fits can result in transiently raised PRL levels, insufficient to cause galactorrhoea.

➲ OHCM 10e, p. 236.

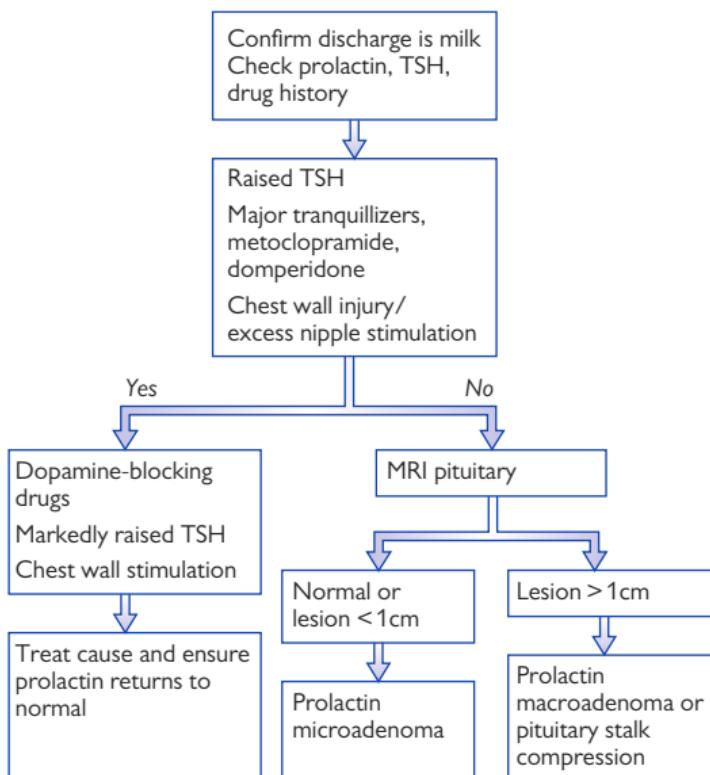


Fig. 2.11 Investigation of galactorrhoea.

Further reading

- McKenna TJ. Should macroprolactin be measured in all hyperprolactinaemic sera? *Clin Endocrinol (Oxf)* 2009; 71: 466–9.
- Melmed S, Casanueva FF, Hoffman AR, et al.; Endocrine Society. Diagnosis and treatment of hyperprolactinemia: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; 96: 273–88.

Impotence/loss of libido/male hypogonadism

Symptoms and signs of hypogonadism in men (low testosterone levels)

- Reduced shaving.
- Loss of libido.
- Impotence.
- Reduced energy/aggression levels.
- Loss of pubic, chest, and axillary hair.
- Gynaecomastia often results due to a lower testosterone/oestrogen ratio.

Note that very low levels of testosterone (at least $<5\text{nmol/L}$, typical normal range $10\text{--}30\text{nmol/L}$) are required to result in symptoms. Mild reductions are common, especially in the elderly, and are rarely of importance. Impotence alone (without loss of libido) can also be caused by neurovascular and psychological causes (e.g. diabetes, spinal damage, urological surgery, atherosclerosis of the aorta, drugs, stress, and psychosexual dysfunction). Where the testosterone level is at the lower limit of normal or SHBG abnormalities are suspected and symptoms are present, measurement of free testosterone or bioavailable testosterone by an established method may be indicated. Such methods may need referral to a reference laboratory.

After history taking for conditions described, investigation of suspected male hypogonadism requires

- PRL.
- Thyroid function.
- LH and FSH.
- Testosterone.

Hyperprolactinaemia or thyrotoxicosis, if present, need to be treated on their own merits. If the testosterone level is clearly low, high gonadotrophins point to testicular failure (e.g. testicular surgery, irradiation or trauma, chemotherapy, crypto-orchidism, previous orchitis, gonadal dysgenesis, including Klinefelter's syndrome XXY). Low gonadotrophin levels with a clearly low testosterone level point to a hypothalamic or pituitary cause (systemic illness, pituitary tumour), which requires further investigations (➔ Hypopituitarism, pp. 128–30). If no cause is found for hypogonadotropic hypogonadism, the likely cause is Kallman's syndrome, especially if associated with anosmia.

➔ OHCM 10e, p. 230.

Further reading

Bhasin S, Cunningham GR, Hayes FJ, et al.; Task Force, Endocrine Society. (2010) Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010; **95**: 2536–59.

Sato N, Katsumata N, Kagami M, et al. Clinical assessment and mutation analysis of Kallmann syndrome 1 (KAL1) and fibroblast growth factor receptor 1 (FGFR1, or KAL2) in five families and 18 sporadic patients. *J Clin Endocrinol Metab* 2004; **89**: 1079–88.

Gynaecomastia

Gynaecomastia results from an excessive effect of oestrogens or a raised oestrogen/testosterone ratio. Causes are summarized in Table 2.12. True gynaecomastia should be associated with palpable breast tissue and distinguished from apparent breast enlargement due to obesity. Though very rare, the most important diagnoses to exclude are hypogonadism and testicular and lung tumours.

Table 2.12 Causes of gynaecomastia

Physiological	Newborn, adolescent, elderly
Hypogonadism	e.g. Klinefelter's syndrome, testicular failure
↑ oestrogen	Testicular tumours, lung cancer producing hCG, liver disease, thyrotoxicosis
Drugs	Including oestrogens, spironolactone, cimetidine, digoxin, testosterone administration

Investigations should include

- LFTs.
- Thyroid function.
- LH and FSH.
- Testosterone.
- Oestradiol.
- hCG.
- AFP.
- CXR.
- Testicular USS.
- Further review of drug history.

Physiological gynaecomastia should only be diagnosed if other causes have been excluded.

➲ OHCM 10e, p. 230.

Delayed puberty

Definition

Puberty is considered delayed in girls if there is no breast development by age 13 (or menses by age 15) and in boys if there is no testicular enlargement by age 14. Note that 3% of normal children will fall into these categories.

Clinical features and initial investigations

A detailed history and examination is required for overt systemic disease, psychosocial stress, and anorexia nervosa, and to assess the child's height, pubertal features (pubic hair, testicular size, breast growth, menses), and any dysmorphic features (e.g. features of Turner's syndrome). Where possible, growth rate should be calculated from sequential height measurements over at least 6 months.

If no obvious cause is identified, baseline investigations should include:

- LH and FSH.
- TSH, FT4, PRL.
- FBC, U&E, bicarbonate (HCO_3^-), CRP, and antigliadin/endomysial antibodies for occult systemic disease.
- Bone age.

This should enable the child to be placed in one of five categories:

1. *Raised LH/FSH (1° gonadal failure).* Causes: Turner's syndrome, Klinefelter's syndrome, ovarian/testicular injury. Proceed to karyotyping (should be performed in all girls with delayed puberty as Turner's syndrome may not be apparent).
2. *Short, low LH/FSH, overt systemic disease.* Causes: asthma, anorexia nervosa, social deprivation, generalized illness, treatment for cancer, including cranial irradiation, dysmorphic (Noonan's syndrome and others).
3. *Short, low LH/FSH, occult systemic disease.* Causes: hypothyroidism, hyperprolactinaemia, renal failure, RTA, coeliac disease, Crohn's disease.
4. *Short, low LH/FSH, no systemic disease.* Causes: constitutional delay of puberty, hypothalamic/pituitary disease.
5. *Not short, low LH/FSH.* Causes: Kallman's syndrome (if anosmia present) or isolated gonadotrophin deficiency. Cannot reliably distinguish from constitutional delay of puberty. Observe.

The investigation of children who fall into the commonest category 'short, low LH/FSH, no systemic disease' is summarized in Fig. 2.12. The onset of puberty after a period of observation is reassuring, but continued observation is required to ensure the process proceeds to completion, including a growth spurt. If not, further investigation for disorders of steroidogenesis, androgen insensitivity, skeletal dysplasia, premature gonadal failure, and, in the ♀, genital tract abnormalities and polycystic ovarian syndrome are indicated.

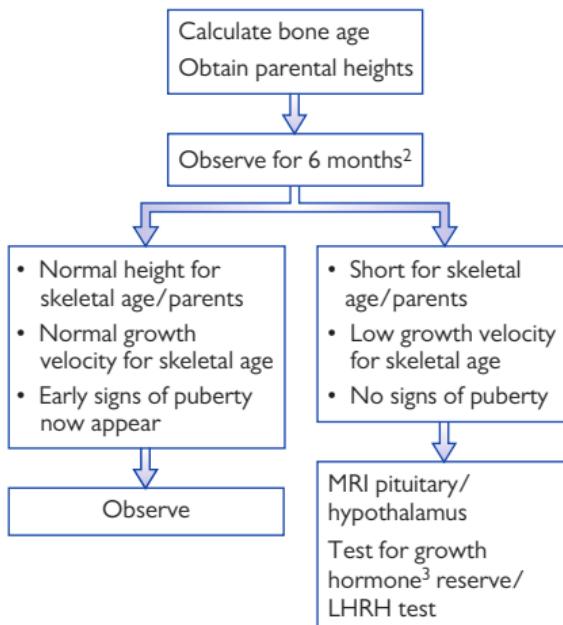


Fig. 2.12 Investigation of delayed puberty in children who are short, with no evidence of systemic disease¹ and low LH/FSH levels.

Notes:

1. Including normal thyroid function and PRL.
2. If develops headache, vomiting, or visual symptoms, proceed immediately to MRI.
3. Refer to a paediatric endocrinologist. Tests used vary, e.g. gonadotrophin response to luteinizing hormone-releasing hormone (LHRH) after androgenic priming and ITT for GH response.

Short stature

Evaluation of children who are below the third growth centile for age or particularly small for their family should include:

- Height for age (percentile).
- Mid-parental height (for girls, mean of father's height minus 12.6cm + mother's; for boys, add 12.6cm to mother's height).
- Bone age (to assess growth potential/height prediction).
- Observation over 3–6 months to determine growth velocity.

Children of short (but normal) parents, who are growing normally, can be observed. Dysmorphic children require further evaluation/specialist assessment. Children who are short for their parental heights (low predicted height), particularly if growing slowly, and short children of pubertal age who have not entered puberty should be investigated as for 'delayed puberty'. Referral for paediatric endocrinological assessment is advised.

Precocious puberty

Definition

Puberty is considered premature if multiple signs, including accelerated growth rate and bone age, appear by age 8 in girls or age 9 in boys. Note that isolated breast development (premature thelarche) or pubic hair (premature adrenarche) are benign conditions if no other evidence of puberty appears. True precocious puberty requires urgent investigation to determine the cause and avoid irreparable loss of final adult height. In girls, it is often idiopathic, but not in boys. The causes are given in Table 2.13.

Table 2.13 Causes of precocious puberty

Central	Gonadotrophin-independent
Idiopathic (especially girls)	Congenital adrenal hyperplasia (♂)
CNS hamartoma (especially pinealoma)	Adrenal/ovarian/hCG-secreting tumour
Other CNS diseases, e.g. hydrocephalus, trauma	McCune–Albright syndrome, hypothyroidism, follicular cyst (♀) familial testotoxicosis (♂)

Investigations

Precocious puberty is confirmed by pubertal levels of sex steroids (oestradiol, testosterone). Testicular enlargement (or ovarian enlargement on USS) and detectable LH/FSH levels suggest central precocious puberty and a CT/MRI scan of the brain is indicated. Gonadal enlargement can also be seen with testotoxicosis, hCG-producing tumours, hypothyroidism, and McCune–Albright syndrome. Further investigation should be performed in combination with a paediatric endocrinologist.

Thyroid function testing: general

In the majority of cases, thyroid function testing and interpretation are straightforward (see Fig. 2.13). However, the following points should be borne in mind.

1. *Which first-line test?*—TSH. TSH levels are the most sensitive indicator of thyroid dysfunction, except in patients with pituitary disease where they are uninterpretable. TSH used alone as a first-line test will miss (levels ‘normal’) unsuspected cases of 2° hypothyroidism, and some laboratories combine TSH and T4 as first-line tests. TSH is also unreliable in patients with recently treated hyperthyroidism, as it can remain suppressed (undetectable) for several weeks after FT4 or FT3 levels have normalized.
2. *Which tests?*—T4/T3. Free T3 and T4 tests (FT3, FT4) are now more reliable and preferred (although more expensive) to total T3 or T4 measurements. Interference in these assays does occur but is increasingly rare. Total thyroid hormone levels are markedly influenced by changes in binding proteins (e.g. due to pregnancy, oestrogen-containing contraceptives).
3. *Thyroid autoantibodies.* These are markers of autoimmune thyroid disease. Antithyroid microsomal antibodies have been identified as antithyroid peroxidase (anti-TPO) antibodies. Anti-TPO antibodies are more sensitive than anti-thyroglobulin (Tg) antibodies and are present in around 45–80% of Graves’ disease and 80–95% of Hashimoto’s disease/atrophic thyroiditis. Increasingly, laboratories are measuring anti-TPO directly as their only antibody test (sometimes just referred to as ‘antithyroid antibodies’). Note that anti-TSH receptor antibodies—the cause of Graves’ disease—require a specific assay request. (For indications for testing,  Anti-TSH receptor antibody testing, p. 184).
4. *Tests should agree.* To confirm thyroid dysfunction, at least two TFTs and, in cases of doubt, all three (TSH, FT3, FT4) should be performed. Results of the tests should be in agreement—if not, assay interference (heterophile antibodies, anti-T4 or anti-T3 antibodies present in the serum) or unusual causes should be suspected.
5. *Avoid thyroid function testing in systemically unwell patients.* In very ill patients, especially in intensive care, a pattern of ‘sick euthyroidism’ is often seen, with low TSH levels, low FT3 levels, and sometimes low FT4 levels. Accurate interpretation of true thyroid status is impossible. A raised FT3 level in a very ill patient suggests significant hyperthyroidism, and a very raised TSH level ($>20\text{mU/L}$) with undetectable FT4 levels suggests profound hypothyroidism. Other changes should be interpreted with extreme caution and the tests repeated after recovery.

 OHCM 10e, pp. 216–17.

	Low TSH	Normal TSH	Raised TSH
Raised FT4/FT3	Thyrotoxicosis		TSH-secreting pituitary tumour Thyroid hormone resistance (receptor defect) Intermittent T4 therapy/acute overdose Interfering anti-T4/T3 antibody Familial dysalbuminaemic hyperthyroxinaemia Acute psychiatric illness
Normal FT4/FT3	Subclinical thyrotoxicosis Levothyroxine ingestion Steroid therapy Non-thyroidal illness Dopamine infusion	Normal	Subclinical hypothyroidism Poor compliance with T4 therapy Interfering (heterophile) antibody Recovery from non-thyroidal illness Hypoadrenalinism
Low FT4/FT3	Non-thyroidal illness Pituitary failure Recent (excessive) treatment for hyperthyroidism		Hypothyroidism

Note: free thyroid hormone assays are assumed—effects of changes in binding proteins on total thyroid hormone assays are not included.

Fig. 2.13 Patterns of TFTs. To use this table, you need the results of both TSH and either free T4 (FT4) or free T3 (FT3) tests. If either FT4 or FT3 are outside the reference range, then FT4/FT3 are considered abnormal in this table. If FT4 and FT3 are abnormal in different directions (e.g. one is low and the other is high), see point 4, 'Tests should agree', pp. 180–1.

Reprinted from *The Lancet*, 357, Dayan CM 'Interpretation of thyroid function tests' 619–24 (2001) with permission from Elsevier.

Further reading

Association for Clinical Biochemistry/British Thyroid Association (2006). UK guidelines for the use of thyroid function tests. London: ACB/BTA. <http://www.acb.org.uk/docs/default-source/guidelines/TFTguidelinefinal.pdf>.

National Academy of Clinical Biochemistry Practice Guidelines (2002). <http://www.aacc.org/members/nacb/LMPG/Pages/default.aspx>.

Hyperthyroidism (thyrotoxicosis)

Clinical features

Hyperthyroidism is rare in childhood but affects all adult age groups. Classic features include weight loss despite ↑ appetite, palpitations, AF, heat intolerance, anxiety, agitation, diarrhoea, tremor, and proximal weakness. Lid retraction and lid lag can be seen in any cause of hyperthyroidism, but proptosis, periorbital oedema, chemosis, diplopia, and optic nerve compression only occur in association with Graves' disease (thyroid eye disease) and occasionally associated with pretibial myxoedema and thyroid acropachy. In the elderly, presentation with isolated weight loss or AF is common. Raised ALP and SHBG, leucopenia, and rarely hypercalcaemia are recognized associations.

Thyroid function testing

An undetectable TSH level and an ↑ free T₃ level are required to diagnose hyperthyroidism. In milder cases, T₄ levels may be in the normal range ('T₃ toxicosis'). Normal TSH levels with ↑ T₄ and T₃ are seen in TSH-secreting pituitary tumours (very rare) or in patients with thyroid hormone resistance (also very rare) (see Fig. 2.13).

Investigation of cause

For an overview of investigation, see Fig. 2.14.

Under the age of 40, Graves' disease is the commonest cause. After this age, Graves' disease, toxic nodular goitre, and toxic nodule all occur. However, a short history (1 month) of symptoms or absence of relevant symptoms (chance blood test finding) raises the possibility of self-resolving (transient) thyroiditis, a diagnosis supported by neck pain and raised ESR (viral/subacute/De Quervain's) or occurrence in the first 9 months post-partum (postpartum thyroiditis—painless). Transient thyrotoxicosis can also occur in patients with subclinical autoimmune thyroiditis ('silent thyroiditis'—painless), especially during cytokine therapy (e.g. interferon for hepatitis C). Graves' disease or other forms of autoimmune thyroiditis are seen in >30% of patients 2–3 years after treatment with alemtuzumab (for MS), in association with lymphocyte repopulation. If self-resolving thyroiditis is suspected, withhold treatment and repeat the tests after 6 weeks. When thyroid eye disease is present, no further tests are required to diagnose Graves' disease. If not, antithyroid antibodies (e.g. anti-TPO antibodies) and isotope thyroid scanning can be useful to distinguish possible causes (see Fig. 2.14 and  Chapter 14). No uptake is seen in transient thyroiditis. Excess thyroid hormone ingestion rarely causes very marked thyrotoxicosis, unless the active form (T₃) is taken (T₃ tablets or desiccated thyroid extract).

Iodine

Iodine has multiple and conflicting effects on the thyroid. Potassium iodide inhibits release of thyroid hormones from the gland and thyroid hormone biosynthesis (Wolff–Chaikoff effect), promoting hypothyroidism. However, escape from these effects occurs in most individuals in a few weeks. In patients with a multinodular goitre, excess iodine (e.g. in amiodarone or radiographic contrast media) can result in thyrotoxicosis by excess provision of substrate (Jod–Basedow effect).

Amiodarone

Has three main effects on the thyroid hormone axis:

- Inhibits T₄ → T₃ conversion, which in the pituitary can result in an asymptomatic mild rise in TSH level (reduced thyroid hormone action) and/or a rise in FT₄ level.
- Can induce true hypothyroidism, usually in the first year of treatment.
- Can induce true hyperthyroidism either via the Jod–Basedow effect in patients with multinodular goitre or by destructive thyroiditis in healthy glands.

Thyrotoxicosis can occur at any time after commencing therapy and can be very difficult to treat. *Interpretation of TFTs on amiodarone*: a raised FT₃ level indicates true hyperthyroidism; a markedly raised TSH level (e.g. >10mU/L), especially if the FT₄ level is low, indicates true hypothyroidism.

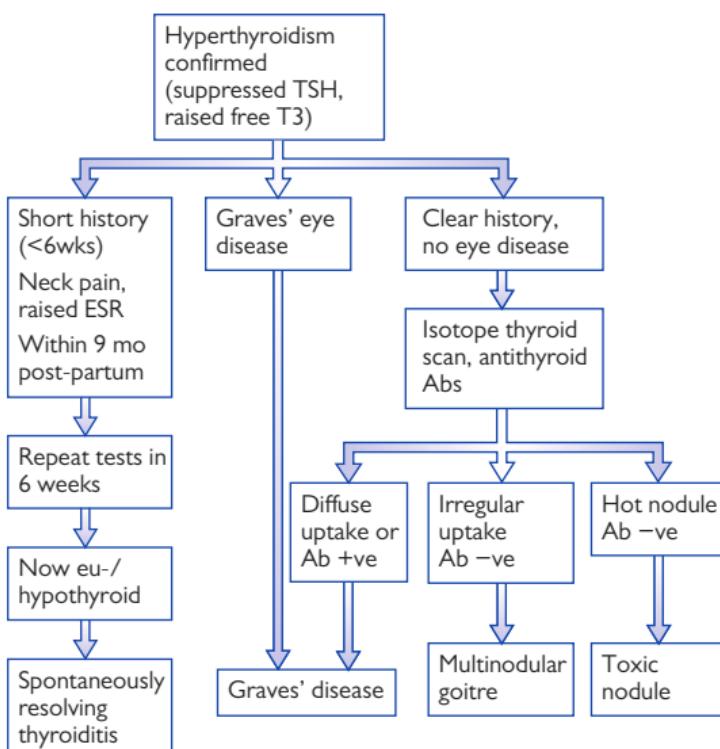


Fig. 2.14 Investigation of the cause of hyperthyroidism.

Hyperthyroidism in pregnancy

Significant hyperthyroidism in pregnancy is generally due to Graves' disease. Mild hyperthyroidism, particularly in association with hyperemesis gravidarum in the first trimester, is often due to a cross-reaction by very high hCG levels with the TSH receptor ('gestational thyrotoxicosis'). In the postpartum period, thyrotoxicosis may be due to postpartum thyroiditis (self-resolving) or a recurrence of Graves' disease (requires treatment). Measurement of anti-TSH receptor antibody levels may be indicated to distinguish these possibilities.

Thyroid storm

This is defined as severe thyrotoxicosis with confusion/delirium not explained by other factors. There is no definitive test and levels of thyroid hormone are not higher than in other thyrotoxic individuals with no features of storm. Severe agitation, tachycardia, and hyperpyrexia are usually seen. Usually precipitated by infection, trauma, or surgery, especially to the thyroid gland. Very rare but tends to occur in individuals who have been poorly compliant in the first few weeks of drug therapy for thyrotoxicosis.

Anti-TSH receptor antibody testing (TBII, TRAbs, TSAb)

This test is increasingly available in local and regional laboratories. In second- or third-generation assays, especially assays for stimulatory antibodies, it is positive in >95% of cases, save other tests, and indicated that the patient is at risk of Graves' eye disease. Indications for anti-TSH receptor antibody testing include distinguishing gestational thyrotoxicosis or postpartum thyroiditis from Graves' disease, indicating the risk of neonatal thyrotoxicosis and (controversial) predicting recurrence after a course of thioamide drug therapy.

➲ OHCM 10e, p. 31, p. 216, p. 562.

Further reading

Barbesino G, Tomer Y. Clinical utility of TSH receptor antibodies. *J Clin Endocrinol Metab* 2013; 98: 2247–55.

Hypothyroidism

Clinical features

Classic clinical features of hypothyroidism include weight gain, cold intolerance, dry skin, constipation, memory loss, lethargy/slow thought/'slowing up', menorrhagia, periorbital/facial oedema, loss of the outer two-thirds of eyebrows, deafness, chest pain, and coma. These are rarely seen nowadays, as TFTs are easy to perform and detect the disease usually at an earlier stage. Weight gain, dry skin, and lethargy are frequently reported, but even biochemically hypothyroid individuals can only confidently be ascribed to thyroid status if they reverse on treatment.

Biochemical diagnosis

↑ TSH with T4 in the normal range is referred to as subclinical hypothyroidism. ↑ TSH with ↓ T4 is overt hypothyroidism. ↓ T4 with TSH in the normal range may also be due to pituitary failure (2° hypothyroidism) and, if persistent, requires pituitary function testing. See Fig. 2.13 for other patterns of TFTs.

Differential diagnosis (causes)

In iodine-sufficient countries, most spontaneous hypothyroidism is due to autoimmune thyroiditis (Hashimoto's disease if goitre present, atrophic thyroiditis if goitre absent)—antithyroid antibodies (anti-TPO, anti-Tg) present in 80–90% of cases. Other common causes are post-thyroidectomy, post-radioiodine therapy, and side effects of amiodarone or lithium. Rarer causes include treatment with cytokines or other drugs (e.g. interferons, granulocytic macrophage colony-stimulating factor (GM-CSF), interleukin-2, tyrosine kinase inhibitors, alemtuzumab), vast excess of iodine intake (iodine drops, water purifying tablets), congenital hypothyroidism (caused by a variety of genetic defects; should be detected by neonatal screening programme), iodine deficiency (urinary iodide excretion <45 µg/day, commonest cause worldwide, especially mountainous areas, S. Germany, Greece, Paraguay—'endemic goitre'), thyroid-blocking substances in the indigenous diet (goitrogens, especially in brassicas and cassava, e.g. in Sheffield, Spain, Bohemia, Kentucky, Virginia, Tasmania—'endogenous goitre' without iodine deficiency), Pendred's syndrome (mild hypothyroidism with sensorineural deafness due to Mondini cochlear defect detectable on MRI, positive perchlorate discharge test). For further information,  Transient hypothyroidism, p. 187.

►► Diagnostic catches ↑ TSH and ↓ T4 always represents hypothyroidism. If the TSH alone is ↑ and the T4 is not even slightly low, a heterophile antibody interfering in the TSH assay may be present in the patient's serum. This is especially likely if there is no change in TSH level after thyroxine treatment, but the T4 level rises (confirming compliance with tablets). For unusual patterns of thyroid function tests, see Fig. 2.14. Note that, within the first 1–3 months (or longer) after treatment of hyperthyroidism, profound hypothyroidism may develop with a ↓ T4, but the TSH may still be suppressed or only mildly raised due to the long period of TSH suppression prior to treatment. Raised TSH alone with disproportionate symptoms of lethargy may be seen in hypoadrenalinism.

Transient hypothyroidism

Transient/self-resolving hypothyroidism, often preceded by hyperthyroidism, is seen in viral thyroiditis, after pregnancy (postpartum thyroiditis), and in some individuals with autoimmune thyroiditis. Treatment temporarily with levothyroxine is only required if the patient is very symptomatic. Thyroid function should return to normal within 6 months.

Subclinical hypothyroidism

A raised TSH (<20mU/L) with normal T4/T3 is very common and seen in 5–10% of women and ~2% of ♂. It is usually due to subclinical autoimmune thyroid disease and is frequently discovered on routine testing. In randomized trials, ~20% of patients obtain psychological benefit from beginning T4 therapy; in many others, it is probably truly asymptomatic. If antithyroid antibodies are detectable, the rate of progression to overt hypothyroidism is ~50% at 20 years, but higher than this with higher initial TSH levels. If the TSH alone is raised with –ve antibodies (or the TSH is normal with raised antibodies alone), overt hypothyroidism develops in 25% at 20 years. A reasonable approach is a trial of levothyroxine for 6 months in symptomatic patients with subclinical hypothyroidism or TSH >10mU/L, and observing the TSH level at 6- to 12-monthly intervals in asymptomatic patients with TSH <10mU/L.

Hypothyroidism and pregnancy

Overt hypothyroidism is associated with poor obstetric outcomes. Recent evidence suggests that subclinical hypothyroidism is associated with an ↑ miscarriage rate and a slight reduction in the baby's intelligence quotient (IQ) and should be treated. Some authorities advocate screening for hypothyroidism in all antenatal patients as early as possible in pregnancy. Patients on T4 need to ↑ their dose by 25–50µg from the first trimester of pregnancy. Maternal levothyroxine can compensate for fetal thyroid failure *in utero*, but congenital hypothyroidism must be detected at birth (screening test) to avoid mental retardation developing. Where the mother and fetus are both hypothyroid—most commonly due to iodine deficiency—mental retardation can develop *in utero* (cretinism). Note that mothers with +ve antithyroid antibodies and/or subclinical hypothyroidism have a 50% chance of developing (transient) postpartum thyroiditis.

➲ OHCM 10e, p. 31, p. 149, p. 203.

Further reading

Cooper DS, Biondi B. Subclinical thyroid disease. *Lancet* 2012; 379: 1142–54.

Hypercalcaemia

Clinical features

Usually asymptomatic if $\text{Ca}^{2+} < 3.0 \text{ mmol/L}$. Typical symptoms include polydipsia/polyuria, constipation, indigestion, pancreatitis, hypertension, tiredness, drowsiness/confusion, abdominal pains, and renal colic. Renal failure can occur due to dehydration (reversible), nephrocalcinosis, and/or stag-horn calculi. Osteitis fibrosa cystica in cases of hyperparathyroidism (with subperiosteal resorption of bone, particularly of the distal phalanges and bone cysts—brown tumours) is now rare, other than in renal failure, but can be associated with bone pain.

Investigation of the cause

Ninety-five per cent of persistent hypercalcaemia is due to either hyperparathyroidism or malignancy (see Fig. 2.15). Asymptomatic 1° hyperparathyroidism is common in 50- to 70-year-old women, and a PTH level (sampled simultaneously with $\uparrow \text{Ca}^{2+}$ and measured in a highly sensitive assay) which is raised or in the upper normal range in the presence of hypercalcaemia confirms the diagnosis. Low normal or low levels of PTH should prompt a search for malignancy, especially breast, prostate, bronchus, kidney, thyroid, or myeloma. Bone-derived ALP levels may be raised in both malignancy and hyperparathyroidism. Bone scan may be useful in disseminated malignancy but can be –ve in cancers releasing PTH-related peptide (NOT detected in routine PTH assays) and in myeloma. If malignancy is not found, the conditions shown in Fig. 2.15 need to be considered. Markedly abnormal renal function is seen in milk-alkali syndrome, myeloma, and tertiary hyperparathyroidism. Sarcoid may be difficult to diagnose but is suggested by a raised serum ACE level (not invariable), a dramatic response to steroids, and a +ve liver or other biopsy for granulomata.

Investigation of established hyperparathyroidism

Familial benign hypocalciuric hypercalcaemia (FBHH) is a very rare condition caused by an inactivating mutation of the calcium-sensing receptor (CaSR). This results in stable, lifelong hypercalcaemia with a raised PTH level, which rarely causes complications. It is inherited in an autosomal dominant fashion.

The hallmark is hypocalciuria—defined as:

$$\text{Urine } [\text{Ca}^{2+}] \times \text{plasma creatinine}/\text{plasma } [\text{Ca}^{2+}] \times \text{urine creat} < 0.01$$

(All in units of mmol/L.)

Although rare, it is important to recognize, as parathyroidectomy is not required.

Further investigation of 1° hyperparathyroidism should include serum creatinine, KUB plain abdominal X-ray to exclude renal stones, and a spot urine $\text{Ca}^{2+}/\text{creatinine}$ to rule out FBHH.

In >80% of cases, 1° hyperparathyroidism is due to adenomatous change in one of the four parathyroid glands. In a minority of cases and in familial hyperparathyroidism associated with MEN-1 (pituitary tumours, endocrine pancreatic tumours, and hyperparathyroidism) or MEN-2 (medullary carcinoma of the thyroid, phaeochromocytoma, and hyperparathyroidism) four gland hyperplasia occurs, requiring resection of at least three-and-a-half glands for treatment. Very rarely (<1% of cases), parathyroid carcinoma is the cause. Lithium therapy may also be associated with (mild) hyperparathyroidism. Once a diagnosis of 1° hyperthyroidism is made, ^{99m}technetium-sestamibi radionucleotide scanning is the most sensitive imaging technique and will show the location of the parathyroid adenoma. In difficult cases, local venous sampling may be required for localizing a parathyroid adenoma, especially if it is outside the neck.

Tertiary hyperparathyroidism

Refers to acquired autonomy of the parathyroid glands leading to hypercalcaemia following chronic vitamin D deficiency, as seen in renal failure or with malabsorption. 2° hyperparathyroidism is associated with hypocalcaemia and is the appropriate response to vitamin D deficiency.

Investigation of hypercalcaemia

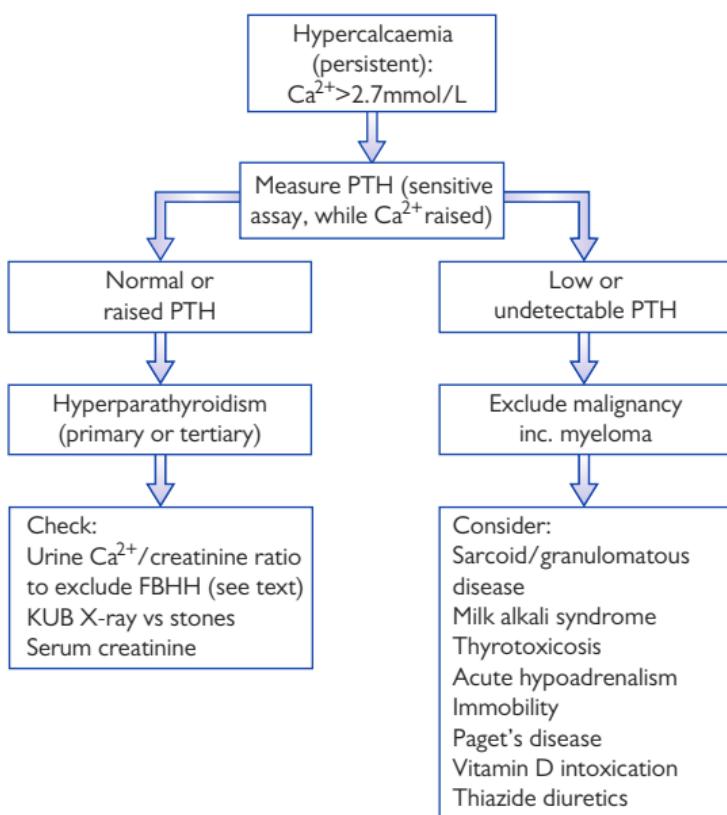


Fig. 2.15 Investigation of hypercalcaemia.

Hypocalcaemia/osteomalacia

Clinical features

Chronic hypocalcaemia is often surprisingly asymptomatic. Symptoms and signs, when present, include muscle spasms, paraesthesiae, especially around the mouth and in fingers, tetany, fits, +ve Chvostek's (VIIth nerve hyperexcitability) and Troussseau's signs (tetany of the hand when BP cuff inflated). Chronic hypocalcaemia is also associated with papilloedema, abnormal dentition (if begins in childhood), cataract, and intracranial calcification (of no clinical consequence). Hypocalcaemia due to vitamin D deficiency is associated with muscle pains, proximal myopathy, and osteomalacia. In some cases of pseudohypoparathyroidism (type Ia), there are phenotypic abnormalities (somatic features), including short fourth metacarpal, bone changes (Albright's hereditary osteodystrophy), mental retardation, short stature, obesity, and resistance to other hormones, e.g. TSH, glucagon, gonadotrophins.

Investigation of cause

Persistent hypocalcaemia (corrected for serum albumin levels) with a normal serum creatinine level is almost always due to either hypoparathyroidism or vitamin D deficiency (osteomalacia). Other causes and distinguishing features are shown in Table 2.14, and a scheme for diagnosis is shown in Fig. 2.16. In failure of PTH action, Ca^{2+} is very low ($<1.8\text{mmol/L}$) and PO_3^{4-} is raised, but ALP is not raised and there is no osteomalacia. If the PTH is found to be raised, then pseudohypoparathyroidism can be diagnosed, which is subclassified as type Ia (paternally inherited a Gs- α defect with somatic features), type Ib ('renally selective' maternally inherited Gs- α defect—no somatic features), or type II. The classic test is the Elsworth–Howard test—measuring urine cyclic adenosine monophosphate (cAMP) response to infused PTH(1–34) analogue. Families with type 1a may also include patients with pseudoseudohypoparathyroidism, characterized by normocalcaemia, but somatic changes of pseudohypoparathyroidism. The mutations are in the same gene. The aetiology of type II pseudohypoparathyroidism (normal renal Ca^{2+} excretion and no other somatic features) remains unclear.

A low or normal PTH in the presence of a Ca^{2+} level $<1.8\text{mmol/L}$ makes hypoparathyroidism the likely diagnosis (see Table 2.14 for possible causes), but an attempt to rule out an activating CaSR mutation with a urine Ca^{2+} /creatinine ratio (see Fig. 2.15) should be made. In this rare genetic condition, Ca^{2+} levels are generally higher (around 1.75mmol/L). Importantly, Ca^{2+} or vitamin D replacement has a high likelihood of causing nephrocalcinosis and is best avoided. Autoimmune hypoparathyroidism in children or young people is particularly seen in association with autoimmune polyglandular syndrome type 1 (chronic candidiasis, coeliac disease, adrenal insufficiency).

In failure of vitamin D action (see Fig. 2.16, Table 2.14), there is a compensatory PTH rise, which partly corrects the Ca^{2+} level but causes a raised ALP. In addition, there is osteomalacia. In the presence of a significantly raised creatinine, renal osteodystrophy (impaired 25-OH vitamin D generation) is the most likely diagnosis; otherwise dietary vitamin D deficiency (low 25-OH vitamin D) or vitamin D resistance must be distinguished (see Table 2.14).

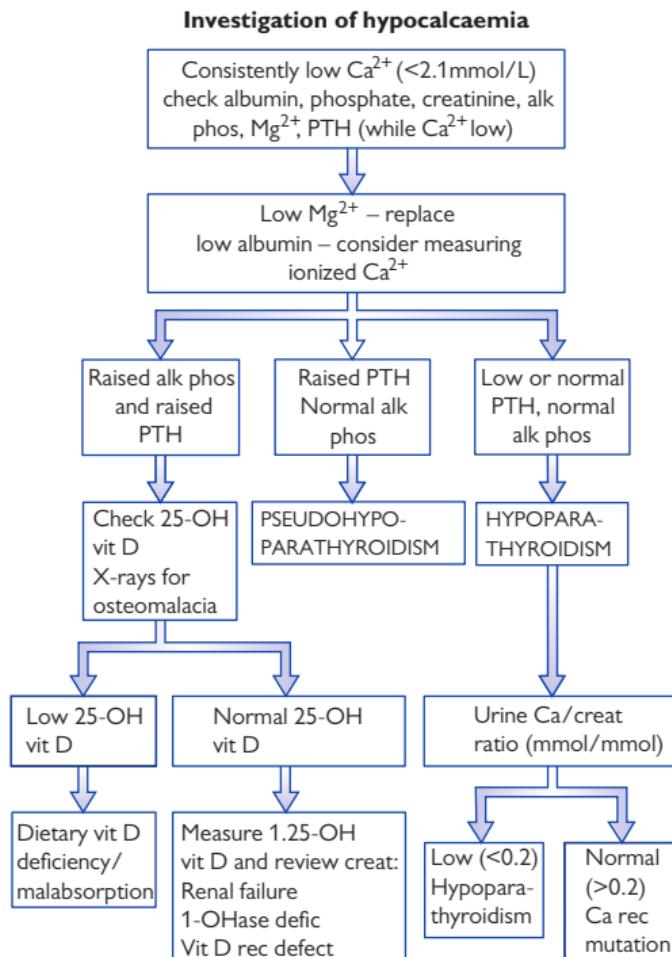


Fig. 2.16 Investigation of hypocalcaemia.

Osteomalacia

Osteomalacia is strictly a histological diagnosis but is suggested by Looser's zones and pseudo-fractures on X-ray (especially pelvis and upper femur). If osteomalacia with muscle weakness occurs in the absence of hypocalcaemia or a raised ALP, hypophosphataemia ('vitamin D-resistant rickets') is likely. Causes of a low PO₄⁴⁻ include intrinsic renal disease, congenital PO₄⁴⁻ leak, acquired PO₄⁴⁻ leak, and oncogenic osteomalacia. This last condition is associated with very difficult-to-find tumours, often benign, typically haemangiopericytomas of the naso-/oropharynx that may take years to become manifest. It is due to secretion of the cytokine fibroblast growth factor 23 (FGF23) by tumours, causing a phosphaturic effect. If suspected, FGF23 levels can be assayed in specialized laboratories. Treatment is PO₄⁴⁻ replacement until the tumour can be resected.

Table 2.14 Causes of hypocalcaemia

Cause	Features
Lack of PTH action	Very low Ca^{2+} , high PO_4^{3-} , normal ALP
Hypoparathyroidism	Autoimmune, post-neck surgery (may be transient), radioiodine, congenital (e.g. di George)
Pseudohypoparathyroidism	Type Ia (paternally inherited Gs- α mutation plus somatic features) Type Ib (maternally inherited, no somatic features, 'renally selective') Type II (no somatic features, normal Ca^{2+} excretion)
Hypomagnesaemia	Inhibits PTH release
Activating CaSR mutation	Indistinguishable from 1° hypoparathyroidism, except present from childhood and urinary Ca^{2+} /creatinine ratio not low
Failure of vitamin D action	Ca^{2+} not very low ($>1.8 \text{ mmol/L}$), ↑ ALP, ↓ PO_4^{3-} , ↑ PTH, osteomalacia
Vitamin D deficiency	Dietary/↓ sunlight, malabsorption, ↑ metabolism (phenytoin, rifampicin)
Renal failure	Failure of 1-hydroxylation of vitamin D
Inherited failure of 1- α -hydroxylase (vitamin D-dependent rickets type I)	Normal 25-OH vitamin D, ↓ 1,25-OH vitamin D levels
Vitamin D receptor defect (vitamin D-dependent rickets type 2*)	Normal 25-OH, normal 1,25-OH vitamin D levels
Other	
Acute pancreatitis	Transient
Hungry bone syndrome—immediately post-parathyroideectomy	Transient
Drugs, e.g. fosfarnet, bisphosphonates, ethylenediamine tetra-acetic acid (EDTA), citrate in blood	Transient
Neonatal (with prematurity)	Transient

* 'Vitamin D-resistant rickets' is rickets with normal Ca^{2+} and vitamin D due to X-linked hypophosphataemia.

Further reading

Ward BK, Magno AL, Walsh JP, Ratajczak T. The role of the calcium-sensing receptor in human disease. *Clin Biochem* 2012; 45: 943–53.

Diabetes mellitus

Diagnosing diabetes mellitus

Diabetes is defined as a state of chronic hyperglycaemia at levels that, if untreated, would result in microvascular complications (e.g. retinopathy). Adverse pregnancy outcomes (and ↑ macrovascular risk) occur at a lower level of glucose and hence lower cut-offs are used in pregnancy. However, since blood glucose levels vary through the day following meals, physical activity, and stress, defining these levels of glucose with a single test or a short dynamic test has proved difficult.

Figure 2.17 provides a scheme for testing for diabetes. Table 2.15 provides the American Diabetes Association (ADA) and WHO criteria for the diagnosis of diabetes, which differ slightly and have been updated since 2013. One of four different criteria can now be used to diagnose diabetes. Note that HbA_{1c} can now be used to diagnose diabetes if performed in a standardized Diabetes Control and Complications Trial (DCCT)-aligned assay and has the advantage that a fasting sample is not required. HbA_{1c} for diabetes diagnosis is not recommended using point-of-care machines (not standardized). In conditions where there is ↑ RBC turnover, such as pregnancy

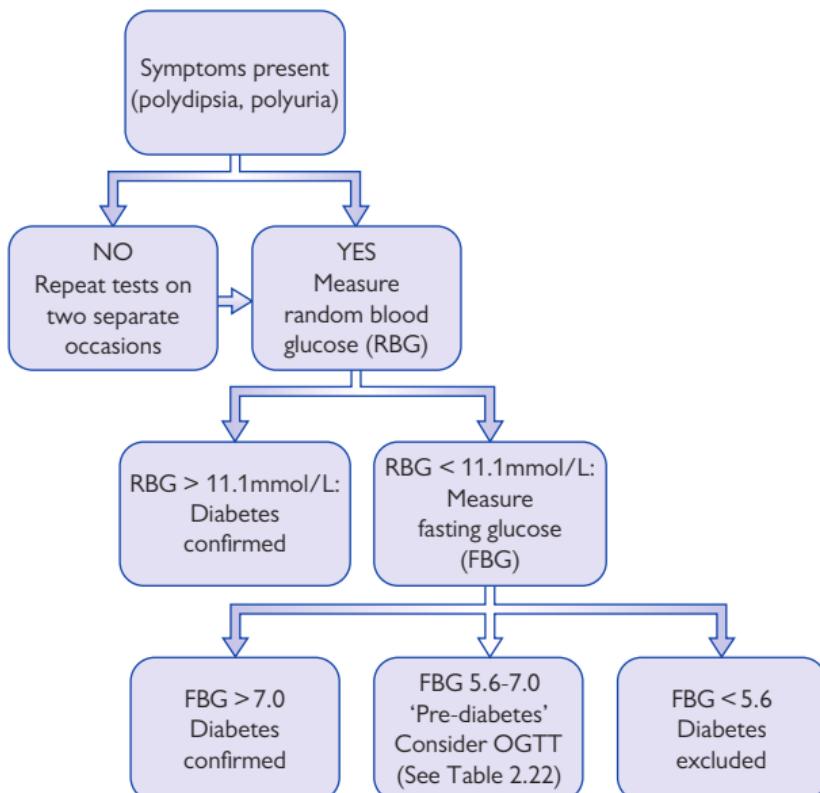


Fig. 2.17 Diagnosis of diabetes.

Table 2.15 ADA and WHO criteria for the diagnosis of diabetes

	ADA (2016)	WHO (2006, 2013)
Normoglycaemia	Fasting plasma glucose <5.6mmol/L (100mg/dL)* OR 2 h post-75g load of plasma glucose <7.8mmol/L (140mg/dL)	Fasting plasma glucose <6.1mmol/L (110mg/dL)* OR 2 h post-75g load of plasma glucose <7.8mmol/L (140mg/dL)
Diabetes	Casual plasma glucose >11.1mmol/L (200mg/dL) ¹ OR fasting plasma glucose >7.0mmol/L (126mg/dL) ¹ OR 2 h post-75g load of plasma glucose >11.1mmol/L (200mg/dL) ² OR HbA _{1c} >48mmol/mol (6.5%)	Casual plasma glucose >11.1mmol/L (200mg/dL) ¹ OR fasting plasma glucose 7.0mmol/L (126mg/dL) ¹ OR 2 h post-75g load of plasma glucose >11.1mmol/L (200mg/dL)
IFG	Fasting plasma glucose 5.6–6.9mmol/L*	Fasting plasma glucose 6.1–7.0mmol/L (126mg/dL) BUT not officially recognized—recommend progress to 2 h 75g glucose tolerance test (GTT)*
Impaired glucose tolerance (IGT)	Not routinely measured 2 h post-75g load of plasma glucose 7.8–11.1mmol/L (140–199mg/dL)	2 h post-75g of glucose 7.8–11.1mmol/L (140–199mg/dL)
↑ risk of diabetes (pre-diabetes)—includes IFG and IGT also	HbA _{1c} : 5.7–6.4% (39–46mmol/mol)	
Gestational diabetes	Casual plasma glucose >11.1mmol/L (200mg/dL) OR fasting plasma glucose 7.0mmol/L (126mg/dL)* OR two or more of the following plasma glucose values after 75g glucose load: fasting >5.3mmol/L; 1h >10mmol/L; 2 h >8.6mmol/L (155mg/dL)*	Casual plasma glucose 11.1mmol/L OR fasting plasma glucose >7.0mmol/L OR one or more of the following: fasting 5.1–6.9mmol*; or following 75g glucose load: 1h post-glucose >10mmol/L; 2 h post-glucose 8.5–11.0mmol/L

* ADA and WHO criteria differ.

¹ If the patient does not have classic symptoms (polyuria, polydipsia, unexplained weight loss), then this test should be repeated on a different day.

² Not recommended by the ADA for routine clinical use.

(second and third trimesters), recent blood loss or transfusion, Epo therapy, or haemolysis, HbA_{1c} should not be used for diagnosis. HbA_{1c} may also be problematic in the presence of haemoglobinopathies, unless specific assays not influenced by abnormal Hb are used. It should also be noted that the four different criteria for diagnosing diabetes do not completely overlap, e.g. a person may have fasting blood sugar of 6.8mmol/L (= impaired fasting glucose (IFG)/↑ diabetes risk) but an HbA_{1c} of 55mmol/mol (= diabetes). Hence only one test should be used.

Oral glucose tolerance testing is rarely required (and only recommended in pregnancy by the ADA) but can sometimes be useful to make the diagnosis of ↑ diabetes risk in borderline cases (see Table 2.15).

Notes

- If symptoms are not present, tests must be repeated on two occasions, ideally more than a week apart, to confirm that levels are indeed chronically raised.
- Values are given for venous plasma glucose. Capillary blood glucose are ~1.0mmol/L higher than venous plasma.
- If the patient has an intercurrent illness (e.g. infection or MI), tests should be repeated once the patient has recovered.
- Different criteria are used to diagnosis diabetes in pregnancy (gestational diabetes).

Blood samples for glucose testing: in unseparated whole blood, glycolysis by red cells reduces glucose levels by 10–15% per h at room temperature, leading to falsely low results. Clotted (serum) samples without preservative can be used for glucose measurements if the sample is separated rapidly; once separated, the glucose level is stable for 8h at room temperature and 72h at 4°C. Alternatively, a tube containing fluoride oxalate to inhibit glycolysis can be used if the sample is to be kept unseparated at room temperature for many hours.

False +ve diagnoses may arise if the subject has prepared inadequately (see Box 2.8).

Box 2.8 Preparation for a fasting blood test

- Refrain from any food or drink from midnight before the morning of the test.
- Water only is permitted.
- Regular medication can generally be deferred until a blood sample has been taken.
- The appropriate sample is taken between 8 a.m. and 9 a.m. the following morning.

This preparation is also required for a 75g oral glucose tolerance test (OGTT) or for measurement of fasting blood lipids. Fasting blood tests should be avoided in insulin-treated patients—risk of hypoglycaemia. Fasting is defined by the ADA as no caloric intake for at least 8h.

Normoglycaemia

The diagnostic criteria for normoglycaemia are given in Table 2.15 (note the difference between ADA and WHO for FPG cut-off). Note that there is NO defined random plasma glucose that confirms normoglycaemia, and this complicates the development of screening strategies for diabetes. However, if a random value is <5.6mmol/L, diabetes is unlikely.

Impaired glucose tolerance

This is the preferred diagnostic category for pre-diabetes used by the WHO. The diagnosis of IGT can only be made using a 75g OGTT; a random blood glucose measurement will often point to the diagnosis when other results are non-diagnostic.

This category denotes a stage intermediate between normal glucose levels and DM. By definition, plasma glucose levels are not raised to DM levels, so typical osmotic symptoms are absent. Although subjects with IGT are not at direct risk of developing chronic microvascular tissue complications, the incidence of macrovascular complications (i.e. coronary heart disease (CHD), cerebrovascular disease, peripheral arterial disease (PAD)) is ↑. Presentation with one of these conditions should therefore alert the clinician to the possibility of undiagnosed IGT (or type 2 DM). Note that up to 25% of individuals who are diagnosed with IGT by an OGTT may revert to normal on re-testing.

Impaired fasting glucose

This is the diagnostic category for pre-diabetes preferred by the ADA and depends on the FPG. Instructions for fasting blood test are shown in Box 2.8. The revised 2005 criteria lowered the lower limit for diagnosing IFG, so the range according to the ADA is 5.6–7.0mmol/L. This category is also usually asymptomatic. To date, cross-sectional studies suggest that IGT and IFG may not be synonymous in terms of pathophysiology and long-term implications, and a proportion of patients will fall into one category, but not the other. Also some patients with fasting blood glucose <7.0mmol/L may have 2h blood glucose on the OGTT of >11.1mmol/L and hence would have diabetes by the WHO criteria. If an OGTT is performed, the 2h value takes precedence over the fasting value in the diagnosis of diabetes if the values do not agree.

Oral glucose tolerance test

The OGTT (see Box 2.9) continues to be regarded as the most relevant means for establishing the diagnosis of diabetes in equivocal cases, although its reproducibility is poor. In borderline cases, the WHO suggests that only when an OGTT cannot be performed does the diagnosis rely on FPG. It is also the preferred approach in pregnancy. OGTTs should be carried out under controlled conditions after an overnight fast.

The interpretation of the 75g GTT is shown in Table 2.16. These results apply to venous plasma. Marked carbohydrate depletion can impair glucose tolerance; the subject should have received adequate nutrition in the days preceding the test.

Box 2.9 Oral glucose tolerance test

- Preparation: 3-day unrestricted CHO intake and activity. No medication on day of test. 8–14h fast. No smoking.
- 75g¹ of anhydrous glucose is dissolved in 250mL of water; flavouring with sugar-free lemon and chilling ↑ palatability and may reduce nausea. The subject sits quietly throughout the test.
- Blood glucose is sampled before (time 0), and at 120min after, ingestion of the drink, which should be completed within 5min.
- Urinalysis may also be performed every 30min, although it is only of interest if a significant alteration in renal threshold for glucose is suspected.

¹ In children, 1.75g/kg, up to 75g.

Table 2.16 Interpretation of the 75g OGTT (WHO)

	Venous plasma glucose (mmol/L)	
	Fasting	120min post-glucose load
Normal	<6.0	<7.8
IFG	6.1–6.9	N/A
IGT	N/A	7.8–11.0
DM	>7.0	>11.1

1. In the absence of symptoms, a diagnosis of diabetes must be confirmed by a second diagnostic test on a separate day.

2. For capillary whole blood, the diagnostic cut-offs for diabetes are >6.1mmol/L (fasting) and 11.1mmol/L (120min). The range for IFG based on capillary whole blood is >5.6 and <6.1mmol/L. In the diagnosis of diabetes, the 2h post-blood value predominates if values do not agree.

Effect of intercurrent illness on glycaemia

Patients under physical stress associated with surgery, trauma, acute MI, acute pulmonary oedema, or stroke may have transient ↑ of plasma glucose—often settles rapidly without antidiabetic therapy. However, the hormonal stress response in such clinical situations is liable to unmask pre-existing DM or to precipitate DM in predisposed individuals. Blood glucose should be carefully monitored and the urine tested for ketones. Sustained hyperglycaemia, particularly with ketonuria, demands vigorous treatment with insulin in an acutely ill patient. Re-testing is usually indicated following resolution of the acute illness—an OGTT at a 4- to 6-week interval is recommended if glucose levels are equivocal.

Screening for diabetes

This remains controversial, and universal screening is not generally advocated, even though rates in the adult population exceed 5% in almost all countries and >10% in many countries. A low threshold for testing in those at high risk is advocated. The ADA suggests that 3-year screening in asymptomatic adults be considered over the age of 45, and especially where the BMI is >25 and there is high-risk ethnicity (including African or South Asian origin), a first-degree relative with diabetes, or women who have had a large baby (>9lb) or a previous diagnosis of gestational diabetes.

Further reading

- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2016; **39** (Suppl 1), S13–22.
- World Health Organization 2006 Guidelines: http://www.who.int/diabetes/publications/Definition%20and%20diagnosis%20of%20diabetes_new.pdf
- World Health Organization (2013). *Diagnostic criteria and classification of hyperglycaemia first detected in pregnancy*. Geneva: World Health Organization. http://apps.who.int/iris/bitstream/10665/85975/1/WHO_NMH_MND_13.2_eng.pdf.

WHO criteria for use of HbA1c.

Diabetes websites

- American Diabetes Association. <http://www.diabetes.org>.
- Diabetes UK. <http://www.diabetes.org.uk>.
- World Health Organization. Diabetes. http://www.who.int/topics/diabetes_mellitus/en/.

Which type of diabetes is it?

Table 2.17 shows the types and causes of diabetes. Type 2 diabetes due to insulin resistance is by far the commonest (accounting for >90% of cases) and is ↑ as the prevalence of obesity and low physical activity in our society rise. However, there are no definitive tests that can distinguish type 1 and type 2 diabetes. Instead, a collection of clinical and laboratory parameters are used (see Table 2.18), but many cases are hard to categorize (sometimes referred to as type 1.5 diabetes!).

Table 2.17 Types and causes of diabetes

Type of diabetes*	Comment
Type 1	β-cell destruction usually leading to absolute insulin deficiency: 1A—immune-mediated; 1B—idiopathic (formerly known as juvenile-onset or IDDM)
Type 2	Predominantly insulin-resistant with relative insulin deficiency (formerly known as maturity-onset or NIDDM)
Gestational diabetes	Diabetes during pregnancy that resolves postpartum. Often an early manifestation of type 2 diabetes
Other specific types	Includes genetic (MODY, mitochondrial, insulin resistance syndromes), pancreatic disease, drug-induced, occurrence in other genetic syndromes, endocrinopathies (e.g. acromegaly)

* Abbreviated from the ADA classification.

Figure 2.18 (guide to the type of diabetes) provides an algorithm for diagnosing the type of diabetes. Although type 2 diabetes remains the commonest diagnosis in adults and is increasingly common in children, *this is a diagnosis for the lifetime of the individuals* and care should be taken to diagnose any underlying conditions as accurately as possible. Note especially:

- *Unusual features*: if any of the unusual features listed in Table 2.19 are present, then the algorithm should not be pursued and an underlying cause for the diabetes investigated.
- *Type 1 diabetes*: it is important to always consider the possibility of type 1 diabetes (see Fig. 2.18), even in older people, as early insulin treatment is essential to avoid the risk of ketoacidosis.
- *Pregnancy*: although the majority of diabetes diagnosed for the first time in pregnancy (gestational diabetes) is part of the spectrum of type 2 diabetes, diabetes due to other causes can occur. The algorithm in Fig. 2.18 should still be considered.
- *Latent autoimmune diabetes of adults (LADA)* refers to diabetes with +ve autoantibodies (anti-glutamic acid decarboxylase (GAD)) developing in adults (typically over the age of 35) but not developing ketosis or with absolute requirement for insulin within 6 months of diagnosis. However, subjects are usually not overweight and develop a need for insulin within a few years. LADA is considered to be a 'slow-onset' version of type 1 diabetes, often initially misdiagnosed as type 2 diabetes.

Table 2.18 Clinical features and laboratory tests used to distinguish type 1 and type 2 diabetes, and maturity-onset diabetes of the young (MODY)

		Type 1	Type 2	MODY
Clinical features	Weight	Slim (BMI <25) + weight loss at diagnosis	Overweight (BMI >25)	Average weight (BMI <30)
	Ketosis	Occurs	Rare	Rare
	Race	Caucasian	↑ risk in South Asians and Afro-Caribbeans	
Laboratory tests	Acanthosis nigricans	Absent	May be present	Absent
	Parent with diabetes	Unusual	Common	Common
	Auto-antibodies	Anti-GAD antibody +ve in around 80%*	-ve	-ve
	Insulin c-peptide	Low (but still present for up to 5 years from diagnosis)	Present	Present
	High-density lipoprotein (HDL) >1.2	Usual	Rare	Usual

* Additional antibody tests can include anti-IA2 and anti-ZnT8 antibodies.

- **Monogenic diabetes—maturity-onset diabetes of the young (MODY):** monogenic disorders resulting in diabetes (see Tables 2.19 and 2.20). Although these account for <1% of cases, they are important to diagnose as they may be easily treated with sulfonylureas (MODY 3), associated with renal disease (MODY 5), or require no treatment (MODY 2). The diagnosis also has important implications for other family members diagnosed with diabetes. Note that it can be very difficult to distinguish type 2 diabetes from MODY without genetic testing, and a high level of suspicion in young people is required (see Fig. 2.18). If suspected, further advice from a genetic testing centre with experience in MODY should be sought (<http://www.diabetesgenes.org>).
- ‘Flatbush’ diabetes refers to patients who present in DKA but subsequently have a course that is more like type 2 diabetes and are able to come off insulin. This is most commonly seen in African-Caribbeans.

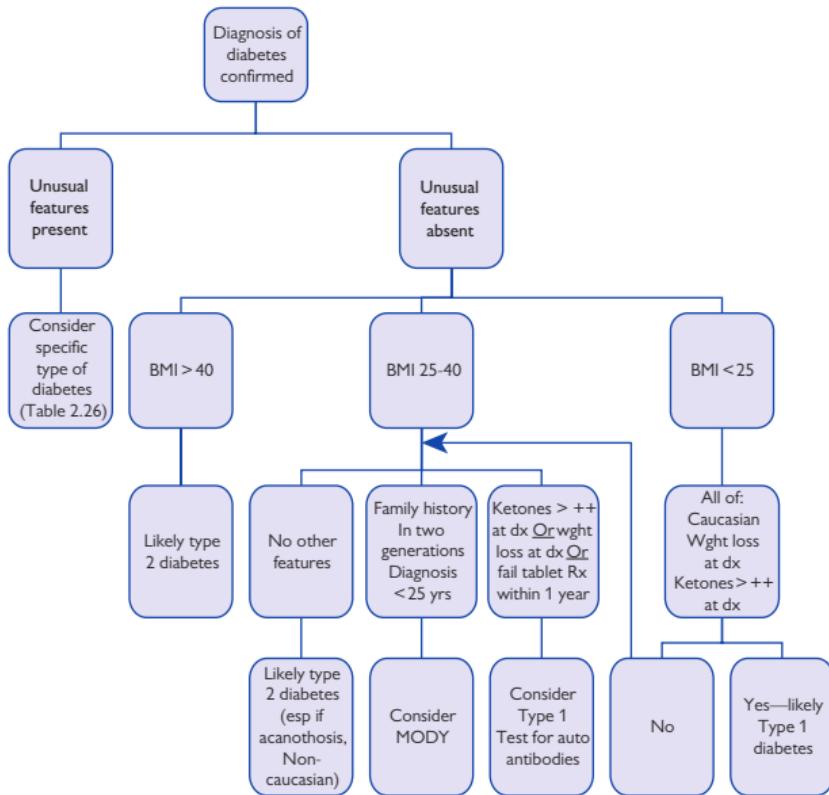


Fig. 2.18 Guide to the type of diabetes.

Table 2.19 Specific features suggestive of an unusual cause of diabetes

Feature	Possible diagnosis
Alcohol excess, history of pancreatic disease	Chronic pancreatitis
Painless jaundice, weight loss in older person	Pancreatic cancer
Cystic fibrosis	Pancreatic disease
On steroids/Cushingoid appearance	Steroid-induced diabetes/Cushing's syndrome
Post-organ transplant	Drug-induced
Specific drugs, e.g. somatostatin analogues, diazoxide	Drug-induced
Severe hypertension	Phaeochromocytoma
Acromegalic appearance	Acromegaly
'Muscular appearance' (lipodystrophy)	Partial lipodystrophy

Table 2.19 (Contd.)

Family history of diagnosis <25 in two or more generations	MODY
Renal cysts, urogenital dysplasia	Hepatic nuclear factor (HNF)1 β MODY
Bilateral deafness (maternal inheritance)	Mitochondrial diabetes
Optic atrophy, Dl	Wolfram syndrome
Diagnosis <6 months old	Kir 6.2 mutation or other forms of neonatal diabetes
Megaloblastic anaemic	Roger's syndrome (thiamine)
Short stature, severe insulin deficiency (>1000U/day)	Insulin receptor defect (leprechaunism, Rabson-Mendenhall syndrome)
Stiff legs/gait/falls: -ve-GAD antibodies	Stiff person syndrome

Table 2.20 Genetically inherited forms of diabetes, including MODY

Gene	% of MODY cases	Comments
Glucokinase	20	MODY 2 'mild' complications rare
HNF-1 α	60	MODY 3 diagnosed later—35 years, progressive β cell failure, but very sensitive to sulfonylureas
HNF-4 α	1	MODY 1
HNF-1 β	1	MODY 5 renal cysts/abnormalities
IPF-1	1	MODY 4
NeuroD1	? <1	MODY 6
Unknown	15	'MODY X'
SUR1	<1%??	Hyperinsulinism in infancy and β cell failure as adult
Mitochondrial	Not MODY	Maternally inherited. May be associated with nerve deafness, lactic acidosis, or syndromes such as DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness)
Lipodystrophy	Not MODY, lamin a/c and others	Associated with localized fat loss

Further reading

DiabetesGenes.org.  <http://www.diabetesgenes.org>.

Monitoring diabetic control

Self-testing and near-patient testing

Self-testing capillary blood glucose (or urine) can be readily performed by the majority of patients, with results available in under 20s. Measurements of longer-term glycaemic control are typically laboratory-based, although increasingly near-patient testing equipment is available for use in clinics that can give results to patients within minutes.

Urine testing

Glycosuria

Semi-quantitative testing for glucose using reagent-impregnated test strips is of limited value and although used to 'screen' for diabetes, it is not recommended for monitoring of glycaemic control. Urinalysis provides retrospective information over a limited period of time. Other limitations are:

- The renal threshold for the reabsorption of glucose in the proximal convoluted tubule (PxCT) is ~10mmol/L on average but varies between individuals. Subjects with a low threshold will tend to show glycosuria more readily, even with normal glucose tolerance ('renal glycosuria'). Children are particularly liable to test +ve for glucose. The renal threshold is effectively lowered in pregnancy. Conversely, a high threshold, common among the elderly, may give a misleadingly reassuring impression of satisfactory control. Fluid intake and urine concentration may affect glycosuria. Renal impairment may elevate the threshold for glucose reabsorption.
- Delayed bladder emptying, e.g. due to diabetic autonomic neuropathy, will reduce the accuracy of the measurements through dilution.
- Hypoglycaemia cannot be detected by urinalysis.

Ketonuria, self-blood testing for ketones

Semi-quantitative test strips for acetoacetate (e.g. Ketostix[®]) are available for patients with type 1 DM but have largely been replaced by self-testing of blood (capillary, fingerprick) for ketones (β -hydroxybutyrate). Useful when intercurrent illness leads to disturbance of metabolic control. The presence of ketonuria on dipstick testing (++) or (+++) or blood ketones (>3.0mmol/L) in association with hyperglycaemia indicates marked insulin deficiency. The patient may be developing ketoacidosis, and ↑ insulin doses and urgent assessment for DKA is required (➔ Diabetic emergencies: diabetic ketoacidosis, hyperosmolar non-ketotic syndrome, and lactic acidosis, pp. 210–11). Note that low-level ketonuria ('+') or blood ketones <0.6mmol/L can occur after a period of fasting, especially in overweight patients, and does not necessarily indicate DKA. Occasionally, patients with type 2 DM develop ketosis during severe intercurrent illness, e.g. major sepsis. Urine testing strips do not detect 3-hydroxybutyrate (although acetone is detected by Acetest[®]). Occasional underestimation of the degree of ketoacidosis using these tests is a well-recognized, albeit uncommon, caveat of alcoholic ketoacidosis but is no longer an issue with the use of blood ketone testing. Blood testing is preferred, as it does not require any delay in obtaining a urine sample and is more accurate. Separate testing strips from glucose testing are required (but often the same meter can be used).

Self-testing of blood glucose

Self-testing of capillary blood glucose obtained by fingerprick has become an established method for monitoring glycaemic control. Frequent testing is a prerequisite for adjusting insulin doses and for safe intensive insulin therapy such as that employed in the DCCT. Use in type 2 diabetes treated by diet or tablets only is not essential. Enzyme-impregnated dry strip methods are available, which are used in conjunction with meter devices and give results in <20s with just 50µL of blood. Adequate training and a system of quality control are important; even when trained health professionals use such systems in clinics or hospitals, misleading results are possible, particularly in the lower range of blood glucose results. Where there is doubt, an appropriate sample (in a tube containing the glycolysis inhibitor fluoride oxalate) should be collected immediately for analysis by the clinical chemistry laboratory. However, acute treatment of hypoglycaemia, where indicated, should not be delayed.

Continuous glucose monitoring systems (CGMS)

Several systems are now available using electrical conductance or microdialysis to provide continuous monitoring of interstitial glucose. Sensors are inserted SC and need to be replaced every 3–7 days. Most systems can display the results in real time (if regularly calibrated against traditional finger stick readings), so that they can be reviewed by the patient, and linked to alarms indicating high and low levels. While these systems, although expensive, are proving increasingly valuable for patients with type 1 diabetes on complex insulin regimes and insulin pump therapy, it must be remembered that the interstitial glucose level is up to 30min ‘behind’ the blood level and if glucose levels are changing rapidly, continuous monitors may ‘miss’ significant hypoglycaemic events. A recent development is sensors that do not require calibration and the reading is made by ‘swiping’ the reader (or an appropriately configured smartphone) over the sensor. These newer sensors have significantly reduced ‘delay’ time, claimed to be <10min vs blood levels.

Laboratory assessment of glycaemic control

Glycated haemoglobin

Measuring HbA_{1c}

HbA_{1c} (comprises 60–80% of total glycated haemoglobin HbA₁) is formed by the slow, irreversible post-translational non-enzymatic glycation of the N-terminal valine residue of the β chain of Hb. The proportion of HbA_{1c}:total Hb (normal non-diabetic reference range ~4–6%) provides a useful index of average glycaemia over the preceding 6–8 weeks. The result is disproportionately affected by blood glucose levels during the final month before the test (~50% of value). Laboratory values have now been aligned to a standard from the DCCT trial (DCCT-aligned), and values are generally consistent between laboratories. Recent recommendations suggest expressing HbA_{1c} in new units (mmol/mol) against a new International Federation of Clinical Chemistry (IFCC) standard (see Table 2.21).

Table 2.21 Guide to HbA_{1c} values in new and old units

DCCT-aligned HbA _{1c} (%)	IFCC-HbA _{1c} (nmol/mol)
6.0	42
6.5	48
7.0	53
7.5	59
8.0	64
9.0	75

Frequency of testing HbA_{1c}

It is suggested that HbA_{1c} is measured every 6 months in stable patients, every 3 months in patients with unstable metabolic control, and every month in pregnancy.

Interpreting HbA_{1c} levels

Average HbA_{1c} levels collected over a longer period (i.e. years) provide an estimate of the risk of microvascular complications. Sustained high concentrations identify patients in whom efforts should be made to improve long-term glycaemic control and ↑ surveillance for long-term complications. Table 2.22 summarizes recent recommendations for target HbA_{1c} levels and capillary glucose measurements in adult subjects with diabetes. Targets need to be modified in pregnancy (see footnote to Table 2.22), in children, in patients with recurrent hypoglycaemia or difficulty complying with medication, and in patients with vascular (especially coronary artery) disease in whom hypoglycaemia may precipitate fatal arrhythmias.

Table 2.22 Target levels of HbA_{1c} and capillary blood glucose values

	Pre-meal (mmol/L)	Post-meal (mmol/L)	HbA _{1c} (nmol/mol)
Non-diabetic	3.5–5.5	<7.8	4–6% (20–42)
Ideal	4–7*	<10*	<7%** (<53)
Suboptimal control			7–10%
Poor control			>10%

* In pregnancy, NICE recommends 3.5–5.9 pre-meal and <7.8 post-meal (1h) HbA_{1c}; aim for <6.1%.

** In type 2 diabetes, NICE recommends a target of 6.5% in patients without vascular disease.

Note: lower target levels (HbA_{1c} <6.5%) have been proposed in subjects with type 2 diabetes treated with lifestyle or metformin alone; Higher targets (e.g. HbA_{1c} <8.0% or higher) are appropriate in subjects with frequent hypoglycaemia or hypoglycaemia unawareness, extensive co-morbid conditions, or limited life expectancy.

Limitations of HbA_{1c} measurements

Although glycated Hb levels are a reliable indicator of recent average glycaemic control, they do not provide information about the daily pattern of blood glucose levels or the frequency of hypoglycaemic episodes; this supplementary information required for logical adjustment of insulin doses is derived from frequent home blood glucose monitoring. More recent changes in glycaemia (i.e. within the preceding 4 weeks or so) will influence HbA_{1c} level more than glucose levels 12 or more weeks ago.

Spurious HbA_{1c} levels may arise in states of:

- Blood loss/haemolysis/reduced red cell survival (low HbA_{1c}).
- Haemoglobinopathy.
 - ↑ levels of HbS (low levels of HbA_{1c})
 - ↑ levels of HbF (high HbA_{1c}).

Modern HbA_{1c} methods are likely to detect haemoglobinopathies without specific testing. Where haemoglobinopathy is present and cannot be adjusted for by an assay method where there is no interference, the HbA_{1c} test is uninterpretable and capillary blood glucose levels or fructosamine must be used.

HbA_{1c} measurements are less reliable in pregnancy where rapid changes in blood glucose levels can occur (e.g. last trimester). They are still used, as they are more reliable than other available methods for estimating overall control, but results should be interpreted with caution.

Uraemia due to advanced diabetic nephropathy is associated with anaemia and ↓ RBC survival, thereby falsely lowering HbA_{1c} levels.

Fructosamine: refers to protein–ketoamine products resulting from the glycation of plasma proteins. The fructosamine assay measures glycated plasma proteins (mainly albumin), reflecting average glycaemia over the preceding 2–3 weeks. This is a shorter period than that assessed using glycated Hb measurements and may be particularly useful when rapid changes in control need to be assessed, e.g. during pregnancy. Levels can be misleading in hypoalbuminaemic states, e.g. nephrotic syndrome. Some fructosamine assays are subject to interference by hyperuricaemia or hyperlipidaemia.

The main indications for fructosamine measurement are currently: (a) the presence of haemoglobinopathy or other interference with the HbA_{1c} assay (☞ Limitations of HbA_{1c} measurements, pp. 207–8) and (b) rapidly changing blood glucose levels (e.g. pregnancy).

Further reading

- American Diabetes Association (ADA). Clinical guidelines on pages for health professionals. ☞ <http://www.diabetes.org>.
- American Diabetes Association. 5. Glycemic targets. *Diabetes Care* 2016; **39**(Suppl 1), S39–46 (ADA standards of medical care).
- National Institute for Health and Care Excellence. Diabetes guidelines. ☞ <http://www.nice.org.uk>.

Diabetic emergencies: diabetic ketoacidosis, hyperosmolar non-ketotic syndrome, and lactic acidosis

DKA should be considered in any unconscious or hyperventilating patient. The hyperosmolar non-ketotic (HONK) syndrome is characterized by marked hyperglycaemia ($>30\text{ mmol/L}$) and dehydration in the absence of significant ketosis or acidosis. Lactic acidosis (LA) associated with metformin is uncommon. A rapid clinical examination and bedside blood tests should allow the diagnosis to be made. Treatment (IV rehydration, insulin, electrolyte replacement) of these metabolic emergencies should be commenced without delay (for details,  Further reading, p. 211).

Confirm diagnosis by bedside measurement of

- Capillary blood glucose.
- Capillary blood testing for ketones (betahydroxybutyrate).
- Urine for nitrites and leucocytes (UTI).

Venous blood for urgent laboratory measurement of

- Plasma glucose (fluoride oxalate; ► true ‘euglycaemic’ DKA is rare).
- U&E (arterial potassium (K^+) can be measured by some gas analysers). Plasma Na^+ may be depressed as a consequence of hyperglycaemia or marked hyperlipidaemia.
- Plasma creatinine (► may be falsely elevated in some assays by DKA).
- Plasma lactate (if indicated—can also be measured by some gas analysers). Indicated if acidosis without heavy ketonuria is present. LA is a complication of tissue hypoxia (type A) and is a rare complication of metformin treatment in patients with type 2 DM (type B).
- Plasma osmolality in HONK—either by freezing point depression or calculated:

$$2 \times [\text{plasma Na}^+] + [\text{plasma K}^+] + [\text{plasma glucose}] + [\text{plasma urea}]$$

- FBC (non-specific leucocytosis is common in DKA).
- Blood cultures (signs of infection, e.g. fever, may be absent in DKA).
- ABGs (corrected for hypothermia) for arterial pH, HCO_3^- , PCO_2 , and PO_2 (if shock or hypotension).

DKA is confirmed by blood glucose $>11\text{ mmol/L}$ (rarely euglycaemic ketoacidosis can occur in pregnancy or subjects on sodium–glucose cotransporter 2 (SGLT2)-inhibiting drugs), ketonaemia $>3.0\text{ mmol/L}$, and acidosis (pH <7.3 or $\text{HCO}_3^- <15\text{ mmol/L}$). Severe DKA is considered to be pH <7.0 , $\text{HCO}_3^- <5\text{ mmol/L}$, or ketones $>6.0\text{ mmol/L}$. Repeat laboratory measurement of blood glucose, electrolytes, and urea at 2, 4, and 6h, and as indicated thereafter. Electrolyte disturbances, renal impairment, or oliguria should prompt more frequent (1–2h) measurements of plasma K^+ . Capillary blood glucose and ketone testing are monitored hourly at the bedside. ► Avoidance of hypokalaemia and hypoglycaemia is most important during therapy. Current therapy is aimed at rapid resolution of ketosis which is considered to be ketonaemia $<0.6\text{ mmol/L}$ ('normal' $<0.3\text{ mmol/L}$) with pH >7.3 .

Other investigations, as indicated

- CXR.
- Microbial culture of urine, sputum, etc.
- ECG: acute MI may precipitate metabolic decompensation; note that serum transaminases and CK may be non-specifically elevated in DKA.
- Sickle-cell test (in selected patients).
- Venous plasma PO₃⁴⁻ (if there is respiratory depression).
- ► Performance of investigations should not delay initiation of treatment and transfer to a high-dependency or intensive care unit (ICU).

Severe metabolic acidosis in the absence of hyperglycaemia (or other obvious cause of acidosis such as renal failure) raises the possibility of

- LA.
- Alcoholic ketoacidosis: this occurs in alcoholics following a binge. Alterations in the hepatic redox state may result in a misleading -ve or 'trace' Ketostix® reaction but detectable on blood ketone strips. A similar caveat may occasionally be encountered when significant LA coexists with DKA. Venous plasma glucose may be normal or ↑.
- Anion gap >15mmol/L. Normally, the anion gap (<10mmol/L) results from plasma proteins, sulfate (SO₄²⁻), PO₄³⁻, and lactate ions. When the anion gap is ↑, measurement of plasma ketones, lactate, etc. usually confirms the aetiology (see Table 2.23).

Table 2.23 Causes of an anion gap acidosis

Ketoacidosis	DKA
	Alcoholic ketoacidosis
	LA (► metformin)
Chronic renal failure	
Drug toxicity	Methanol (metabolized → formic acid)
	Ethylene glycol (metabolized → oxalic acid)
	Salicylate poisoning

Further reading

Joint British Diabetes Societies (JBDS) guidelines (2013). Available at: <http://www.diabetologists-abcd.org.uk>.

Investigation of hyperlipidaemia

1° dyslipidaemias are relatively common and contribute to an individual's risk of developing atheroma (e.g. CHD, CVD). Prominent examples include familial combined hyperlipidaemia (FCHL; ~2–3% of UK population) and heterozygous familial hypercholesterolaemia (FH; UK incidence 1 in 500). Major hypertriglyceridaemia also predisposes to pancreatitis. The key features of familial FH, FCHL, and diabetic dyslipidaemia are considered later.

Investigations

Although many subtle alterations in plasma lipids have been described, therapeutic decisions rest on measurement of some or all of the following in serum or plasma (plasma being preferred, since it can be cooled rapidly):

- Total cholesterol (may be measured in non-fasting state in first instance, since levels are not greatly influenced by meals).
- Triglycerides (TGs) (after 12h fast).
- LDL-cholesterol (calculated using the Friedewald formula when TGs are <4.5mmol/L):

$$\text{LDL-cholesterol} = [(\text{total cholesterol} - \text{HDL-cholesterol}) - \text{TGs}] / 2.19$$

Note that because of the unreliability of this calculation, especially at higher TG levels, it has been recommended to report results also as 'non-HDL-cholesterol', which is typically around 0.7mmol/L higher than calculated LDL-cholesterol.

- HDL-cholesterol (regarded as the 'cardioprotective' subfraction)—HDL particles are synthesized in the gut and liver and thought to be involved in 'reverse transport' of cholesterol from peripheral tissues to the liver where it can be excreted as bile salts.

Notes on sampling in relation to lipoprotein metabolism

- TGs (triacylglycerols) are measured after a ~12h overnight fast in order to clear diet-derived chylomicrons.
- Alcohol should be avoided the evening prior to measurement of TGs (can exacerbate hypertriglyceridaemia).
- A weight-maintaining diet is recommended for 2–3 weeks before testing.
- Lipid measurements should be deferred for 2–3 weeks after minor illness and 2–3 months after major illness, surgery, or trauma since cholesterol levels may be reduced. Following acute MI, it is generally accepted that plasma cholesterol is reliable if measured within 24h of the onset of symptoms.
- The effects of certain drugs on lipids should be considered (see Box 2.10).
- Glycaemic control should be optimized wherever possible before measuring plasma lipids in patients with diabetes.

Important additional considerations are

- Day-to-day variability: generally, decisions to treat hyperlipidaemia should be based on >1 measurement over a period of 1–2 weeks. This is especially true for patients with mixed hyperlipidaemia (i.e. including hypertriglyceridaemia).

Box 2.10 Causes of secondary hyperlipidaemia**Hypercholesterolaemia**

- Hypothyroidism (even minor degrees of 1° hypothyroidism)
- Cholestasis (raised lipoprotein X levels)
- Nephrotic syndrome
- Anorexia nervosa
- Diuretics
- Immunosuppressive agents
- Hepatoma
- Dysglobulinaemias

Hypertriglyceridaemia

- Obesity
- Diabetes (especially type 2 DM)
- Lipodystrophic syndromes (of diabetes and HIV-associated)
- Alcohol excess (note: moderate alcohol consumption may raise HDL-cholesterol)
- Renal failure
- Antiretroviral agents
- Oestrogens (especially oral preparations in some women)
- Corticosteroids
- β -adrenergic blockers
- Retinoids

Recommended investigation for exclusion of 2° hyperlipidaemia: U&E, plasma creatinine, fasting venous glucose, LFTs, and TFTs. For patients on statins, check LFTs and CK periodically (► measure urgently if myositis occurs—a rare, but potentially fatal, complication).

- Exclusion of 2° hyperlipidaemia—many common conditions, drugs, and dietary factors can influence plasma lipids (see Box 2.10).
- Family members should also have their plasma lipids measured if familial hyperlipidaemia is suspected in a proband.

Both cholesterol and TGs may be affected to some degree by these factors, but one often predominates. Pre-existing 1° hyperlipidaemias may be exacerbated.

Clinical features

For example, xanthelasma, tendon xanthomas, etc. should always be sought. A detailed family history, drug history, and medical history (for diabetes and other cardiovascular risk factors such as hypertension) should always be obtained. Certain endocrine disorders and impaired hepatic or renal function can influence circulating lipid composition and cardiovascular risk. A classification of the major familial dyslipidaemias is presented in Table 2.24.

► Specialist advice should be sought in the management of major or resistant hyperlipidaemias. TG levels $>11\text{mmol/L}$ (1000mg/dL) are considered 'very high' and require therapy because of the risk of pancreatitis.

Table 2.24 Familial hyperlipoproteinæmias

Genetic disorder	Defect	Presentation	Cholesterol	Triglycerides	Phenotype
Familial LPL deficiency	Absence of LPL activity	Eruptive xanthomata; hepatosplenomegaly	↑	↑↑↑	I
Familial apo C-II deficiency	Absence of apo C-II	Pancreatitis	↑	↑↑↑	I or V
Familial hypercholesterolaemia	LDL receptor deficiency	Tendon xanthomata; premature atheroma	↑↑↑	↑ or N	IIa or IIb
Familial dysbeta-lipoproteinæmia	Abnormal apo E and defect in TG metabolism	Tubo-eruptive and palmar xanthoma; premature atheroma	↑↑↑	↑↑↑	III
Familial combined hyperlipidaemia	Uncertain	Premature atheroma	↑ or N	↑ or N	IIa, IIb, or IV
Familial hypertriglyceridaemia	Uncertain; eruptive xanthomata; hepatosplenomegaly; pancreatitis	N	↑	↑↑↑	IV
					V

↑, ↑↑, and ↑↑↑, mildly, moderately, and severely raised; respectively cholesterol and triglycerides refer to concentrations in plasma; phenotype refers to Fredrickson classification (I to V, see Table 2.25); apo, apoprotein; LPL, lipoprotein lipase; n, normal; TG, triglycerides.

Table 2.25 Phenotypic (Fredrickson) classification of hyperlipidaemias

Type	Cholesterol	Triglycerides	Particle excess	Usual underlying cause
I	↑	↑↑↑	Chylomicrons	LPL or apo C-II deficiency
IIa	↑↑	↑	LDL	LDL receptor defect, LDL overproduction
IIb	↑↑	↑↑	VLDL, LDL	VLDL or LDL overproduction or ↓ clearance
III	↑↑	↑↑	IDL—dysbeta lipoproteinaemia	Impaired remnant removal may be due to certain apo E phenotypes or apo E deficiency
IV	N or ↑	↑↑	VLDL	VLDL overproduction or ↓ clearance
V	↑	↑↑↑	Chylomicrons VLDL	Diabetes

↑, ↑↑, and ↑↑↑, mildly, moderately, and severely raised, respectively; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; LPL, lipoprotein lipase; apo, apoprotein; IDL, intermediate-density lipoproteins.

Further reading

- JBS3 Board. Joint British Societies' consensus recommendations for the prevention of cardiovascular disease (JBS3). *Heart* 2014; **100** Suppl 2: ii1–67.
- National Heart, Lung, and Blood Institute, National Cholesterol Education Programme Guidelines (2013). *Managing blood cholesterol in adults: systematic evidence review from the Cholesterol Expert Panel*. <http://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cholesterol-in-adults>.

Test protocols

Insulin tolerance test (insulin stress test)

- **Indication:** suspected ACTH or GH deficiency.
- **Contraindications:** patients with epilepsy, CHD (check ECG).
- **Children:** use no more than 0.1U/kg. Considerable care should be exercised; the test should only be performed in a centre with expertise.
- **Alternatives:** short Synacthen® test for hypocortisolism; stimulation tests for GH deficiency, e.g. growth hormone-releasing hormone (GHRH) arginine test (► Hypothalamus/pituitary function, see pp. 128–30).
- **Preparation:** patient fasting overnight. Bed required (although day-case procedure). Patient must be accompanied home and may not drive. OMIT morning hydrocortisone or other steroid hormone replacement if patient is taking this and previous day's GH. Physician must be present throughout the test. Requires written consent.
- **Procedure:** early morning outpatient test in fasting patient. Indwelling venous cannula and constant medical supervision required throughout. Cannula is kept patent by running a saline infusion with a three-way tap for sampling. Discard initial 2–3mL when each sample is taken. Label all samples clearly with time and patient details. Near-patient testing glucometer required.
 1. Take baseline blood for glucose, cortisol, and GH. Check IV access is working well. Review the test with the patient, and explain symptoms s/he is likely to experience (see point 5).
 2. Draw up 25mL of 50% glucose for immediate administration IF REQUIRED.
 3. Give soluble (regular) insulin as an IV bolus in a dose of 0.15U/kg after an overnight fast. Consider 0.1U/kg (lower dose) if suspected profound hypocortisolism. ► This appears a very small dose, e.g. typically around 10U. CHECK DOSE CALCULATION CAREFULLY. Usually an insulin syringe is used to draw it up and then transfer it to a 2mL syringe containing saline.
 4. Take blood at 15min intervals (0, 15, 30, 45, 60min) for glucose, cortisol, and GH.
 5. Observe for symptoms and signs of hypoglycaemia. The first sign is usually profuse sweating. The patient may then be aware of symptoms such as palpitations, hunger, and paraesthesiae. This typically occurs 30–45min into the test. Check near-patient glucose to confirm $<3.5\text{ mmol/L}$. Continue to talk to, and reassure, the patient. If the patient becomes very drowsy or unrousable, then give 25mL of 50% glucose. This does not invalidate the test, as the hypoglycaemic stimulus has already occurred. Continue blood sampling at standard times.
 6. If the patient has not experienced hypoglycaemia by 45min and near-patient glucose is $>4\text{ mmol/L}$, give a further IV bolus of 0.15 or 0.3U/kg if the patient is known to be very insulin-resistant (e.g. acromegalic). Repeat sampling at 15min intervals for 60min after this second bolus.
 7. At the end of procedure (usually 60min), give IV 25mL of 50% dextrose if the patient still has symptoms of hypoglycaemia.
 8. Give the patient a meal including complex carbohydrate (e.g. sandwiches or lunch), and observe for a minimum of 1h further before accompanied discharge.

- **Unwanted effects:** severe hypoglycaemia with depressed level of consciousness or convulsion requires immediate termination of the test with 25mL of 50% glucose IV. Repeat if necessary and follow with 5% or 10% glucose infusion. Continue to collect samples for hormone and glucose measurements.
- **Interpretation:** the test is only interpretable if adequate hypoglycaemia is achieved (<2.2mmol/L). Normal maximal cortisol response >550nmol/L. (Note: may be lower, depending on local assay range—check with the laboratory). Normal GH response >20mU/L. Impaired responses (if hypoglycaemic stimulus adequate) denote corticotrophin (assuming adrenal glands are normal) or GH deficiency or both. Peak GH response <10mU/L is sufficient to consider GH replacement; peak GH response <5mU/L is severe GH deficiency.

Combined arginine-growth hormone-releasing hormone test

- **Indication:** GH deficiency. Now preferred to ITT.
- **Contraindications:** previous reaction to stimulatory hormones. Administer with caution to patients with severe liver or renal disease.
- **Alternatives:** ITT. Other stimulation tests now outdated (e.g. glucagon, exercise). Serum IGF-1 levels give an idea of the GH status but are unreliable at low levels.
- **Preparation:** order GHRH and arginine from pharmacy. Omit GH injections for a minimum of 24h. The patient arrives in the morning after fasting for 10h (overnight). Water is allowed and patients should take all their routine medications in the morning (but not GH). Informed consent must be obtained and documented. Warn patients about possible side effects of IV GHRH such as flushing lasting <5min.
- **Procedure:**
 - Weigh the patient.
 - Insert an indwelling IV cannula for blood sampling, administration of bolus GHRH and arginine infusion (keep patent with heparinized saline).
 - Patients should rest throughout the test.
 - Take basal samples for GH at -30 and 0min.
 - Give GHRH as an IV bolus of 1 μ g/kg body weight at 0min; at the same time, start infusing 30g of 12.5% arginine solution over 30min, preferably using an infusion pump (children: 0.5g/kg as a 12.5% solution, to a maximum of 30g).
 - Take blood samples for GH at 30, 45, 60, 75, 90, 105, and 120min.
 - Patients are allowed home after a full lunch.
- **Interpretation:** the diagnosis of adult GH deficiency is confirmed if the peak GH concentration is <15mIU/L (by ITT, <12mU/L in GHRH testing). Severe GH deficiency (as defined by NICE)—peak <9mIU/L. Cut-offs by BMI range have been proposed (34.5mU/L for those with a BMI <25kg/m², 24mU/L for a BMI of 25–30kg/m², and 12.6mU/L for those with a BMI >30kg/m²) but are not universally accepted.

Note: conversion factor: $\mu\text{g}/\text{L} \times 3.0 = \text{mIU}/\text{L}$.

Combined anterior pituitary function testing

- *Indication:* assessment for anterior pituitary hypofunction.
- *Contraindications:* previous reaction to stimulatory hormones.
- *Alternatives:* ITT for GH and adrenal axis; metyrapone test for adrenal axis. Other stimulation tests for GH, e.g. GHRH–arginine test.
- *Preparation:* test usually performed in morning for basal sampling.
- *Procedure:* IV cannula inserted. Basal blood samples taken for cortisol, oestradiol (♀) or testosterone (♂), FT4, and IGF-1. Hypothalamic hormones are given sequentially IV, each as a bolus, over around 20s: LHRH 100 μg , TRH 200 μg , and ACTH 250 μg . Additionally, GHRH (1 $\mu\text{g}/\text{kg}$ body weight) may be given. (Reduce doses in children.) Samples are drawn at 0, 20, 30, 60, and 120min for LH, FSH, TSH, cortisol, and PRL. If GHRH is given, samples are drawn at the same time points for GH.
- *Interpretation:* normal values as follows:
 - *TRH*—suspect 2° hypothyroidism if peak response (at 20min) <20mU/L. (Note: low levels also seen in hyperthyroidism—ensure FT4 or total T4 not raised.)
 - *ACTH*—peak cortisol response >550nmol/L at 30 or 60min. (Note: may be lower, depending on local assay range—check with the laboratory.)
 - *LHRH*—peak LH/FSH response 2–5 times the basal value. LH, peak at 20min; FSH later.
 - *GHRH*—normal GH peak response >15mU/L.

Water deprivation test

- **Indication:** diagnosis of DI and to distinguish cranial and nephrogenic DI.
- **Contraindications:** none if carefully supervised. For correct interpretation, thyroid and adrenal deficiencies should be replaced first. Interpretation in the presence of DM and uraemia can be difficult.
- **Alternatives:** morning urine osmolality of $>600\text{mOsmol}$ excludes significant degrees of DI. No other definitive test for DI.
- **Patient preparation:** usually an outpatient procedure. Correct thyroid and adrenal insufficiencies in advance. Renal function and blood glucose should have been checked in advance. Steroid and thyroid hormone replacement should be taken as normal on the day of the test. If the patient is on desmopressin, omit the dose on the evening before the test (or, if not possible, halve this dose). Free fluids, but not to excess, up to 7.30 a.m. on the day of the test. No alcohol on the night before the test or on the morning of the test. Light breakfast, but no tea, coffee, or smoking on the morning of the test. Empty the bladder before attending for the test. If urine volume is $<3\text{L/day}$ ('mild cases'), ask the patient to have no fluids or food from 6.00 p.m. on the evening before the test ('prolonged water deprivation test').
- **Requirements for test:** accurate weighing scales. Supervision for the whole test (up to 8h). Desmopressin for injection ($2\mu\text{g}$). Immediate access to serum electrolyte, plasma, and urinary osmolality assays. Access to a plasma arginine vasopressin (AVP) (ADH) assay desirable.
- **Procedure:** 7.30 a.m.
 1. Weigh the patient, and calculate 97% of the body weight.
 2. Mark this target on the chart.
 3. No food or fluid for the next 8h.
 4. Insert a cannula for repeated blood sampling, and flush.
- **Procedure:** 8 a.m.
 5. Obtain plasma for Na^+ and osmolality, and urine for osmolality.
 6. Then collect urine hourly for volume and osmolality, and plasma every 2h for Na^+ and osmolarity.
 7. Weigh the patient before and after passing urine if unobserved.
 8. If patient loses 3% of the body weight, order urgent plasma osmolality and Na^+ .
 9. If plasma osmolality $>300\text{mOsmol}$ ($\text{Na}^+ >140\text{mmol/L}$), stop the test; allow the patient to drink, and give desmopressin (see point 14).
 10. If plasma osmolality $<300\text{mOsmol}$, the patient may have been fluid-overloaded before the test, and water deprivation can continue.
 11. Stop the test at 8h (4.00 p.m.), and take final recordings of urine and plasma.
 12. Save an aliquot of plasma for vasopressin levels in case of difficulties in test interpretation.
 13. Ideally urine osmolalities will have reached a plateau ($<30\text{mOsmol rise between samples}$).
 14. Now give $2\mu\text{g}$ of desmopressin intramuscularly (IM) (or $20\mu\text{g}$ intranasally), and collect urine samples only for a further 2h. Allow free fluids at this stage.

- *Interpretation:* normal response: plasma osmolality remains in the range of 280–295mmol; urine osmolality rises to >2 times plasma (>600mOsmol). If urine volumes during water deprivation do not reduce and yet the plasma does not become more concentrated (rising osmolality) and weight does not fall, suspect surreptitious drinking during the test. For interpretation of abnormal results,  Table 2.2, p. 136

Diagnostic trial of desamino D-arginyl vasopressin

- *Indication:* distinction of partial DI from 1° polydipsia.
- *Contraindications:* cardiac failure; current diuretic use (test uninterpretable). Note that this test may precipitate severe hyponatraemia in 1° polydipsia and should be performed in an inpatient unit with clinical and biochemical regular review.
- *Preparation:* admission to an assessment unit. First-line tests for polydipsia/polyuria should have been performed (Polydipsia and polyuria: diabetes insipidus, pp. 134–6).
- *Procedure:*
 1. 24h urine volume, morning urine osmolality, weight, fluid intake (as far as possible), serum osmolality, Na^+ , urea, and creatinine should all be performed daily and the results reviewed the same day.
 2. Subjects should have access to fluid *ad libitum* but should be reminded that they should only drink if they are thirsty.
 3. After an initial 24h period of observation, desmopressin is administered at a dose of 2 μg bd SC for 3 days.
 4. Stop the test if serum Na^+ falls to <130mmol/L.
- *Interpretation:* reduction in urine volume to <2L/day and in urine osmolality to >600mOsmol/L without a fall in serum Na^+ to <140mmol/L suggests central DI. Reduction in urine volume with no ↑ in urine osmolality >600mOsmol/L and without a fall in serum Na^+ suggests partial nephrogenic DI. Limited reduction in urine volume, with some ↑ in urine osmolarity, but a fall in serum Na^+ , suggests 1° polydipsia.

Low-dose dexamethasone suppression test

- *Indication:* to distinguish hypercortisolism from normality. The dexamethasone-suppressed CRH test is believed to have less false +ves in cases of alcoholic or depressive pseudo-Cushing's syndrome.
- *Patient preparation:* patients should not be on oral steroids or drugs that ↑ steroid metabolism.
- *Overnight dexamethasone suppression test:* 1mg of dexamethasone is taken by mouth (PO) at midnight. A serum sample for cortisol is taken the following morning between 8 a.m. and 9 a.m.
 - *Interpretation:* serum cortisol should suppress to <140nmol/L (usually <50nmol/L). Values of 140–175nmol/L are equivocal and suggest a 2-day test should be performed. There is 10–15% false +ve rate.
- *Two-day low-dose dexamethasone suppression test (preferred):* dexamethasone 0.5mg is given PO every 6h for eight doses (2 days), starting in the early morning. Ideally tablets are taken strictly at 6-hourly intervals (6 a.m., 12 noon, 6 p.m., 12 midnight), which may necessitate an inpatient stay. A 24h collection for UFC is taken on the second day of the test, and serum cortisol is measured at 6 a.m. on the third day, 6h after the last dose. IV administration of dexamethasone can be used if there are concerns over absorption or compliance.
 - *Interpretation:* serum cortisol 6h after the last dose should be <140nmol/L, usually <50nmol/L. UFC on the second day should be <70nmol/L, normally <30nmol/L. The 2-day test strictly performed has less false +ves than the overnight test.
- *Dexamethasone-suppressed CRH test:* dexamethasone 0.5mg is given PO every 6h for nine doses (2 days), but starting at midnight and ending at 6 a.m. Tablets are taken strictly at 6h intervals (12 midnight, 6 a.m., 12 noon, 6 p.m.), which may necessitate an inpatient stay. The last dose is taken at 6 a.m., and an injection of CRH (100 μ g IV or 1 μ g/kg) is given at 8 a.m. A blood sample for cortisol is taken at 8.15 a.m. (i.e. 15min later).
 - *Interpretation:* serum cortisol level should be <38nmol/L (normal).

Further reading

Yanovski JA, Cutler GB Jr, Chrousos GP, Nieman LK. Corticotrophin-releasing hormone stimulation following low-dose dexamethasone administration: a new test to distinguish Cushing's syndrome from pseudo-Cushing's states. *JAMA* 1993; **269**: 2232–8.

High-dose dexamethasone suppression test

- *Indication:* to distinguish between patients with Cushing's disease (ACTH-secreting pituitary tumour) and ectopic ACTH production in patients with established hypercortisolism.
- *Patient preparation:* as low-dose test, except that the test can be performed immediately following the 2-day low-dose test.
- *Procedure:*
 1. $2 \times 24\text{h}$ UFC collections are made to calculate the mean basal 24h UFC.
 2. Baseline serum cortisol measurement is also taken before the first dexamethasone dose, ideally at 6 a.m. If the low-dose test is performed first, the baseline values (urine and blood) must be taken prior to the low-dose test (i.e. any doses of dexamethasone).
 3. Dexamethasone 2mg is given PO every 6h for eight doses (2 days), starting in the early morning. Ideally tablets are taken strictly at 6h intervals (6 a.m., 12 noon, 6 p.m., 12 midnight), which may necessitate an inpatient stay.
 4. A 24h urine collection for UFC (final) is taken on day 2, and a blood sample is taken for (final) cortisol 6h after the last dexamethasone dose (6 a.m. on day 3). Creatinine excretion should be measured and compared between urine samples to confirm true 24h collections.
- *Interpretation:* the percentage suppression of basal cortisol is calculated as follows:

$$\text{(Basal cortisol} - \text{final cortisol})/\text{basal cortisol} \times 100$$

The same calculation is made for basal and day 2 UFC. Fifty per cent suppression is suggestive of pituitary-dependent disease; 90% suppression ↑ the likelihood (strict criteria). Thymic carcinoids and phaeochromocytomas releasing ACTH are sources of false +ves.

Short Synacthen® test

- *Indication:* suspected adrenal insufficiency. Will not detect recent-onset 2° adrenal insufficiency.
- *Contraindication:* asthma/allergy to ACTH—risk of allergic reaction (can be performed with careful medication supervision of the patient).
- *Preparation:* the patient must not take hydrocortisone on the morning of the test, as this will be detected in the cortisol assay. The test can be performed on low-dose dexamethasone, but the morning dose should be omitted until after the test. May have some value in patients on higher-dose steroid therapy to indicate the degree of suppression of adrenocortical function.
- *Procedure:* 250µg of synthetic ACTH (Synacthen®) given IM or IV. Blood taken at times 0, 30, and 60min for serum cortisol.
- *Low-dose test:* the test can be performed with a very low dose of ACTH (e.g. 1µg). This may detect more subtle degrees of hypoadrenalism, but the clinical significance of these findings remains uncertain.
- *Interpretation:* a value at any time of >550nmol/L makes the diagnosis very unlikely. (Note: may be lower, depending on local assay range—check with the laboratory).

Long (depot) ACTH test

- *Indication:* distinguishing 1° and 2° adrenal failure.
- *Patient preparation:* a short Synacthen® test should be performed prior to the test to diagnose adrenal failure. If the patient is on steroid replacement, change to dexamethasone 0.5mg/day.
- *Procedure:* blood is taken at 9 a.m. for basal cortisol; 1mg of depot synthetic ACTH (Synacthen®) is then given IM on two consecutive days, and blood collected 5h after each dose (2 p.m.). A final cortisol sample is taken at 9 a.m. on the third day.
- *Interpretation:* serum cortisol should rise to >1000nmol/L on the last day and, if adrenal failure previously indicated by a short Synacthen® test, such a rise indicates 2° adrenal failure (pituitary/hypothalamic cause, including suppressive drugs).

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Full blood count

Called *complete blood count* (CBC) in the United States (USA).

Before the advent of modern haematology blood analysers, the blood count consisted of a haemoglobin (Hb) concentration (estimated using a manual colorimetric technique), a white cell count, and a manual platelet count. Other parameters, such as mean cell volume (MCV), had to be mathematically calculated (*derived*) using the measured variables Hb, red cell count (RCC), and packed cell volume (PCV).

Modern analysers use a variety of methods to provide a huge range of full blood count (FBC) variables, including electronic impedance, laser light scatter, light absorbance, and staining characteristics. The resultant FBC provides measured variables such as Hb, PCV, and RCC, along with derived (mathematically) MCV, mean cell Hb (MCH), and mean corpuscular Hb concentration (MCHC). These machines also provide automated platelet counts and a 5-part differential white blood count (WBC).

Sample: peripheral blood ethylenediamine tetra-acetic acid (EDTA); the sample should be analysed in the laboratory within 4h, if possible.

Main parameters measured

- Hb concentration.
- RCC.
- MCV.
- MCH.
- MCHC.
- Haematocrit (Hct) or PCV.
- Red cell distribution width (RDW).
- White cell count.
- WBC differential.
- Platelet count.

Some machines are even more sophisticated and will measure *reticulocyte counts*, in addition to determination of reticulocyte Hb and MCV.

Role of full blood count

Why ask for an FBC? How will this aid the diagnosis or management of the patient? The FBC assesses several different parameters and can provide a great deal of information. The red cell variables will determine whether or not the patient is anaemic. If anaemia is present, the MCV is likely to provide clues as to the cause of the anaemia. The white cells are often raised in infection—neutrophilia in bacterial infections and lymphocytosis in viral (but not always so). Platelets (size or number) may be abnormal either as a direct effect of an underlying blood disease or simply reflecting the presence of some other underlying pathology. Most of us take a somewhat cursory glance at the FBC when the report arrives on the ward or in clinic, but a more detailed look may reveal a great deal more!

Further reading

↗ http://www.bbc.co.uk/health/talking/tests/blood_full_blood_count.shtml.

↗ http://www.rcpa.edu.au/pathman/full_blo.htm.

Red cell parameters

Haemoglobin concentration

Units: g/dL or g/L (Europe uses SI units; the USA uses g/dL or grams %).

Defines anaemia (Hb < lower limit of normal, adjusted for age and sex). Values differ between ♂ and ♀, since androgens drive RBC production and hence an adult ♂ has higher Hb, PCV, and RCC than an adult ♀.

Red cell count

Unit: $\times 10^{12}/\text{L}$.

Useful in the diagnosis of polycythaemic disorders (\uparrow production of microcytic, hypochromic erythrocytes) and thalassaemias.

Causes of low red cell count

- Hypoproliferative anaemias, e.g. iron, vitamin B₁₂, and folate deficiencies.
- Aplasias, e.g. idiopathic or drug-induced (do not forget chemotherapy).
- Parvovirus B₁₉ infection-induced red cell aplasia resulting in transient marked anaemia.

Causes of high red cell count

- PRV.
- Thalassaemia.

Mean cell volume

Unit: femtolitre (fL), 10^{-15}L .

Provided as part of the derived variables or, if you know the PCV and RCC, can be calculated as (PCV/RCC), e.g. if the PCV is 0.45 and RCC $5 \times 10^{12}/\text{L}$, then the MCV is 90fL.

This index provides a useful starting point for the evaluation of anaemia (see Table 3.1).

Table 3.1 MCV index

MCV ↓	MCV normal	MCV ↑
Iron deficiency	Blood loss	B ₁₂ or folate deficiency
Thalassaemia homo-/heterozygotes	Myelodysplasia	Myelodysplasia
Sideroblastic anaemia	Anaemia of chronic disease	

Mean cell haemoglobin

Unit: pg.

- High (for range, Normal ranges, inside front cover): macrocytosis.
- Low: microcytosis, e.g. iron deficiency anaemia.

Mean cell haemoglobin concentration

Unit: g/dL or g/L.

- Of value in evaluation of microcytic anaemias.
- High: severe prolonged dehydration, hereditary spherocytosis, cold agglutinin disease.
- Low: iron deficiency anaemia, thalassaemia.

Haematocrit or packed cell volume

These are not entirely synonymous terms (but they are more or less). If blood is placed in a microcapillary tube and centrifuged, the red cells are spun down to the bottom, leaving the plasma above. The RBCs will occupy about 40% of the blood in the tube—the blood will have a PCV of 0.4 (or 40%). The Hct is similar, but derived, using automated blood counters.

PCV unit: L/L (although the units are seldom cited in reports).

- High PCV: polycythaemia (any cause).
- Low PCV: anaemia (any cause).

Red cell distribution width

Measures the range of red cell size in a sample of blood, providing information about the degree of red cell anisocytosis, i.e. how much variation there is between the sizes of the red cells. Of value in some anaemias, e.g.:

- ↓ MCV with normal RDW suggests β-thalassaemia trait.
- ↓ MCV with high RDW suggests iron deficiency.

(Probably noticed more by haematology staff than those in general medicine!)

Hypochromic red cells (% HRCs)

Defined as red cells with Hb <280g/L. This is useful in identifying patients with functional iron deficiency and monitoring response to erythropoietin-stimulating agent (ESA) Epo therapy and the requirement for IV iron.

Reticulocyte mean cell haemoglobin (CHr), reticulocyte haemoglobin content (Ret-He)

Predict iron deficiency anaemia and functional iron deficiency, and predict response to IV iron in those on haemodialysis.

Assessment of iron status, pp. 244–7.

White cells

The automated differential white cell count is provided as part of the FBC. The RBCs in the sample are lysed before WBCs are counted. A typical FBC will show the total WBC and the *5-part differential white cell count*, broken down into the five main white cell subtypes in peripheral blood which include:

- Neutrophils.
- Lymphocytes.
- Monocytes.
- Eosinophils.
- Basophils.

The printed FBC usually shows the % of each type of white cell, but unless the absolute WBC (as $\times 10^9/\text{L}$) is known, this % count is of little value.

► As a general rule, ignore the % count—you cannot detect abnormalities, such as neutropenia, unless you have the absolute values.

Abnormalities of the WBC, e.g. neutrophilia, neutropenia, etc., are discussed in  OHCM 10e, p. 330.

Platelet count

- Unit: $\times 10^9/L$.
- Normal: $150\text{--}400 \times 10^9/L$.

Platelets (*thrombocytes* in the USA) are the smallest cells in peripheral blood. Traditional counting methods with a microscope and counting chamber have now been replaced by automated counting with haematology analysers.

Platelet distribution width

This is analogous to the RDW and provides information about the range of platelet sizes in a blood sample.

- The platelet distribution width (PDW) will be high if there are giant platelets in the presence of normal-sized platelets, e.g. essential thrombocythaemia (one of the myeloproliferative disorders).
- The PDW will be normal in a reactive thrombocytosis (where the platelet count is ↑, but they are all of normal size).

Platelet clumping

This is an *in vitro* artefact in some individuals. Platelets clump in EDTA and the blood analyser will report spurious thrombocytopenia. The actual *in vivo* count is normal and the platelets function normally. Taking blood into citrate or heparin will show the patient's platelet count to be normal. The presence of even a small blood clot in an EDTA sample may also reduce the platelet count (the haematology technical staff will usually check to see whether the sample contains a small clot before sending out the report).

Peripheral blood film

Examining a stained peripheral blood smear under the microscope allows the examination of red cells, white cells, and platelets (see Fig. 3.1). In addition, the blood film will help detect parasites (e.g. malaria, trypanosomes) or abnormal cells in the blood.

When to request a blood film examination

The haematology laboratory will usually examine a peripheral blood film if the patient's indices are abnormal (unless there has been no major change from previous FBCs). If you suspect an underlying blood disorder, you should request a film. Note: the laboratory staff may not make a film if the indices are completely normal.

Method

A fingerprick blood sample may be spread onto a glass slide (the phlebotomist may do this for you), air-dried, fixed, and stained. Alternatively, a drop of EDTA blood may be treated in the same manner (the haematology laboratory staff will make the film). Beware: old EDTA samples produce strange artefacts such as extreme red cell crenation—if a film is required, it should be made from a fresh blood sample.

- Sample: EDTA (as fresh as possible).

Information from blood film

Red cells

- Size.
- Shape (e.g. sickling).
- Membrane changes (e.g. oxidative membrane damage).
- Colour.
- Basophilic stippling.
- Inclusions, e.g. Howell–Jolly bodies, malarial parasites, haemoglobin C (HbC) crystals, etc.

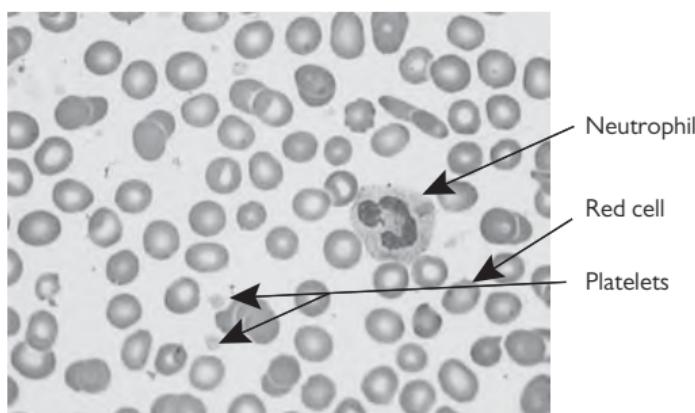


Fig. 3.1 Normal peripheral blood film showing a neutrophil with its typical lobulated nucleus, numerous red cells, and a few platelets.

White cells

- Number.
- Morphology.
- Abnormalities such as toxic granulation, dysplastic changes.
- Presence of abnormal cells, e.g. leukaemic blasts or lymphoma cells.

Platelets

- Number.
- Size.
- Shape.

Other features on the film

- Parasites.
- Red cell rouleaux (stacking effect—seen, e.g. when ESR is ↑).
- Nucleated red cells.
- Plasma cells.
- Occasionally see circulating carcinoma cells.

⇒ OHCM 10e, p. 328.

Red cell morphology

In health, the normal RBC is a pink, biconcave disc-shaped cell, and most red cells are roughly the same size, shape, and colour. They should be roughly the size of a small lymphocyte nucleus. Many diseases and deficiency disorders alter the RBC appearance by either reducing its Hb content or altering the membrane such that characteristic morphological abnormalities are produced. Examples include target cells, sickle cells, bite cells, burr cells, and many others (see Table 3.2). Most of the morphological features are not absolutely specific for one particular disorder, but rather they suggest a range of conditions that may be associated with the RBC feature (see Fig. 3.2). This should prompt you to look for conditions which might account for the abnormality.

Table 3.2 Peripheral blood film in anaemias

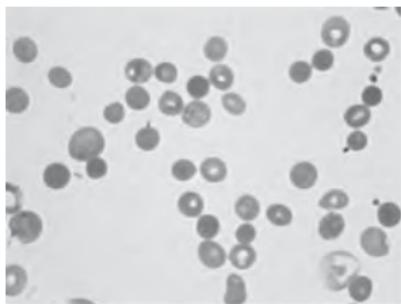
Microcytic RBCs	Fe deficiency, thalassaemia trait and syndromes, congenital sideroblastic anaemia, ACD (if long-standing)
Macrocytic RBCs	Alcohol/liver disease (round macrocytes), MDS, pregnancy and newborn, compensated haemolysis, B ₁₂ or folate deficiency, hydroxyurea and antimetabolites (oval macrocytes), acquired sideroblastic anaemia, hypothyroidism, chronic respiratory failure, aplastic anaemia
Dimorphic RBCs	Two populations, e.g. Fe deficiency responding to Fe, mixed Fe and B ₁₂ /folate deficiencies, sideroblastic anaemia, post-red cell transfusion
Hypochromic RBCs	Reduced Hb synthesis, e.g. Fe deficiency, thalassaemia, sideroblastic anaemia
Polychromatic RBCs	Blood loss or haematinic treatment, haemolysis, marrow infiltration
Spherocytes	Hereditary spherocytosis, haemolysis, e.g. warm AIHA, delayed transfusion reaction, ABO, HDN, DIC, and MAHA, post-splenectomy
Pencil/rod cells	Fe deficiency anaemia, thalassaemia trait and syndromes, PK deficiency
Elliptocytes	Hereditary elliptocytosis, MPD, and MDS
Fragmented RBCs	MAHA, DIC, renal failure, HUS, TTP
Teardrop RBCs	Myelofibrosis, metastatic marrow infiltration, MDS
Sickle cells	Sickle-cell anaemia, other sickle syndromes (not sickle trait)
Target cells	Liver disease, Fe deficiency, thalassaemia, HbC syndromes
Crenated RBCs	Usually storage or EDTA artefact. Genuine RBC crenation may be seen post-splenectomy and in renal failure (→ burr cells)

Table 3.2 (Contd.)

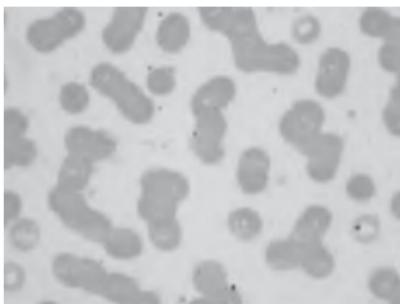
Burr cells	Renal failure
Acanthocytes	Hereditary acanthocytosis, α-β-lipoproteinaemia, McLeod red cell phenotype, PK deficiency, chronic liver disease (especially Zieve's syndrome)
Bite cells	G6PD deficiency, oxidative haemolysis
Basophilic stippling	Megaloblastic anaemia, lead poisoning, MDS, liver disease, haemoglobinopathies, e.g. thalassaemia
Rouleaux	Chronic inflammation, paraproteinaemia, myeloma
↑ reticulocytes	Bleeding, haemolysis, marrow infiltration, severe hypoxia, response to haematinic therapy
Heinz bodies	Not seen in normals (removed by spleen), small numbers seen post-splenectomy, oxidant drugs, G6PD deficiency, sulfonamides, unstable Hb (Hb Zurich, Köln)
Howell-Jolly bodies	Composed of DNA, removed by the spleen, seen in dyserythropoietic states, e.g. B ₁₂ deficiency, MDS, post-splenectomy, hyposplenism
H bodies	HbH inclusions, denatured HbH (β_4 tetramer), stain with methylthioninium chloride (methylene blue), seen in HbH disease (—/—α), less prominent in α-thalassaemia trait, not present in normal subjects
Hyposplenic film	Howell-Jolly bodies, target cells, occasional nucleated RBCs, lymphocytosis, macrocytosis, acanthocytes. Infectious mononucleosis, any viral infection, toxoplasmosis, drug reactions

ABO, ABO blood groups; ACD, anaemia of chronic disease; AIHA, autoimmune haemolytic anaemia; DIC, disseminated intravascular coagulation; Fe, iron; G6PD, glucose-6-phosphate dehydrogenase; HDN, haemolytic disease of the newborn; HUS, haemolytic uraemic syndrome; MAHA, microangiopathic haemolytic anaemia; MPD, myeloproliferative disease; PK, pyruvate kinase; TTP, thrombotic thrombocytopenic purpura.

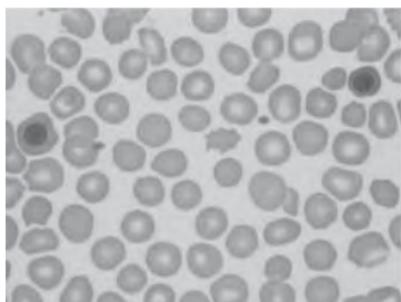
► Pay attention to the peripheral blood film comment (inserted on the report by the haematology laboratory staff or automated blood counter)—it should help you decide which tests to carry out next. Conversely, cryptic laboratory comments like 'anisopoikilocytosis noted' do not help the clinician much. (Note: *aniso* = unequal; *poikilo* = varied.)



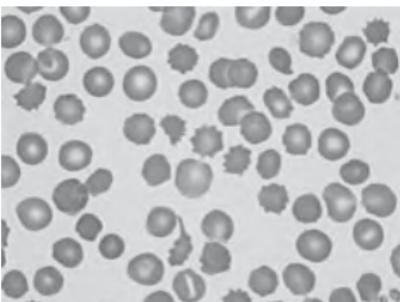
Stippled red cells in haemolysis.



Marked rouleaux in myeloma.



Single nucleated red cell (on left).



Crenated red cells.

Fig. 3.2 Red cell abnormalities in various disease states.

Parasites on blood film and marrow

Blood film

Although there are now highly sensitive monoclonal antibody (MoAb) kits for the diagnosis of diseases such as malaria, a well-stained blood film can often make the diagnosis more easily and more cheaply. Blood films are useful for confirming a diagnosis of:

- Malaria.
- Trypanosomiasis.
- Microfilaria.

Parasites in bone marrow

Some diseases, such as leishmaniasis, require bone marrow aspiration and staining (in fact, there are many infections that can be diagnosed using bone marrow):

- *Leishmania donovani*.
- TB.
- *Tropheryma whippleii* (Whipple's disease).
- *Cryptococcus neoformans*.
- *Penicillium*.
- *Histoplasma capsulatum*.
- *Candida albicans*.
- *Toxoplasma gondii*.

See Fig. 3.3 for examples.

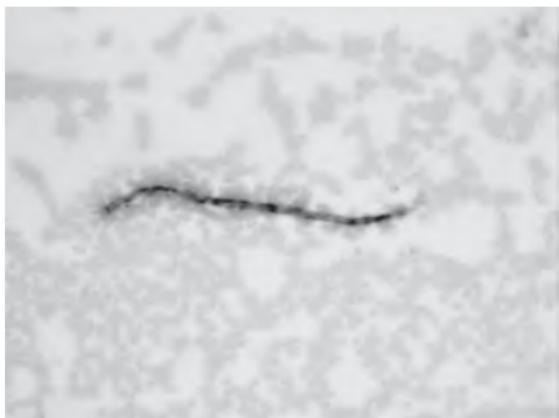
Further reading

MedlinePlus (2016). CBC blood test.  <http://www.nlm.nih.gov/medlineplus/ency/article/003642.htm>.

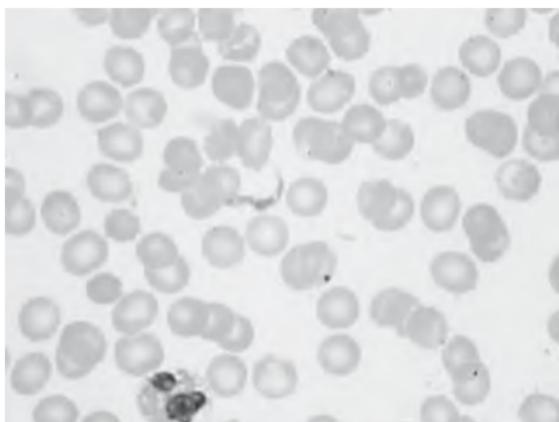
Thomas DW, Hinchliffe RF, Briggs C, Macdougall IC, Littlewood T, Cavill I; British Committee for Standards in Haematology. Guideline for the laboratory diagnosis of functional iron deficiency. *Br J Haematol* 2013; **161**: 639–48.



Falciparum malaria (blood film)



Loa loa (bone marrow)



Trypanosome (blood film)

Fig. 3.3 Parasites, such as malaria, loa loa, and trypanosomes, may be seen on a stained blood film.

White blood cell morphology

In much the same way as RBC morphology provides clues about underlying disease, so too does microscopical examination of stained peripheral blood WBCs. Modern counters enumerate WBCs, and our greater reliance on modern technology means that visual inspection of blood films is becoming a dying art. A well-stained blood film may provide the diagnosis much more cheaply (see Fig. 3.4).

Blood film when WBC is decreased

- Sometimes difficult to determine the diagnosis since so few WBCs.
- May suggest B_{12} or folate deficiency (are the RBCs normal or large?).
- *Aplastic anaemia*: are the platelets and Hb normal?
- *Underlying leukaemia*: are there any leukaemic blasts* present?
- *Overwhelming infection*: may see toxic granulation (large, dark granules in the cytoplasm—not diagnostic, but suggestive).
- *May be immune or post-viral*: atypical lymphocytes may be seen; other indices usually normal.

Note: a blast (*) is a primitive cell seen in the marrow in large numbers in leukaemia. We all have some blasts in our marrows, but these should be <5% of the total nucleated bone marrow cells in health.

Blood film when WBC is increased

What cell predominates?

- *Lymphocytes*: suggests viral, CLL, acute leukaemia (lymphoblastic).
- *Granulocytic?* (neutrophils, eosinophils, basophils)—may be reactive or CML.
- *Abnormal-looking WBC?* Look for Auer rods (\equiv AML), smear cells (CLL), bilobed neutrophils (pseudo-Pelger cells seen in MDS).

(See Table 3.3.)

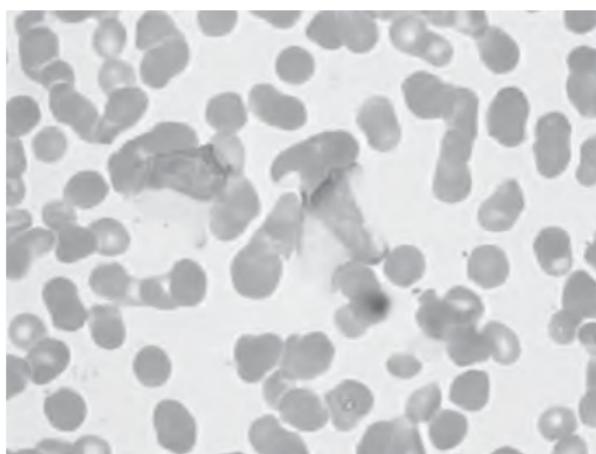


Fig. 3.4 Blood film: atypical WBCs (this was from a patient with glandular fever, but these cells may be seen in any viral illness).

Table 3.3 Some WBC abnormalities seen on FBC reports

Atypical lymphocytes	Infectious mononucleosis, any viral infection, toxoplasmosis, drug reactions
Auer rods	Seen in myeloblasts; pathognomonic of AML Prominent in AML M3 subtype (acute promyelocytic leukaemia)
Pelger–Huët anomaly	Bilobed neutrophils. May be hereditary (neutrophils are functionally normal) or acquired, e.g. MDS (pseudo-Pelger cells)
Left-shifted	Immature WBCs seen in peripheral blood. Seen in severe infections, inflammatory disorders, DKA, marrow 'stress', MPD, CML
Right-shifted	Hypermature WBCs seen in, e.g. megaloblastic anaemia and iron deficiency
Toxic granulation	Coarse granules in neutrophils. Seen in severe infection, post-operatively, and inflammatory disorders
Smear cells	Lymphocytes in which the cell membrane has ruptured when making the blood film—there are no smear cells <i>in vivo</i> ! Seen in CLL

Diagnosis must be made in context

How old is the patient?

- Viral illnesses often produce bizarre films in children, but beware of complacency (acute leukaemia may be the cause).
- MDS and malignancies like CLL and CML are diseases of older individuals.

Is the patient well?

- May be worth repeating the FBC and film to see if abnormalities have resolved.
- If the patient is unwell or has lymphadenopathy or hepatosplenomegaly, then an underlying disease must be excluded.

Assessment of iron status

Anaemia of iron deficiency is caused by defective synthesis of Hb, resulting in red cells that are smaller than normal (microcytic) and contain reduced amounts of Hb (*hypochromic*). The diagnosis of iron deficiency anaemia is generally straightforward, but it may be confused with anaemia of chronic disease (ACD) or other hypochromic anaemias (see Table 3.4 and Fig. 3.5).

Iron plays a pivotal role in many metabolic processes, and the average adult contains between 3 and 5g of iron, of which two-thirds are present in the O₂-carrying molecule Hb. Somewhat surprisingly, there is no specific excretion mechanism in humans. Iron balance is controlled at the level of gut absorption and relies on two iron-sequesering proteins *transferrin* (iron transport and recycling of iron) and *ferritin* (safeguards iron entry into the body and maintains surplus iron in a safe and readily accessible form).

Ferritin

This is the 1° iron storage protein, consisting of 24 apoferritin subunits forming a hollow sphere (each can hold up to 4500 iron atoms).

Haemosiderin

Haemosiderin, located predominantly in macrophages, is a water-soluble protein–iron complex with an amorphous structure.

Transferrin and its receptor

Transferrin contains only 4mg of iron and is the principal iron transport protein with >30mg of iron transported round the body daily. Synthesis of transferrin is inversely proportional to the body iron stores, with ↑ transferrin concentration when iron stores are reduced.

The transferrin receptor (TfR) is a disulfide-linked dimer, composed of two identical 85kDa subunits. The serum TfR (sTfR) concentration is elevated in iron deficiency. However, sTfR may also ↑ in any condition in which there is ↑ erythropoiesis, e.g. haemolytic anaemias, thalassaemia, polycythaemia vera, and other myeloproliferative disorders.

Assessment of iron status

Several parameters are available

- Hb concentration.
- Serum ferritin.
- Serum iron and transferrin (as TIBC).
- % hypochromic cells in peripheral blood.
- Reticulocyte MCH (CHe) and reticulocyte Hb content (Ret-He).
- Red cell protoporphyrin assay (not widely available).
- Bone marrow aspirate (stained for iron)—the ‘gold standard’.
- Soluble TfR assay (STfR).

Remember, iron deficiency is not an ‘all-or-nothing’ phenomenon. In progressive deficiency, there is a gradual loss of iron with subtle alterations of iron-related parameters, during which the red cells may look entirely normal. In the initial stages of developing iron deficiency, macrophages become depleted of iron and the serum ferritin ↓ to the lower end of the normal

range; during this 'latency' period, the Hb is normal. As the deficiency progresses, plasma iron levels ↓ and TIBC ↑. Free RBC protoporphyrin levels ↑ as it accumulates, and eventually hypochromic RBCs appear in the peripheral blood. At this stage, an FBC will usually show ↓ Hb, MCV, MCH, and MCHC, and the peripheral blood film will show microcytic hypochromic red cells.

Table 3.4 Hypochromic anaemias—may be confused with iron deficiency

Disorder	Example
Disorders of iron metabolism	Iron deficiency anaemia: <ul style="list-style-type: none"> • Blood loss • Reduced iron intake • Impaired iron transport
Anaemia of chronic disorders	Chronic inflammatory diseases Malignant disease
Disorders of haem synthesis	Sideroblastic anaemias: <ul style="list-style-type: none"> • Hereditary • Idiopathic • 2°: <ul style="list-style-type: none"> • Drugs • Alcohol • Lead poisoning
Globin synthesis disorders	Thalassaemias: <ul style="list-style-type: none"> • β-thalassaemia • α-thalassaemia

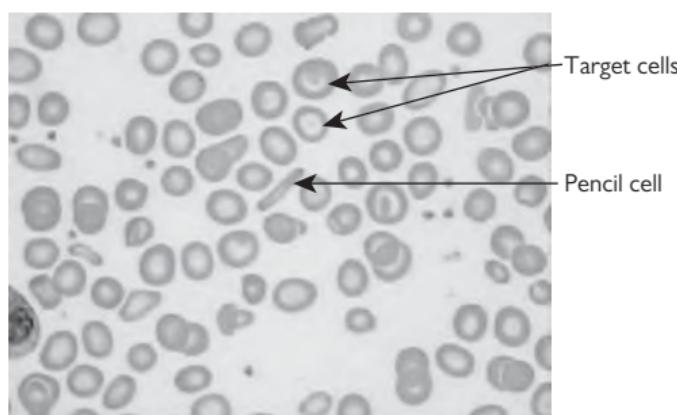


Fig. 3.5 Blood film of iron deficiency anaemia. Note the variation in cell size and shape.

Confirmation of simple iron deficiency anaemia

(See Fig. 3.6.)

- Hb ↓.
- Serum ferritin will be ↓.
- Serum iron ↓ and TIBC ↑ (generally unhelpful and little used today).
- STfR ↑.
- MCV ↓.
- MCH and MCHC ↓.
- Microcytic and hypochromic RBCs on blood film.
- Absent marrow iron.

► Beware: serum ferritin is an acute phase protein and may be normal or even ↑ in inflammatory, malignant, or liver disease. During the inflammatory response, the iron/TIBC are unlikely to be of any value (iron ↓ and TIBC will be ↓). If an inflammatory process is suspected, an alternative test is required, e.g. STfR, which is not affected by inflammatory disorders.

Functional iron deficiency

This is the situation where iron is retained in body stores and, although stores are adequate, delivery to the bone marrow is inadequate for erythropoiesis. It occurs in inflammatory disease, being one component of ACD. Functional iron deficiency also occurs during the use of Epo where response is improved with the use of IV iron.

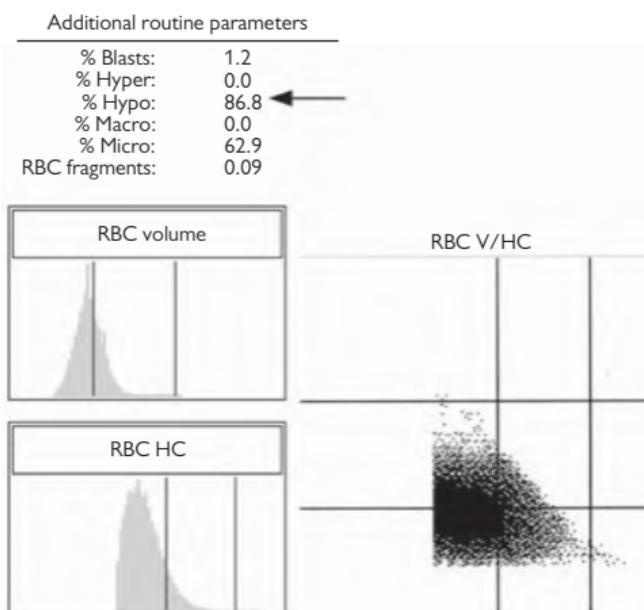


Fig. 3.6 The % hypochromic red cells (provided by some automated counters) helps in the diagnosis of iron deficiency. Note that the RBC volume and Hb content (HC) are both shifted to the LEFT (= small, pale red cells).

Iron overload

Can be iatrogenic or genetic (haemochromatosis).

Ferritin and transferrin saturation are elevated. Investigations to assess the degree of cardiac and hepatic iron accumulation are required.

⇒ *OHCM* 10e, p. 326.

Further reading

Iron Disorders Institute. *Iron deficiency anemia*. <http://www.irondisorders.org/iron-deficiency-anemia>.

Iron Panel. <http://www.ironpanel.org.au/AIS/AISdocs/adultdocs/Acontents.html>.

Provan D, Weatherall D. Acquired anaemias and polycythaemia. *Lancet* 2000; **355**: 1260–8.

Assessment of vitamin B₁₂ and folate status

Measurement of serum B₁₂ and red cell folate levels is necessary in the investigation of macrocytic anaemia and certain other situations (see below). Serum folate levels are an unreliable measurement of body stores of folate—the red cell folate level is probably more meaningful.

- B₁₂ unit: ng/L.
- Serum and red cell folate units: µg/L.
- Sample: clotted blood sample (serum B₁₂ and folate) and peripheral blood EDTA (red cell folate).

Deficiency of either vitamin leads to megaloblastic anaemia where there is disruption of cell division in all actively dividing cells (includes the bone marrow and gut). In the marrow, there is nuclear:cytoplasmic asynchrony where the nuclei are immature despite a mature, well-haemoglobinized cytoplasm. In the peripheral blood, there may be anaemia, often with pancytopenia; the red cells show oval macrocytic changes with basophilic stippling and occasionally nucleated red cells. Neutrophils typically become hypersegmented (they have >5 lobes).

Until recently, B₁₂ and folate assays were tedious microbiological assays, but these have now been replaced by automated techniques using radio-isotopic methods, which allow large numbers of samples to be batched and tested fairly cheaply.

Deficiencies of B₁₂ or folate do not always cause macrocytic anaemia

In the past, deficiency of either B₁₂ or folate was synonymous with macrocytic anaemia, but deficiency of either vitamin may present without anaemia or macrocytosis—remember, these are late features of the disease. However, in most cases of deficiency, the marrow will show characteristic megaloblastic change (nuclear asynchrony with giant metamyelocytes). ► Deficiency of B₁₂ may cause neurological problems in the absence of anaemia.

Whom should you test?

- Patients with gastrointestinal tract (GIT) disease, glossitis, abnormalities of taste, previous surgery, or radiotherapy to the stomach or small bowel.
- Neurological disease, e.g. peripheral neuropathy, demyelination.
- Psychiatric disturbance, e.g. confusion, dementia.
- Malnutrition, e.g. growth impairment in children; vegans.
- Alcohol abuse.
- Autoimmune disease of the thyroid, parathyroid, or adrenals.
- Patients with a family history of pernicious anaemia.
- Others, e.g. drugs that interfere with vitamin absorption or metabolism such as nitrous oxide, phenytoin, etc.

Look for blood film abnormalities

- B₁₂ and folate deficiencies produce similar clinical and laboratory features:
- Oval macrocytes.
- Hypersegmented neutrophils (also seen in renal failure, iron deficiency, and MDS).

(See Fig. 3.7.)

Which test next?

Make sure you have the following

- FBC.
- Blood film.
- Serum B₁₂ level.
- Serum and red cell folate levels.
- Intrinsic factor antibodies (IFA), +ve in 50–75% of patients with PA.
- Consider the bone marrow (helps exclude MDS, myeloma, and other pathologies that give rise to macrocytic anaemia, but seldom performed today since it is easy to get B₁₂ and folate results back quickly).

Interpretation of results: vitamin B₁₂

Normal ranges are based on two standard deviations either side of the mean, so there will be 'normal' people who have 'abnormal' B₁₂ (or folate) levels.

- B₁₂ < normal:
 - Deficiency.
 - Altered metabolism.
 - 'Normal'.

The lowest levels are seen in those most deficient. What matters is whether there is tissue deficiency (leads to marrow and neurological changes).

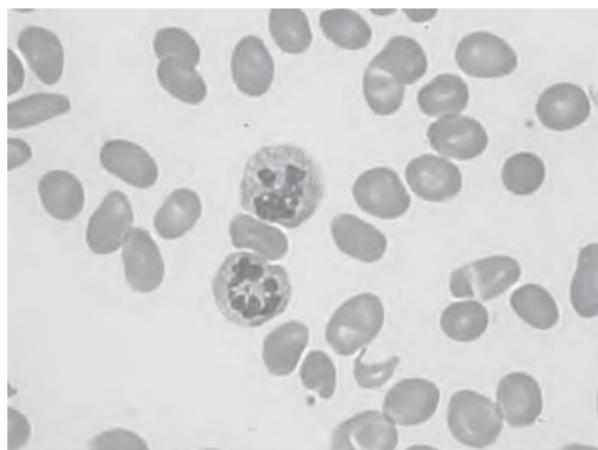


Fig. 3.7 Blood film of megaloblastic anaemia. There are large oval macrocytes and two hypersegmented neutrophils (the nucleus has >5 lobes).

Mild decrease in B₁₂ level?

Difficult, but common! Probably worth repeating the test and reviewing the patient and other results. If no evidence of tissue deficiency, can probably observe the patient. If there is evidence of tissue deficiency, then the patient will require treatment.

Detecting tissue deficiency

The most reliable method is probably the measurement of serum homocysteine (accumulates in vitamin B₁₂ and folate deficiency).

Beware B₁₂ not associated with tissue deficiency

- Folate deficiency.
- Pregnancy.
- Myeloma.
- Transcobalamin I deficiency (very rare).

Folate

↓ level seen in hospitalized patients due to -ve folate balance.

The B₁₂ level is low—what next?

Available tests for the cause of B₁₂ deficiency include

- Parietal cell (+ve in serum of 90% of patients with PA, but also found in other disorders and 15% of the normal elderly) and intrinsic factor antibodies (IFA now preferred—if +ve, confirms diagnosis of PA).
- Schilling test (urinary excretion method where the addition of intrinsic factor (IF) restores B₁₂ absorption in PA, but not in intestinal, e.g. ileal, disease), seldom performed now due to lack of required radioisotope, or
- Whole body B₁₂ counting.
- Endoscopy with duodenal biopsy.
- Other gastroenterology tests for malabsorption (Gastroenterology, p. 522).

The folate level is low—what next?

- Check dietary history.
- Endoscopy with duodenal biopsy.
- Other gastroenterology tests for malabsorption (Gastroenterology, p. 522).

(OHC M 10e, p. 266.

Further reading

Devalia V, Hamilton MS, Molloy AM; British Committee for Standards in Haematology. Guidelines for the diagnosis and treatment of cobalamin and folate disorders. *Br J Haematol* 2014; **166**: 496–513. Guidelines and Protocols Advisory Committee (2012). Cobalamin (vitamin B12) deficiency: investigation and management. <http://www2.gov.bc.ca/gov/content/health/practitioner-professional-resources/bc-guidelines/vitamin-b12>.

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Provan D, Weatherall D. Acquired anaemias and polycythaemia. *Lancet* 2000; **355**: 1260–8.

Erythrocyte sedimentation rate

This simple, but very useful, qualitative test measures how fast a patient's red cells fall through a column of blood. It is a sensitive, but non-specific, index of plasma protein changes that result from inflammation or tissue damage. The ESR is affected by Hct variations, red cell abnormalities (e.g. poikilocytosis, sickle cells), and delay in analysis, and it is therefore less reliable than measurement of plasma viscosity (PV). The ESR is affected by age, sex, menstrual cycle, pregnancy, and drugs (e.g. OCP, steroids) (see Table 3.5).

The ESR is widely used in clinical medicine and, despite attempts (by haematology departments) to replace the ESR with PV, the ESR has remained in use and appears to retain a valuable place in the armoury of disease diagnosis and monitoring.

- *Sample:* peripheral blood EDTA; the sample should be analysed in the laboratory within 4h.

Many factors influence the ESR, causing a high or low result:

- *High ESR (significant*—look for a cause):*
 - Any inflammatory disorder, e.g. infection, rheumatoid, TB.
 - MI (the ESR ↑ as an early response).
 - Anaemia.

Note: (*) depends on exactly how high. An ESR of 30 probably means little, but >100 is highly significant and indicates something seriously wrong.

- *Low ESR (rarely important, but useful for exams):*
 - Polycythaemia.
 - Hypofibrinogenaemia.
 - CCF.
 - Poikilocytosis.
 - Spherocytosis.
 - Sickled cells.

►► A normal ESR does not exclude organic disease.

⇒ OHCM 10e, p. 372.

Table 3.5 Normal range (upper limits)

Age	Men	Women
17–50 years	10mm/h	12mm/h
51–60	12	19
>60	14	20

Further reading

Brigden M. Clinical utility of the erythrocyte sedimentation rate. *Am Fam Physician* 1999; 60: 1443–50.

⇒ <http://www.aafp.org/afp/991001ap/1443.html>.

Harris GJ. Plasma viscometry and ESR in the elderly. *Med Lab Technol* 1972; 29: 405–10.

Lewis SM. Erythrocyte sedimentation rate and plasma viscosity. *Ass Clin Pathol Broadsheet* 1982; 94: 1–6.

Plasma viscosity

This test is a sensitive, but non-specific, index of plasma protein changes, which result from inflammation or tissue damage. Provides much the same information as the ESR. The ESR and PV tend to rise in parallel, but the PV is *unaffected* by Hct variations (e.g. severe anaemia or polycythaemia) and delay in analysis up to 24h, and it is therefore more reliable than the ESR. It is not affected by sex but is affected by age, exercise, and pregnancy. It is constant in health and shows no diurnal variation. There is a suggestion that the PV may be a more sensitive indicator of disease severity than the ESR.

- **Sample:** peripheral blood EDTA. The sample is centrifuged and the plasma removed.
- **Normal range:** 1.50–1.72cP (or mPA/s at 25°C).

High and low plasma viscosity

A high PV generally signifies some underlying pathology, e.g. inflammatory states, paraproteinaemias such as MGUS or myeloma; low PV can be ignored.

Note: despite the advantages outlined, the PV has not been adopted by all medical staff (who still prefer the ESR as a measure of inflammation). The PV is better for monitoring hyperviscosity syndromes, e.g. Waldenström's macroglobulinaemia. The fact that both tests are still used shows that there is a role for both.

➲ OHCM 10e, p. 373.

Further reading

Cooke BM, Stuart J. Automated measurement of plasma viscosity by capillary viscometer. *J Clin Pathol* 1988; 41: 1213–16.

Tests for glandular fever

This infection is caused by EBV. Infected cells produce so-called heterophile antibodies (these are IgM molecules that agglutinate horse and sheep RBCs but do not agglutinate ox RBCs and do not react at all with guinea pig RBCs).

There are various kits available that can detect the presence of heterophile antibodies and, in the right clinical context, will confirm a diagnosis of EBV infection. The Monospot test is probably the commonest in current use. The Paul–Bunnell test was the first to demonstrate the presence of heterophile antibodies in patients with EBV infection.

Clinical features

Glandular fever often affects young adults (12–25 years) and results in malaise, fever, tonsillitis, petechial haemorrhages on the palate, and lymphadenopathy. Splenomegaly is fairly common. A similar clinical picture is seen in CMV, *Toxoplasma*, and early HIV infections.

- Sample: EDTA.

Positive Monospot

- EBV infection.

False positives

- Toxoplasmosis.
- CMV infection.
- Rheumatoid.
- Malaria.

Further reading

Hoff G, Bauer S. A new rapid slide test for infectious mononucleosis. *JAMA* 1996; **275**: 351–3.

Investigation of haemolytic anaemia

The normal red cell has a lifespan of ~120 days. Anaemia resulting from ↓ RBC lifespan is termed *haemolytic*. May be inherited or acquired, and the basic underlying mechanisms may involve abnormalities of the RBC membrane, RBC enzymes, or Hb.

Extravascular vs intravascular

Extravascular haemolysis implies RBC breakdown by the reticuloendothelial system (RES) (e.g. liver, spleen, and macrophages at other sites), whilst intravascular haemolysis describes RBC breakdown in the circulation itself (see Fig. 3.8). There are many investigations available that will help determine the predominant site of destruction, which, in turn, will help define the underlying cause of haemolysis, which is why we do the tests in the first place.

Detection of haemolysis itself

The main question is whether the patient's anaemia is due to haemolysis or some other underlying mechanism such as blood loss, marrow infiltration, etc.

→ OHCM 10e, p. 336.

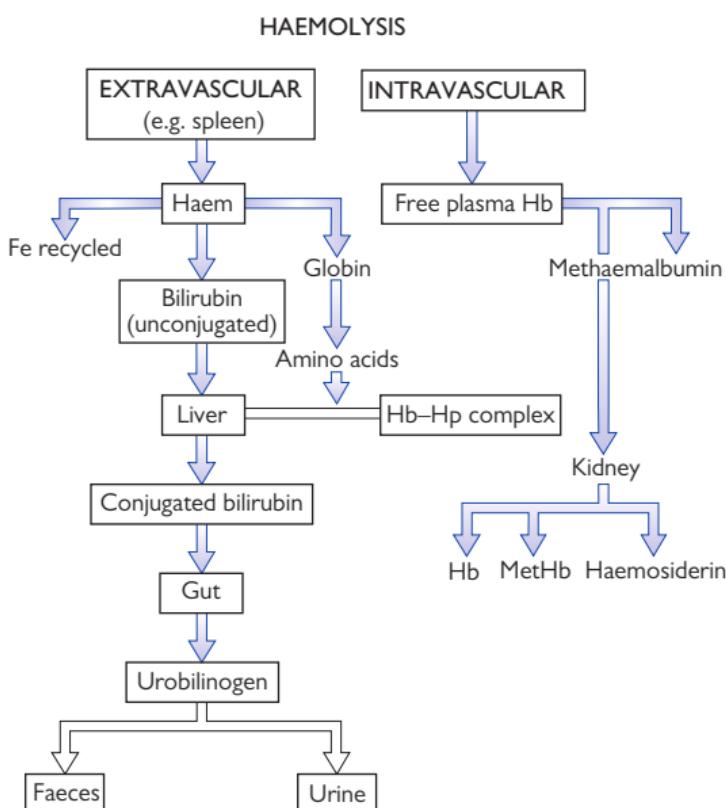


Fig. 3.8 Increased red cell breakdown may be extravascular (outside the circulation, predominantly the spleen, liver, and marrow) or intravascular (within the vessels).

Modified from Lewis SM, Bain BJ & Bates I, eds. (2001) Dacie & Lewis Practical Haematology, 11th edition, Churchill Livingstone, Edinburgh.

General tests of haemolysis

Is haemolysis actually occurring? Suggestive features

- Evidence of ↑ red cell destruction.
- Evidence of ↑ red cell production (to compensate for red cell loss).
- Evidence of autoantibody in the patient's serum.

Evidence of red blood cell destruction

- ↑ serum bilirubin (split conjugated/unconjugated is useful).
- ↑ serum LDH (reflecting ↑ RBC turnover).
- Spherocytes or other abnormal RBCs, e.g. fragments on blood film.
- Plasma haptoglobins may be ↓ or absent.
- ↑ faecal and urinary urobilinogen (faecal not measured).
- ↓ RBC lifespan (seldom measured nowadays).

Evidence of increased red blood cell production

- ↑ reticulocytes (on film, manual, or automated count). Not absolutely specific, will ↑ in brisk acute bleed, e.g. GIT.
- ↑ MCV (reticulocytes are larger than mature RBCs, and do not forget folate deficiency, which occurs in haemolytic disorders).

Is it mainly intravascular?

- ↑ plasma Hb.
- Methaemalbuminaemia.
- Haemoglobinuria.
- Haemosiderinuria.

What is the cause?

Genetic

- RBC morphology (e.g. spherocytes, elliptocytes).
- Hb analysis.
- RBC enzyme assays.

Acquired

- Immune—check DAT.
- Non-immune: check RBC morphology (e.g. TTP/HUS).
- Is there some other underlying disease?
- Consider PNH (rare).

Further reading

Hill QA, Stamps R, Massey E, Grainger JD, Provan D, Hill A; British Society for Haematology Guidelines. Guidelines on the management of drug-induced immune and secondary autoimmune, haemolytic anaemia. *Br J Haematol* 2017; **177**: 208–20.

Hill QA, Stamps R, Massey E, Grainger JD, Provan D, Hill A; British Society for Haematology. The diagnosis and management of primary autoimmune haemolytic anaemia. *Br J Haematol* 2017; **176**: 395–411.

Schick P (2016). *Hemolytic anemia*. <http://www.emedicine.com/med/topic979.htm>.

Reticulocytes

These are immature RBCs formed in the marrow and found in small numbers in normal peripheral blood. They represent an intermediate maturation stage in the marrow between the nucleated RBC and the mature RBC (the reticulocyte lacks a nucleus but retains some nucleic acid). Measuring the number of reticulocytes in the blood may help determine whether the anaemia is due to ↓ RBC production. The reticulocyte count is also a useful measure of response to haematinic (iron, B₁₂, or folate) replacement therapy.

Detection and measurement

- Modern automated blood counters using laser technology measure the number of reticulocytes directly.
- 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 (see Fig. 3.9).
- Usually expressed as a percentage of total red cells, e.g. 5%, though absolute numbers can be derived from this and the total red cell count.
- Sample: EDTA.
- Normal range: 0.5–2.5% ($50\text{--}100 \times 10^9/\text{L}$).

Causes of increased reticulocyte counts

Marrow stimulation due to

- Bleeding.
- Haemolysis.
- Response to oral iron therapy.
- Infection.
- Inflammation.
- Polycythaemia (any cause).
- Myeloproliferative disorders.
- Marrow recovery following chemotherapy or radiotherapy.
- Epo administration.

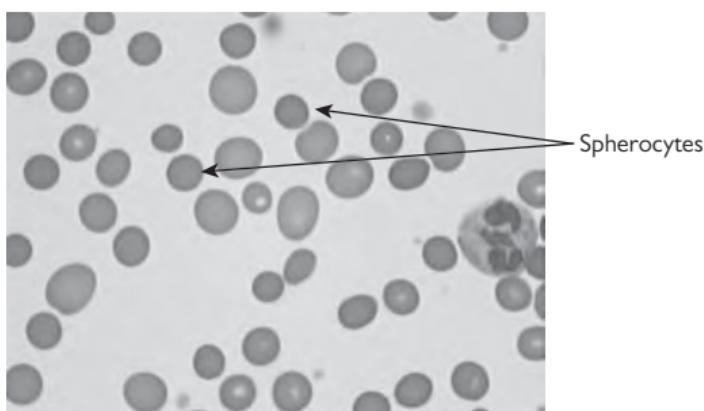


Fig. 3.9 Blood film of numerous spherocytes (small, darker red cells) and reticulocytes (larger red cells) in autoimmune haemolytic anaemia.

Causes of decreased reticulocyte counts

Marrow infiltration due to

- Leukaemia.
- Myeloma.
- Lymphoma.
- Other malignancy.

Marrow underactivity (hypoplasia) due to

- Iron, folate, or B₁₂ deficiency. Note: the return of reticulocytes is the earliest sign of response to replacement therapy.
- Immediately post-chemotherapy or radiotherapy.
- Autoimmune disease, especially refractory anaemia (RA).
- Malnutrition.
- Uraemia.
- Drugs.
- Aplastic anaemia.
- Red cell aplasia.

Further reading

Howells MR, Jones SE, Napier JA, Saunders K, Cavill I. Erythropoiesis in pregnancy. *Br J Haematol* 1986; 64: 595–9.

Serum haptoglobins

Haptoglobins (Hps) are plasma proteins synthesized by the liver, whose function is the removal of free plasma Hb. Hp molecules bind free Hb and are taken up by the RES for degradation. Hp–Hb complexes do not appear in the urine because their large size prevents them from passing through the renal tubules.

The Hp–Hb complex is cleared by the RES at a rate of 15mg/100mL/h, which means that even very mild haemolysis will cause the disappearance of Hps from the circulation. Serum Hps should be measured in patients with suspected intravascular haemolysis. However, the Hp level is frequently reduced in patients with extravascular haemolysis, and the Hp level cannot be used to determine whether the basic haemolytic process is intra- or extravascular. It should generally be accompanied by estimation of serum methaemalbumin, free plasma Hb, and urinary haemosiderin.

- *Sample:* clotted blood.
- *Normal range* (expressed as Hb-binding capacity): 30–250mg/dL.

Conditions with decreased haptoglobins

Haemolysis including

- Incompatible blood transfusion.
- AIHA.
- Sickle-cell disease.
- Thalassaemia major.
- PNH.

Others

- 1% of the population have a genetic lack of Hps.
- Lower levels in infancy.

Note: it takes about 1 week after haemolysis has stopped for Hp levels to return to normal.

Conditions with increased haptoglobins (acute phase protein, like ferritin)

- Any disorder with ↑ ESR.
- Carcinoma, especially if bony 2°.
- Any inflammatory disorder.
- Trauma.
- Surgery.
- Steroid therapy.
- Androgen therapy.
- DM.

Further reading

Rougemont A, Dumbo O, Bouvier M, et al. Hypohaptoglobinaemia as an epidemiological and clinical indicator for malaria. Results of two studies in a hyperendemic region in West Africa. *Lancet* 1988; 2: 709–12.

Serum bilirubin

Two forms are found: pre-hepatic bilirubin (unconjugated) and bilirubin conjugated to glucuronic acid (conjugated). Generally, serum bilirubin levels are 17–50 $\mu\text{mol/L}$ in haemolysis (mainly unconjugated).

► Beware: serum bilirubin levels may be normal, even if haemolysis is present; a level of >85 $\mu\text{mol/L}$ suggests liver disease.

Serum bilirubin levels may be modestly ↑ (e.g. 20–30 $\mu\text{mol/L}$) in dyserythropoietic disorders, such as vitamin B₁₂ or folate deficiency, or myelodysplasia, due to ineffective erythropoiesis where RBCs are destroyed in the marrow before ever being released into the circulation.

Urobilin, urobilinogen, and urinary haemosiderin

Urobilinogen is the reduced form of urobilin, formed by bacterial action on bile pigments in the GIT. Faecal and urinary urobilinogen ↑ in haemolytic anaemias.

Urinary haemosiderin

Usage

The most widely used and reliable test for detection of chronic intravascular haemolysis. Results from the presence of Hb in the glomerular filtrate.

Principle

Free Hb is released into the plasma during intravascular haemolysis. The Hb-binding proteins become saturated, resulting in the passage of haem-containing compounds into the urinary tract of which haemosiderin is the most readily detectable.

Method

1. A clean-catch sample of urine is obtained from the patient.
2. The sample is spun down in a cytocentrifuge to obtain a cytospin preparation of urothelial cells.
3. Staining and rinsing with Perl's reagent (Prussian blue) are performed on the glass slides.
4. Examine under the oil-immersion lens of a microscope.
5. Haemosiderin stains as blue dots within urothelial cells.
6. Ignore all excess stain and staining outside cells or in debris, all of which are common.
7. True +ve only when clear detection within urothelial squames is seen.

Caution

An iron-staining +ve control sample should be run alongside the test case to ensure the 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 the test in 3–7 days if –ve. Conversely, haemosiderinuria may persist for some time after the haemolytic process has stopped. A repeat in 7 days should confirm.

Causes of haemosiderinuria

(See Table 3.6.)

Table 3.6 Causes of haemosiderinuria

Common causes	Red cell enzymopathies, e.g. G6PD and pyruvate kinase deficiencies, but only during haemolytic episodes <i>Mycoplasma</i> pneumonia with anti-I cold haemagglutinin Sepsis Malaria Cold haemagglutinin disease TTP/HUS Severe extravascular haemolysis (may cause intravascular haemolysis)
Rarer causes	PNH Prosthetic heart valves Red cell incompatible transfusion reactions Unstable Hb March haemoglobinuria

Plasma haemoglobin

In health, Hb is contained within RBCs, but during intravascular haemolysis, excessive quantities of Hb may be released from ruptured RBCs. Normally Hps mop up free Hb. If there are insufficient Hps to cope with the free Hb, the kidneys clear the Hb, leading to haemoglobinuria. Some Hb may be broken down in the circulation to haem and globin; haem can bind to albumin, producing methaemalbumin (\rightarrow methaemalbuminaemia).

► The finding of free Hb in plasma is *highly suggestive* of intravascular haemolysis.

- *Sample:* sodium citrate (but discuss with the haematology laboratory before sending sample).
- *Normal range:* 10–40mg/L (up to 6mg/L).
- *Pitfalls:* any RBC damage occurring during blood sampling may result in an erroneously high reading. Great care must be taken during venepuncture.

Causes of increased plasma haemoglobin

(See Table 3.7.)

Table 3.7 Causes of increased plasma haemoglobin

Mild ↑ (50–100mg/L)	Moderate ↑ (100–250mg/L)	Severe ↑ (>250mg/L)
Sickle/thalassaemia	AIHA	Incompatible blood transfusion
Haemoglobin C disease	Sickle-cell disease Thalassaemia major Haemoglobin SC Prosthetic heart valve March haemoglobinuria	PNH Paroxysmal cold haemoglobinuria Blackwater fever

Further reading

Crosby WH, Dameshek W. The significance of hemoglobinemia and associated hemosiderinuria, with particular reference to various types of hemolytic anemia. *J Clin Lab Med* 1951; **38**: 829–41.

Schumm's test

- Use: detection of methaemalbumin (seen after all Hps used up in a haemolytic process; usually implies haemolysis is predominantly intravascular).

This spectrophotometric test for methaemalbumin (which has a distinctive absorption band at 558nm) should be requested in patients with suspected intravascular haemolysis and may be abnormal in patients with significant extravascular (generally splenic) haemolysis. It should be accompanied by an estimation of the serum Hp level, free plasma Hb, and urinary haemosiderin.

- Sample: heparinized or clotted blood.

Positive result in

- Intravascular haemolysis.
- Mismatched blood transfusion.
- RBC fragmentation syndromes.
- G6PD deficiency with oxidative haemolysis.
- PNH.
- March haemoglobinuria.
- Unstable Hb.

Further reading

- Hoffbrand AV, Lewis SM, Tuddenham EGD (eds.). *Postgraduate Haematology*, 4th edn. Oxford: Butterworth-Heinemann, 2000.
Winstone NE. Methemalbumin in acute pancreatitis. *Br J Surg* 1965; 52: 804–8.

Hereditary haemolytic anaemias

There are many inherited causes for haemolytic anaemia, which fall into three major groups, shown in Table 3.8.

Table 3.8 Inherited causes for haemolytic anaemias

Mechanism	Example
Red cell membrane disorders	Hereditary spherocytosis Hereditary elliptocytosis
Red cell enzyme disorders	G6PD deficiency Pyruvate kinase deficiency
Hb disorders	Sickle-cell anaemia Thalassaemia

Red cell membrane disorders

Hereditary spherocytosis

This is the best known inherited membrane abnormality leading to a reduced red cell lifespan and sometimes severe anaemia. Inheritance is usually autosomal dominant and there is often a +ve family history.

Hereditary elliptocytosis

Usually autosomal dominant. Rarely causes symptomatic anaemia.

Hereditary pyropoikilocytosis

Autosomal recessive, often compound heterozygotes. Often severe haemolysis.

South East Asian ovalocytosis

Heterozygotes asymptomatic.

Hereditary stomatocytosis

Heterogeneous group with often severe haemolysis.

EMA binding test

Immunophenotyping using the reagent EMA to bind to red cell transmembrane proteins. Altered fluorescence is seen with red cell membrane disorders.

SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis)

Gel electrophoresis of membrane proteins. Only available in reference laboratories.

Osmotic fragility test

Principle of the test

The test measures the ability of red cells to take up water before rupturing (lysing). This is determined by the volume:surface area ratio. Normal red cells can ↑ their volume by up to 70% before lysing (because they are disc-shaped and have the capacity to take in extra water easily). Spherocytic red cells have an ↑ volume:surface area ratio and are able to take up less water than normal red cells before lysing (they are spheres and, as such, they are 'full' already).

- Sample: EDTA (need a normal control sample sent at the same time).

(See Fig. 3.10.)

Method

- RBCs are incubated in saline at various concentrations. This results in cell expansion and eventually rupture.
- Normal RBCs can withstand greater volume ↑ than spherocytic RBCs.

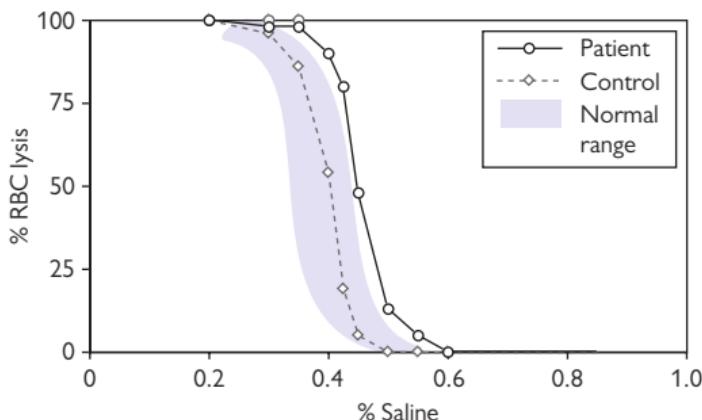


Fig. 3.10 Test and control samples subjected to varying concentrations of saline. The broad grey band represents the normal range. The red cells in the test sample (from a patient with hereditary spherocytosis) lyse at higher concentrations of saline than that seen in the normal control, which suggests that hereditary spherocytosis is a likely diagnosis.

- A +ve result (confirming hereditary spherocytosis) seen when RBCs lyse in saline at near to isotonic concentration, i.e. 0.6–0.8g/dL (normal RBCs will simply show swelling with little lysis).
- Osmotic fragility is more marked in patients who have not undergone splenectomy and if the RBCs are incubated at 37°C for 24h before performing the test.

Other supportive tests

- There will be a +ve family history of hereditary spherocytosis in many cases.
- The blood film shows ↑↑ spherocytic RBCs.
- Anaemia, ↑ reticulocytes, ↑ LDH, unconjugated bilirubin, urinary urobilinogen with ↓ Hps.
- DAT –ve.

► Beware: this test is not diagnostic of hereditary spherocytosis but will be +ve in any condition in which there are ↑ numbers of spherocytic red cells. Use this test in conjunction with a history, blood film, and family studies (hereditary spherocytosis is inherited as autosomal dominant, so one of the parents and some siblings should be affected).

Most testing laboratories use a combination of the above tests. No one screening test can detect all cases of hereditary spherocytosis.

Further reading

- Bolton-Maggs PHB, Langer JC, Iolascon A, Tittensor P, King MJ. Guidelines for the diagnosis and management of hereditary spherocytosis – 2011 update. *Br J Haematol* 2012; **156**: 37–49.
 Parpart AK, Lorenz PB, et al. The osmotic resistance (fragility) of human red cells. *J Clin Invest* 1947; **26**: 636–40.
 Weatherall DJ, Provan AB. Red cells I: inherited anaemias. *Lancet* 2000; **355**: 1169–75.

Red cell enzyme assays

Numerous red cell enzymes are responsible for maintaining the integrity of the RBC in order to allow it to function efficiently in O₂ delivery and CO₂ removal. RBC enzyme defects lead to shortened RBC survival (i.e. haemolysis) and anaemia. Although there are numerous enzymopathies that may cause haemolysis, the most useful starting assays are for G6PD and pyruvate kinase (PK).

Of course, one should start by taking a detailed history from the patient, asking about previous haemolytic episodes, family history, ethnic origin, and possible drug toxicities.

- **Sample:** fresh EDTA or heparin. The enzymes are stable for 6 days at 4°C and 24h at 25°C.
- **Methods:** these are too numerous and complex to list here.

Essentially there are three methods for analysis of G6PD:

1. Brilliant cresyl blue decolorization test.
2. Methaemoglobin reduction test.
3. Ultraviolet (UV) spot test.

- **Normal range:** varies between laboratories (check with your local laboratory).
- **Pitfalls:** during a haemolytic episode in patients with G6PD deficiency, the oldest RBCs are destroyed first. Younger RBCs (and especially reticulocytes) have higher levels of the enzyme than older cells. It follows therefore that if the enzyme level is assayed during an acute episode, the G6PD level obtained may be falsely normal. This will rise further as reticulocytes pour into the peripheral blood, as happens during recovery from the acute attack. It is better to wait until the acute attack is over and the patient is in steady state.

Further reading

- Arya R, Layton DM, Bellingham AJ. Hereditary red cell enzymopathies. *Blood Rev* 1995; 9: 165–75.
Beutler E. The molecular biology of G6PD variants and other red cell enzyme defects. *Annu Rev Med* 1992; 43: 47–59.
World Health Organization Scientific Group (1967). *Standardization of procedures for the study of glucose-6-phosphate dehydrogenase*. Technical Report Series, No. 366. Geneva: World Health Organization.

Haemoglobin abnormalities

There are two main classes of Hb abnormalities (see Table 3.9).

Table 3.9 Main classes of Hb abnormalities

Abnormality	Example
Structural Hb variants	Sickle Hb, HbD, HbE
Imbalanced globin production	Thalassaemias (α , β , etc.)

Structural haemoglobin variants

If the amino acid change results in an electrical charge difference, this may be detected by protein electrophoresis (separates proteins on the basis of charge). Investigation requires a full clinical history, FBC, blood film, and Hb electrophoresis.

Thalassaemias

β -thalassaemia is diagnosed from the blood indices, blood film, HbA₂, and HbF levels. For α -thalassaemia, the investigation is more complex, requiring DNA analysis to detect α -globin deletions. Globin chain synthesis, which examines the ratio of α : β -globin production, is performed less with the advent of DNA-based methods.

Further reading

Weatherall DJ, Provan AB. Red cells I: inherited anaemias. *Lancet* 2000; 355: 1169–75.

Haemoglobin analysis

Haemoglobin electrophoresis

Electrophoresis is an electrical method for separating molecules on the basis of size (for DNA fragments) or overall electrical charge (for proteins). Hb electrophoresis allows the separation of different Hb, *providing* they have differing charges (Hb molecules with the same charge will move together on the gel and cannot be distinguished). The methods used take advantage of the fact that amino acid side chains on the globin molecules can be ionized. The net overall charge of a protein depends on the pH of the solution in which it is and the pKs of the amino acids (the pK is the pH at which half of the side chains are ionized).

Normal adult haemoglobins

- HbA: 97% total.
- HbA₂: 2.0–3.2%.
- HbF: 0.5%.

Electrophoretic methods used

- Cellulose acetate (at pH 8.6).
- Citrate agar (at pH 6.0).
- Isoelectric focusing (IEF).
- High-performance liquid chromatography (HPLC).

Due to space limitations, each of these methods will be discussed only briefly. Other texts deal with this topic in considerable detail.

- Sample: peripheral blood EDTA.

Cellulose acetate

This test is commonly performed in the diagnosis of abnormal Hb production (haemoglobinopathies or thalassaemia). Because some Hb have the same net charge, they will run together, e.g. HbS will run in the same band as HbD and HbG, and HbC will run with HbE. To resolve these bands, electrophoresis is next carried out at acid pH.

Citrate agar

This is similar to cellulose acetate where Hb molecules are separated at an acid pH (pH 6.0) to separate out Hb that run together at alkaline pH.

Isoelectric focusing

This is a high-resolution method for separating different Hb molecules. The basic principle of the test relies on the fact that all proteins and amino acids have a pH at which their net charge is zero. This is termed the isoelectric point. At this pH, there is no net movement in the presence of an externally applied electric field. The Hb molecules are subjected to a pH gradient. This method has the advantage of high resolution but is more expensive than standard electrophoresis (see Fig. 3.11).

High-performance liquid chromatography

This chromatographic technique has been around for 20 years or more and is being increasingly used for analysis of Hb molecules. Hb molecules are passed through a matrix column and eluted from the column at varying times, during which their absorbance is measured. Detection of standard Hb variants is simple; the advantage of HPLC is that novel Hb variants can also be detected, and HPLC can separate proteins that cannot be resolved using other means. HPLC is more expensive than all the techniques mentioned above (see Figs 3.12 and 3.13).

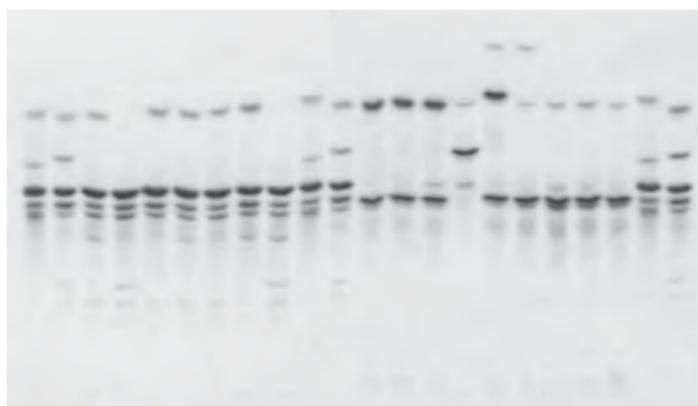


Fig. 3.11 Isoelectric focusing of haemoglobin.

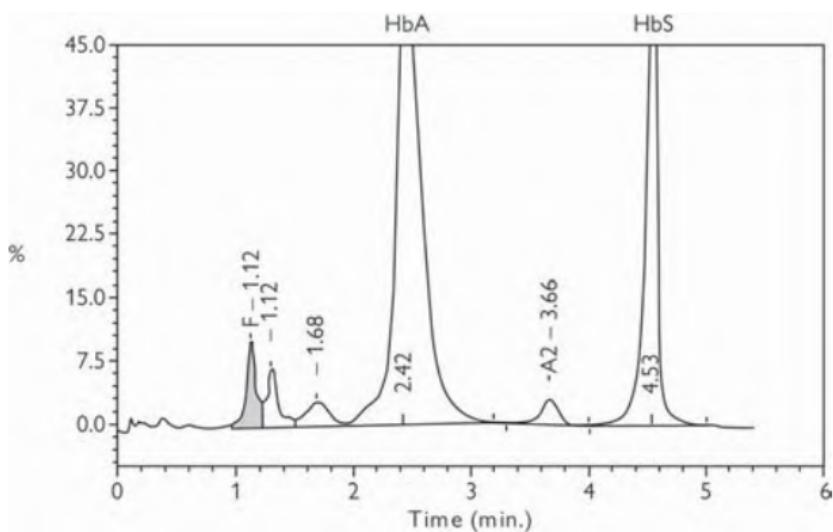


Fig. 3.12 HPLC analysis showing sickle trait (HbA + HbS).

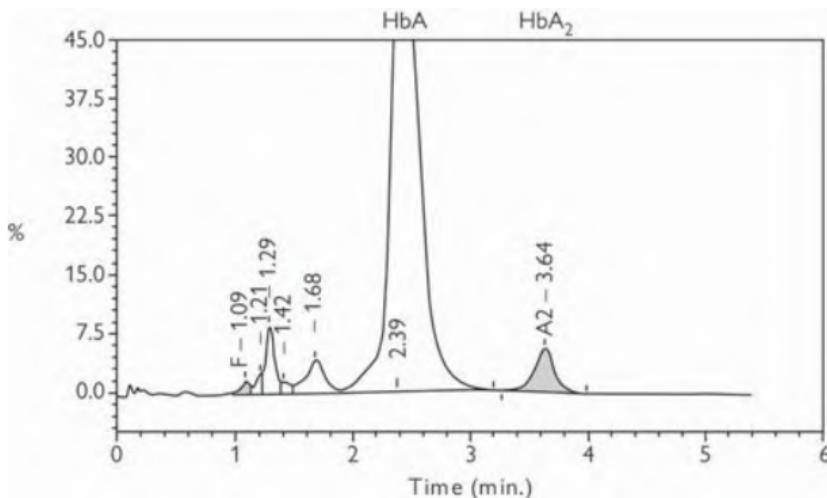


Fig. 3.13 HPLC analysis showing β -thalassaemia trait (elevated HbA₂).

When should you request these tests?

Haemoglobin analysis is usually carried out

- When the MCV is $\downarrow\downarrow$, but Hb normal or slightly \downarrow .
- In patients from ethnic groups known to be associated with high levels of Hb disorder, e.g. sickle or thalassaemia.

Investigation of possible thalassaemia

1. Check the FBC and look at the MCV.
2. Is the MCV normal ($>76\text{fL}$)? If so, thalassaemia is unlikely.
3. Does the FBC show anything else? ↑ RCC with ↓ MCV and MCH are likely in thalassaemia.
4. Measure the HbA_2 ; this is generally ↑ in β-thalassaemia trait (carrier).
5. Carry out HPLC.
6. Measure HbF level.
7. Look at the distribution of HbF in RBCs (HbF is present in *all* RBCs in African hereditary persistence of fetal Hb (HPFH), but not present in all cells in carriers for ↓ β-thalassaemia).
8. Assess the iron status (common cause of ↓ MCV—do not miss this!).
9. Look for RBC inclusions (e.g. H bodies in α-thalassaemia or Heinz bodies in unstable Hb disorders).
10. Carry out DNA analysis, examining both α- and β-globin genes.

Further reading

Information Center for Sickle Cell and Thalassemic Disorders. *Thalassemia*.  http://sickle.bwh.harvard.edu/menu_thal.html.

Investigation of sickle haemoglobin

Sickle Hb is the result of a point mutation in the β -globin gene, resulting in a Glu \rightarrow Val switch at position 6 of the β -globin protein. Sickled Hb (HbS) forms long filaments (tactoids), reducing its solubility when O_2 tension is reduced. This forms the basis of the sickle solubility test (see Fig. 3.14).

- Sample: any anticoagulant.

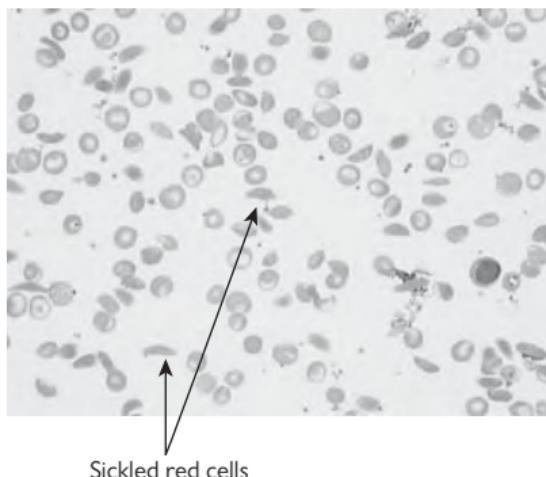


Fig. 3.14 Blood film of homozygous sickle-cell anaemia (HbSS). Note the sickle-shaped (crescent) red cells.

The patient's blood is mixed with sodium dithionite solution and left to stand. A +ve sickle sample should be used as a control. When the tubes are examined, a clear solution implies that there is no sickle Hb; a turbid solution confirms the presence of HbSS in the patient's sample.

► A +ve result will be obtained for sickle carriers (HbAS) and sickle-cell homozygotes (HbSS). If a +ve result is obtained, Hb electrophoresis must be carried out to determine whether the patient is a carrier or has homozygous sickle-cell anaemia.

Molecular diagnosis of sickle-cell disease

This is useful for prenatal diagnosis. The β -globin genes of the fetus are amplified using PCR; cells are obtained by amniocentesis or chorionic villus sampling (CVS) and digested with a bacterial restriction enzyme, e.g. *Mst* II. If the sickle mutation is present, no digestion will occur (the mutation removes the restriction site).

Neonatal haemoglobin screening

- Obtain blood from the neonate (e.g. heel prick) and in babies at risk of sickle or β -thalassaemia major (e.g. the mother has a gene for HbS, C, D^{Punjab}, E, O^{Arab}, β - or ↓ β -thalassaemia).
- Universal neonatal screening is generally used in areas where there is a high incidence of haemoglobinopathy.

⇒ OHCM 10e, p. 340.

Further reading

Information Center for Sickle Cell and Thalassemic Disorders. *Sickle cell disease*.  http://sickle.bwh.harvard.edu/menu_sickle.html.

Steinberg MH, Forget BG, Higgs DR, Weatherall DJ (eds.). *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management*. Cambridge: Cambridge University Press, 2001.

Estimation of haemoglobin A₂ ($\alpha_2\delta_2$)

Normal adults have three types of Hb: HbA, HbA₂, and HbF. HbA ($\alpha_2\beta_2$) is the major Hb, and HbA₂ is a minor adult Hb, which is very useful for the diagnosis of the β -thalassaemia trait. HbA₂ levels are ↑ in the heterozygote (carrier state), and this is a specific test for this genotype. The test is carried out using a column chromatography method.

- *Sample:* EDTA.
- *Normal range:* 2.0–3.2%.

Causes of increased HbA₂

- β -thalassaemia trait (HbA₂ level is ~3.9–6.5%).

Causes of decreased HbA₂

- Iron deficiency.
- δ -thalassaemia.

Estimation of fetal haemoglobin

Fetal Hb (HbF) makes up >50% of the total Hb at birth but ↓ to ~5% by 5 months of age (as γ chain production is replaced by β chains). HbF levels may be raised in some haemoglobinopathies.

- *Sample:* EDTA.

Increased HbF found in

- β -thalassaemia trait.
- β -thalassaemia major.
- Hereditary persistence of HbF.
- Homozygous sickle-cell disease (HbSS).
- Sickle/ β^+ thalassaemia (some cases).
- Sickle/ β^0 thalassaemia (some cases).
- Juvenile CML.
- Multiple myeloma (uncommon and never measured).
- Acquired aplastic anaemia.

Haemoglobin H bodies (β_4)

HbH, consisting of a tetramer of β -globins (β_4), is found in α -thalassaemia. The β chains form tetramers due to the relative lack of α -globins with which to pair. The demonstration of HbH allows the detection of the α -thalassaemia trait (either $-\alpha/-\alpha$ or $--/\alpha\alpha$) and HbH disease ($--/\alpha$).

Method

The HbH body test involves staining RBCs with brilliant cresyl blue; HbH bodies are seen as large, dark inclusions in the red cells.

- *Sample:* fresh EDTA.

► Note: the presence of HbH confirms α -thalassaemia, but the absence of HbH bodies does not exclude the diagnosis.

Heinz bodies

These are red cell inclusions made up of insoluble denatured globin protein. Heinz bodies are seen when RBCs are stained with methyl violet or new methylthioninium chloride (methylene blue) stain.

- *Sample:* fresh EDTA.
- *Interpretation:* Heinz bodies are seen close to the RBC membrane. These are normally removed by the spleen and are therefore more frequent following splenectomy.

Causes of Heinz bodies

Oxidative haemolysis

- Chlorates, phenacetin, other drugs.
- G6PD and PK deficiencies, and other enzymopathies.
- Unstable Hb.

Further reading

Sevitt S, Stone P, Jackson D, Baar S, Pollock A. Acute Heinz-body anaemia in burned patients. *Lancet* 1973; 2: 471–5.

Testing for unstable haemoglobins

Globin gene mutations may lead to amino acid substitutions that render the Hb molecule unstable, leading to haemolysis. Most mutations causing unstable Hb are autosomal dominant, and >80% affect the β chain. Affected individuals are heterozygotes. Heinz bodies in RBCs are intracellular Hb precipitates. Unstable Hb can be detected electrophoretically or by using the heat precipitation test, in which lysed RBCs are heated to 50°C for 1h.

- *Sample:* fresh EDTA.
- *Interpretation:* normal fresh haemolysates should be stable for 1h at 50°C. If there is an unstable Hb, a precipitate will be seen in the tube.

Examples

- Hb Köln.
- Hb Gun Hill.

Molecular tests for diagnosis of thalassaemia

Although most haematology laboratories can diagnose β -thalassaemia trait and β -thalassaemia major, there are occasions when molecular tests are required, e.g. antenatal diagnosis where a couple are at risk of having a child with β -thalassaemia major or hydrops fetalis (absence of α -globin, usually lethal). In addition, the diagnosis of α -thalassaemia is difficult and requires DNA analysis, either using Southern blotting or PCR amplification of globin genes.

β -thalassaemia

There are >100 β -globin mutations now known, but fortunately each population tends to have its own group of mutations (this avoids having to test for all known mutations). It is important that you include the ethnic group on the request form, since this will assist the laboratory who will then screen for mutations commonly found in the ethnic group of the patient. Details of these mutations can be found in the β - and δ -thalassemia repository.

Methods used for molecular diagnosis of β -thalassaemia

The methods used are complex and outwith the scope of this small book.^{1–3}

How the ARMS PCR technique works

- This is amplification refractory mutation system PCR.
- Specific point mutations are known for the β -globin mutations.
- PCR primers are designed to bind with the mutated sequence.
- If the patient has the mutation, there will be PCR amplification.
- If the patient lacks the mutation, there is no binding of the primers to the patient's DNA and no amplification.
- So a band on the gel means the mutation is present (and the reverse is true—if the band is absent, then that particular mutation is absent).

Other techniques, including reverse dot blots and DNA sequencing, are sometimes needed if ARMS PCR fails.

Methods used for molecular diagnosis of α -thalassaemia

Whereas β -thalassaemia is usually the result of point mutations (single base changes), the α -thalassaeemias are usually the result of deletions of chunks of DNA in the region of the α -globin genes. Southern blotting is useful in detecting deletions, since the DNA band sizes after digestion with restriction enzymes will differ to the wild type (i.e. normal).

1 Bowden DK, Vickers MA, Higgs DR. A PCR-based strategy to detect the common severe determinants of alpha thalassaemia. *Br J Haematol* 1992; **81**: 104–8.

2 Lewis SM, Bain BJ, Bates I (eds.). *Dacie & Lewis Practical Haematology*, 9th edn. Edinburgh: Churchill Livingstone, 2000.

3 Huisman TH, Carver MF. The beta- and delta-thalassemia repository. *Hemoglobin* 1988; **22**: 169–95.

UK Haemoglobinopathy Reference Laboratory

This is based at the John Radcliffe Hospital in Oxford (UK). Difficult cases (e.g. α -thalassaemia) can be sent to this laboratory (after discussing the case first); they will perform α -globin gene analysis and send a detailed report containing the genotype of the patient. See end of the chapter for contact details (⇒ Specialized haematology assays, pp. 330–1).

Acquired haemolytic anaemias

Determining the cause of haemolytic anaemia can be a complex process. Having excluded inherited disorders of Hb, RBC membrane, or enzymes, we are left with a diverse group of disorders with a common phenotype of ↑ RBC destruction (and ↓ RBC lifespan; see Table 3.10).

Table 3.10 Acquired haemolytic anaemias: mechanisms

Mechanism	Examples
Autoimmune	Warm antibody (IgG mainly): <ul style="list-style-type: none"> Idiopathic haemolytic anaemia 2° to other autoimmune diseases (e.g. SLE), lymphoid malignancies (e.g. CLL), infections, drugs (e.g. penicillins, methyldopa) Cold antibodies (IgM mainly): <ul style="list-style-type: none"> Cold agglutinin syndromes, cold haemagglutinin disease (CHAD), 2° to infection
Alloimmune	Haemolytic transfusion reactions, HDN
Infections	Many, including malaria, meningococcal, pneumococcal, viral
Chemical or physical	Drugs, burns, drowning
RBC fragmentation syndromes	Mechanical heart valves, MAHA (seen in DIC, HUS, TTP, pre-eclampsia, SLE, carcinoma)
Membrane disorders	Examples are liver disease, PNH

Immune

- Autoimmune (1°, or 2° to SLE or CLL).
- Alloimmune (e.g. transfusion reactions, haemolytic disease of the newborn (HDN)).
- Antibody can be warm (IgG) or cold (IgM usually).

Red blood cell damage

- Drugs.
- Poisons.
- Burns.

Red blood cell fragmentation syndromes

- DIC.
- Thrombotic microangiopathies (TMAs)
- March haemoglobinuria.

Investigations

There is little point investigating the cause of haemolytic anaemia until you have shown that haemolysis is *actually* occurring.

Look for the acquired cause

- FBC and peripheral film:
 - Spherocytes (suggests warm antibody; also present in hereditary spherocytosis).
 - ↑ WBC, e.g. might suggest an underlying lymphoproliferative disorder such as CLL.
 - RBC fragments (suggests physical damage to the RBC, e.g. microangiopathic haemolytic anaemia (MAHA), TTP/HUS, burns, March haemoglobinuria, mechanical heart valves).
 - Parasites, e.g. malaria.
 - Infections, e.g. *Clostridium*, *Bartonella*, *Babesia*.
- Antiglobulin test (DAT):
 - IgG or IgG + complement (C3d) on RBC.
 - DAT is usually +ve in immune-mediated haemolysis.
- Renal function (often abnormal in TTP/HUS).
- Coagulation screen (DIC with RBC fragmentation).
- LFTs (abnormal in Zieve's syndrome).
- USS for splenomegaly.
- *Cold agglutinins*: IgM, usually against I or i proteins, RBC membrane proteins.
- Immunophenotype if suspect PNH.

Immunophenotyping for GPI-linked proteins

PNH is a rare acquired red cell membrane disorder. Loss of glycosyl phosphatidyl inositol (GPI)-linked surface proteins make the red cells vulnerable to complement-mediated lysis, causing episodes of marked intravascular haemolysis, with free Hb in the urine (haemoglobinuria). Immunophenotyping shows cells to be deficient in the GPI-anchored proteins CD55 and CD59 (erythrocytes) and CD16, CD24, and FLAER (granulocytes),

The technique of immunophenotyping is described later in this chapter.

Bleeding time

This is a test of 1° haemostasis, and mainly of platelet function *in vivo*, rather than a laboratory test. You will generally need to arrange this test through the haematology department who will carry out the test for you.

Procedure

A disposable spring-loaded blade is used to make two incisions of fixed depth into the skin of the forearm, whilst a sphygmomanometer is inflated to 40mmHg. Blood from the incisions is mopped up using circular filter paper (care needs to be taken to avoid disturbing the clot that forms on the cut surface).

- *Normal range:* up to 7min (varies, depending on the method used; >9min is abnormal). Longer in ♀.
- *Uses:* previously felt to be the best screen for acquired or congenital functional or structural platelet disorders. If bleeding time is normal and history –ve (i.e. no major bleeding problems in the past), this excludes an underlying platelet disorder.

Precautions

► Do not carry out bleeding time if the platelet count is $<100 \times 10^9/L$ (will be prolonged). Aspirin will interfere with the test—ask patients to stop aspirin 7 days before the test is carried out.

Causes of prolonged bleeding time

- Low platelet count.
- Platelet function defect (acquired, e.g. aspirin, paraprotein, MDS).
- von Willebrand's disease (vWD).
- Vascular abnormalities, e.g. Ehlers–Danlos.
- Occasionally low factor V or XI.
- Afibrinogenaemia.

Pitfalls

Highly operator-dependent, with low reproducibility. Because of this, the test is seldom used now.

Further reading

Mielke CH Jr. Aspirin prolongation of the template bleeding time: influence of venostasis and direction of incision. *Blood* 1982; **60**: 1139–42.

Parkin JD, Smith IL. Sex and bleeding time. *Thromb Haemost* 1985; **54**: 731.

Prothrombin time

This tests the extrinsic coagulation pathway and is useful for detecting coagulation deficiencies, liver disease, and DIC. The prothrombin time (PT) is also the main monitor for coumarin therapy (e.g. warfarin), expressed as a ratio—the *international normalized ratio* (INR). The test measures the clotting time of plasma in the presence of a tissue extract, e.g. brain (thromboplastin). The test measures prothrombin, but also factors V, VII, and X (see Fig. 3.15).

- Sample: citrate.

Increased prothrombin time

- Oral anticoagulation therapy (vitamin K antagonists).
- Fibrinogen deficiency (factor I).
- Prothrombin deficiency (factor II).
- Deficiency of factors V, VII, or X (in V or X deficiency, the activated partial thromboplastin time (APTT) will be ↑).
- Liver disease, especially obstructive.
- Vitamin K deficiency.
- DIC.

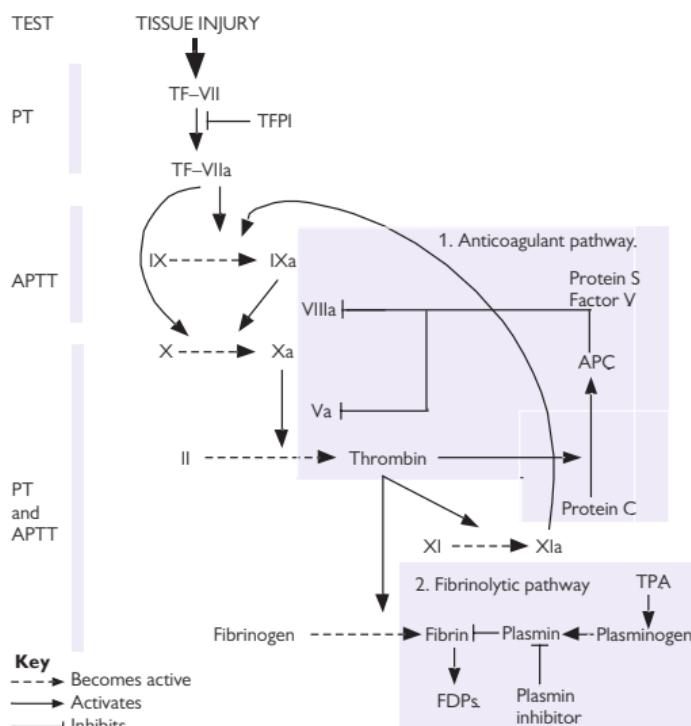


Fig. 3.15 Coagulation cascade showing the factors assayed using the various clotting tests.

Modified from Provan D, et al. *Oxford Handbook of Clinical Haematology*, 2nd edn. Oxford: Oxford University Press, 2004.

Further reading

Provan D, Singer CRJ, Baglin T, Lilleyman J. *Oxford Handbook of Clinical Haematology*, 2nd edn. Oxford: Oxford University Press, 2004.

Activated partial thromboplastin time

Other terms: somewhat confusingly, the APTT may be called kaolin cephalin clotting time (KCCT) or partial thromboplastin time with kaolin (PTTK).

What is APTT testing?

This is a test of the intrinsic coagulation system and depends on contact factors + factors VIII and IX, and reactions with factors X, V, II, and I. The APTT is sensitive to circulating anticoagulants (e.g. lupus anticoagulant) and heparin.

- Sample: citrate.

Uses

1. Heparin monitoring.
2. Screening for haemophilia A and B (VIII and IX deficiencies, respectively).
3. Screening for coagulation inhibitors.
 - Normal range: 26.0–33.5s (often expressed as activated partial thromboplastin time ratio (APTR)).

Increased activated partial thromboplastin time

- DIC.
- Liver disease.
- Massive blood transfusion.
- Heparin treatment.
- Circulating anticoagulant.
- Modest ↑ in patients taking oral anticoagulants.
- Haemophilia.

Is there an inhibitor present?

The APTT will be long if there is an inhibitor, such as the lupus anticoagulant, present. This can be determined by mixing the patient's plasma with an equal volume of normal control plasma and repeating the APTT. This is known as a 50:50 mix. If the APTT is long because of an inhibitor, it will not fully correct when normal plasma is added. However, if the APTT is long because of a deficiency, it will be corrected with the normal plasma.

Further reading

- Denson KW. Thromboplastin—sensitivity, precision and other characteristics. *Clin Lab Haematol* 1988; **10**: 315–28.
- Lab Tests Online (2012). Coagulation cascade.  http://www.labtestsonline.org/understanding/analytes/coag Cascade/coagulation_cascade.html.
- Turi DC, Peerschke EI. Sensitivity of three activated partial thromboplastin time reagents to coagulation factor deficiencies. *Am J Clin Pathol* 1986; **85**: 43–9.
- van den Besselaar AM, Meeuwisse-Braun J, Jansen-Grüter R, Bertina RM. Monitoring heparin therapy by the activated partial thromboplastin time—the effect of pre-analytical conditions. *Thromb Haemost* 1987; **57**: 226–31.

Thrombin clotting time

This is affected by the concentration of factor I (fibrinogen) and the presence of fibrin or fibrinogen degradation products and heparin.

- Sample: citrate.

Increased thrombin clotting time

- Low fibrinogen, e.g. DIC.
- ↑ FDPs/cross-linked fibrin degradation products (XDPs)/D-dimers.
- Heparin.*
- Dysfibrinogenaemia (inherited; mutation in the fibrinogen gene leads to amino acid change and non-functional fibrinogen).

Note: (*) if suspected, check reptilase time, similar to thrombin clotting time (TCT), but not affected by heparin.

D-dimers

D-dimers are produced during polymerization of fibrinogen as it forms fibrin. Measurement of D-dimer levels is more specific for this process than the older FDP test and is now being used to detect the presence of DIC and other coagulation disorders. The test measures fibrin lysis by plasmin and is a sensitive indicator of coagulation activation (e.g. such as that seen in DIC). The assay uses an MoAb specific for D-dimers; it will not cross-react with fibrinogen or fibrin.

- Sample: citrate (clotting screen bottle).

Increased D-dimers seen in

- DIC.
- DVT.
- PE.

Summary of clotting tests in a variety of disorders

(See Table 3.11.)

Table 3.11 Summary of clotting tests in a variety of disorders

PT	APTT	TCT	Platelets	Diagnosis
N	N	N	N	Platelet function defect, XIII deficiency, normal
↑	N	N	N	VII deficiency, early oral anticoagulation
N	↑	N	N	VIIIC/IX/XI/XII deficiencies, vWD, circulating anticoagulant, e.g. lupus
↑	↑	N	N	Vitamin K deficiency, oral anticoagulant, V/VII/X/II deficiencies
↑	↑	↑	N	Heparin, liver disease, fibrinogen deficiency
N	N	N	↓	Thrombocytopenia (any cause)
↑	↑	N	Low	Massive transfusion, liver disease
↑	↑	↑	Low	DIC, acute liver disease

Modified from Dacie & Lewis Practical Haematology, 8th edn. Edinburgh: Churchill Livingstone, 1995.

►► Disseminated intravascular coagulation

DIC is a medical and haematological emergency. It may be seen in a variety of situations and is characterized by generalized bruising and bleeding, usually from venepuncture sites, post-operatively, and spontaneously.

Diagnosis requires FBC, clotting screen, and evidence of rapid consumption of fibrinogen. Classic (acute) DIC, where the test results fit the bill, is easy to spot. The situation may be more subtle and you are strongly advised to discuss the case with a haematology registrar or consultant if you are in any doubt about the diagnosis of DIC.

►► Laboratory diagnosis

FBC	↓ platelets ± red cell fragments seen on blood film
PT	↑ in moderately severe DIC
APTT	Usually ↑
Fibrinogen	↓ (falling levels significant—but remember this is an acute phase protein, so levels may be normal, even in florid DIC)
D-dimers	↑

Conditions associated with DIC

(See Table 3.12.)

Table 3.12 Conditions associated with DIC

Disorder	Example
Infectious disease	Septicaemia Viraemia
Obstetric emergency	Placental abruption Eclampsia Amniotic fluid embolism Placenta praevia Septic abortion
Surgical	Cardiac bypass
Malignant disease	Metastatic cancer Acute leukaemia (especially AML M3, i.e. acute promyelocytic leukaemia)
Shock	Trauma Severe burns
Transfusion	ABO-mismatched transfusion
Miscellaneous	Snake bites (some) Liver cirrhosis

Further reading

Levi MM (2016). Disseminated intravascular coagulation. <http://www.emedicine.com/emerg/topic150.htm>.

Platelet function tests

These are specialized tests carried out by the coagulation laboratory for the investigation of patients with suspected platelet dysfunction. Because of their complexity, platelet function tests will not be described in detail here.

Patients generally present with bleeding or bruising problems and have had normal coagulation results. Because of the labour-intensive nature and cost of these assays, you will need to arrange these tests after discussion with your local haematology medical staff.

- **Sample:** blood collection needs to be optimal with non-traumatic venepuncture, rapid transport to the laboratory with storage at room temperature and testing within a maximum of 2–3 h.

Current tests

- Platelet count.
- Morphology.
- Adhesion.
- Aggregation.
- Platelet release.
- Bleeding time.

Platelet count

Normal range $150\text{--}400 \times 10^9/\text{L}$. Adequate function is maintained, even when the count is <0.5 normal level but progressively deteriorates as it drops. With platelet counts $<20 \times 10^9/\text{L}$, there is usually easy bruising and petechial haemorrhages (although more serious bleeding can occur).

Morphology

Large platelets are biochemically more active; ↑ mean platelet volume (MPV >6.5) is associated with less bleeding in patients with severe thrombocytopenia. Altered platelet size is seen in inherited platelet disorders.

Platelet adhesion

Adhesion to glass beads now rarely performed in routine laboratory practice, but potentially useful in vWD diagnosis.

Platelet aggregation

Most useful of the special tests; is performed on a fresh sample using an aggregometer.

Aggregants used

- Adenosine 5-diphosphate (ADP) at low and high concentrations.
Induces two aggregation waves: the 1° wave may disaggregate at low concentrations of ADP; the second is irreversible.
- Collagen has a short lag phase, followed by a single wave, and is particularly affected by aspirin.
- Ristocetin-induced platelet aggregation (RIPA) is carried out at high (1.2mg/mL) and lower concentrations and is mainly used to diagnose vWD.
- Arachidonic acid.
- Adrenaline (epinephrine), not uncommonly reduced in normal people.

Platelet release

Enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) are used to measure the granule proteins β -thromboglobulin (β -TG) and heparin neutralizing activity (HNA). These are sensitive markers of platelet hyper-reactivity and beyond the scope of the routine laboratory.

Practical application of tests

Their main role is in diagnosis of inherited platelet functional defects. In acquired platelet dysfunction 2° to causes such as renal and hepatic disease, DIC, and macroglobulinaemia, platelet function is rarely tested.

Further reading

Yardumian DA, Mackie IJ, Machin SJ. Laboratory investigation of platelet function: a review of methodology. *J Clin Pathol* 1986; 39: 701–12.

Thrombophilia screening

Thrombophilia describes acquired or inherited disorders that predispose to arterial or venous thromboembolism (VTE). Thrombophilia should be considered in young patients who have apparent strong family history of VTE.

- Causes: Recurrent thrombosis, p. 92.

Which patients should be screened for possible thrombophilia?

- Arterial thrombosis, consider antiphospholipid antibody and lupus anticoagulant testing.
- Venous thrombosis:
 - Patients <40 years from thrombosis-prone families.
 - Family history of thrombosis with high-risk thrombophilia in first-degree relative.
 - Unusual site, e.g. mesenteric, portal vein thrombosis.
 - Children with purpura fulminans.
 - Recurrent miscarriage (three or more).
 - VTE in pregnancy and the OCP.

Screen

- Exclude medical causes (check ESR, LFTs, AIP, fasting lipids).
- FBC (exclude thrombocytosis).
- Clotting screen for acquired defects (PT, APTT, LA/anticardiolipin antibody (ACL), ↑ fibrinogen).
- Screen for inherited thrombophilia:
 - First-line protein C (PC), protein S (PS), antithrombin (AT), activated protein C resistance (APCR).
 - Check for the presence of the factor V Leiden mutation in APCR +ve patients (DNA analysis).
 - Consider testing plasminogen, fibrinogen, homocysteine levels, prothrombin variant.
- DNA analysis for prothrombin gene mutation.

Thrombophilia investigations are time-consuming and expensive, and you should discuss with the local haematology medical or laboratory staff before sending samples. Note: some thrombophilia tests cannot be carried out in the 'acute' phase of a VTE event or whilst the patient is taking an anticoagulant.

Further reading

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- Cattaneo M, Monzani ML, Martinelli I, Falcon CR, Mannucci PM. Interrelation of hyperhomocyst(e)tinemia, factor V Leiden, and risk of future venous thromboembolism. *Circulation* 1998; **97**: 295–6.
- Chong LY, Fenu E, Stansby G, Hodgkinson S. Management of venous thromboembolic diseases and the role of thrombophilia testing: summary of NICE guidance. *BMJ* 2012; **344**: e3979.
- Dahlback B. Resistance to activated protein C as risk factor for thrombosis: molecular mechanisms, laboratory investigation, and clinical management. *Semin Hematol* 1997; **34**: 217–34.
- Lane DA, Mannucci PM, Bauer KA, et al. Inherited thrombophilia: Part 1. *Thromb Haemost* 1996; **76**: 651–62.
- Lane DA, Mannucci PM, Bauer KA, et al. Inherited thrombophilia: Part 2. *Thromb Haemost* 1996; **76**: 824–34.

Antithrombin and proteins C and S

These proteins are the body's *natural anticoagulants*; hence, deficiencies may lead to thromboembolic disease.

Antithrombin

Used to be called ATIII, but there was never an ATI or ATII, so now abbreviated to AT. A useful measure in thrombophilia screening since low levels of AT are found in 4.5% of patients with unexplained VTE.

Decreased antithrombin levels

- Hereditary (40–60% normal level), autosomal dominant.
- Chronic liver disease.
- Protein wasting disorders.
- Heparin therapy.
- Third trimester of pregnancy.
- Acute leukaemia.
- Burns.
- Renal disease.
- Gram –ve sepsis.

Protein S

- Reduced levels predispose to VTE. Individuals with 30–60% normal level may suffer recurrent thrombosis.
- ↓ PS.
- Inherited (autosomal dominant).
- Pregnancy.
- Oral anticoagulants, e.g. warfarin.
- Nephrotic syndrome.
- Liver disease.

Protein C

- Similar to PS; autosomal dominant inheritance in genetic cases.
- ↓ PC.
- Hereditary.
- Liver disease.
- Malignancy.
- Warfarin therapy.
- Pregnancy.

Bone marrow examination

This is a key investigation in haematology. It may be diagnostic in the follow-up of abnormal peripheral blood findings and is an important staging procedure in defining the extent of disease, e.g. lymphomas. It is a helpful investigative procedure in unexplained anaemia, splenomegaly, or selected cases of POU.

- Preferred sites: the posterior iliac crest is the usual site (allows an aspirate and a biopsy to be obtained). The sternum is suitable only for marrow aspiration and is not a test for the squeamish.

The marrow aspirate provides

- Cytology of nucleated cells.
- Qualitative and semi-qualitative analysis of haematopoiesis.
- Assessment of iron stores (if Perls' iron stain used).
- Smears for cytochemistry (helps in the diagnosis of leukaemias).

Marrow cells can also be used for

- Chromosomal (cytogenetic) analysis.
- Immunophenotype studies using MoAbs.

Marrow trephine biopsy provides information about

- Marrow cellularity.
- Identification and classification of abnormal cells.
- Immunohistochemistry on infiltrates.

Contraindications

None, other than physical limitations, e.g. pain or restricted mobility. Avoid sites of previous radiotherapy (inevitably grossly hypocellular and not representative). (See Table 3.13.)

Table 3.13 Tests carried out on bone marrow

Test	Purpose
Chromosomes	Standard cytogenetic analysis, looking for rearrangements suggestive of acute or chronic leukaemias and myelodysplasia
	Fluorescence <i>in situ</i> hybridization (FISH) looking for additions or losses of chromosomes, as well as more subtle changes seen in leukaemias and lymphomas
DNA or RNA analysis	Using PCR to look for mutations or translocations that help classify leukaemias and lymphomas; also useful for monitoring disease levels in some disorders
Immunophenotype	Cell surface marker profile helps in the diagnosis of most leukaemias and lymphomas; may also be used to monitor disease levels post-treatment
Microbiology	For example, TB culture (not routine, but occasionally useful in cases of POU)
Cytochemical stains	To help define type of leukaemia

Procedure

1. BM aspiration may be performed under local anaesthetic (LAn) alone, but short-acting IV sedative (e.g. midazolam) is preferred when a trephine biopsy is performed. General anaesthetic used in children.
2. Place the patient in the (left) lateral position, or use the right side if s/he cannot lie on the left side.
3. Infiltrate the skin and periosteum over the posterior iliac spine with LAn.
4. Make a small cutaneous incision before introducing the aspirating needle, which should penetrate the marrow cortex 3–10mm before removal of the trocar.
5. Aspirate no more than 0.5–1mL of marrow initially (to avoid dilution of the sample with blood).
6. Make smears promptly (► the sample clots rapidly!).
7. If further samples are needed, e.g. for immunophenotyping, cytogenetics, etc., these can be aspirated after making initial slides.
8. For trephine biopsy, use an Islam or Jamshidi needle.
9. Advance the needle through the same puncture site to penetrate the cortex.
10. Remove the trocar and, using firm hand pressure, rotate the needle clockwise and advance as far as possible.
11. Remove the needle by gentle anticlockwise rotation.
12. Following the procedure, apply simple pressure dressings.
13. Minor discomfort at the location may be dealt with by simple analgesia such as paracetamol.

Cytochemistry tests (leukaemia diagnosis)

These staining methods have been around for many years (for decades, they were all that was available) but remain extremely useful in the diagnosis and classification of leukaemias. Modern technologies, such as flow cytometry and nucleic acid analysis, have refined leukaemia and lymphoma diagnosis, but the examination of well-stained cytochemistry BM smears remains the cornerstone of good haematology practice.

After performing a BM aspirate and spreading the material onto glass slides, the air-dried, unfixed microscope slides are passed to the cytochemistry laboratory that will fix and stain the slides according to the likely diagnosis (e.g. stains for AML differ to those for ALL; see Tables 3.14 and 3.15). Positive results with particular stains will point to a specific diagnosis. This will then be augmented by flow cytometric or molecular assays (see Fig. 3.16).

Table 3.14 Cytochemical stains

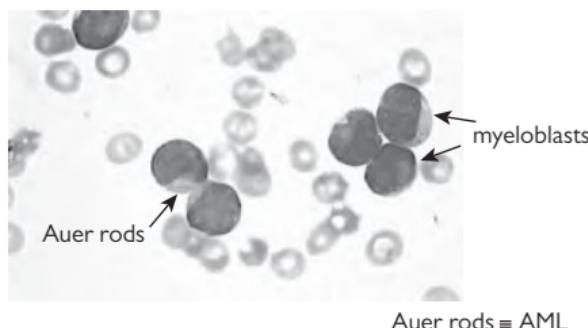
Cytochemical stain	Substrate/cell
Myeloperoxidase (MPO)	Lysosomal enzyme found in neutrophils and monocytes
Sudan black (SB)	Phospholipids in neutrophil granules
Chloroacetate esterase	Stain-specific esterase in granulocytes and mast cells. Makes it easier to diagnose AML M4 subtype
α -naphthyl acetate esterase (ANAE)	Esterase stain, useful for diagnosis of AML subtypes
Acid phosphatase	Enzyme found in many different WBCs. Useful for T-cell malignancies
Periodic acid–Schiff (PAS)	Detects glycogen in cells. Granulocytes have diffuse staining, whereas lymphocytes staining is much coarser

Table 3.15 Cytochemical stains in acute leukaemia

	Acute lymphoblastic leukaemia		Acute myeloid leukaemia		
	B lineage	T lineage	M1–3	M4–5	M6–7
MPO	–	–	+/++	+	–
SB	–	–	+/++	+	–
Chloroacetate esterase	–	–	-/+	–	–
ANAE	–	–	–	++	–
Acid phosphatase	–	+ (focal)	–	+ (diffuse)	+ (focal)
PAS	+ (blocks)	–	–	+ (fine granular)	+

+, positive; ++, strongly positive; –, negative.

From Hoffbrand AV, Lewis SM, Tuddenham EGD. *Postgraduate haematology*, fourth edition, Oxford: Butterworth-Heinemann, 2000.



Auer rods ≡ AML

Fig. 3.16 AML marrow showing myeloblasts (leukaemic cells) and an Auer rod in one cell. Auer rods are pathognomonic of AML, since they do not occur in any other disorder.

Further reading

Cytochemistry. <https://clinicalgate.com/cytochemistry/>

Neutrophil alkaline phosphatase

Uses

This is a cytochemical stain used to demonstrate the presence and quantity of the neutrophil enzyme ALP. Historically, the NAP score was of value in differentiating 'reactive' states from myeloproliferative disorders such as CML, PRV, etc.—now more often it features in examination multiple choice questions! (Note: sometimes termed leucocyte alkaline phosphatase, LAP.)

Procedure

Best performed on fresh blood films, made without the use of anticoagulant. EDTA samples may be used but are less satisfactory. The film should be made, air-dried, fixed, and then stained—all within 30min. Positive NAP activity is indicated by the presence of bright blue granules in the neutrophil cytoplasm (the nucleus is stained red; see Fig. 3.17).

- **Scoring:** films are scored from 0 to 4 on the basis of stain intensity:
 - 0: -ve, no granules seen.
 - 1: weak +ve, few granules.
 - 2: +ve, few to moderate numbers of granules.
 - 3: strongly +ve.
 - 4: very strong.

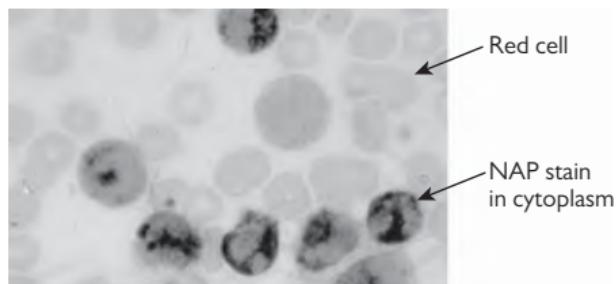


Fig. 3.17 NAP-stained blood film showing positively stained neutrophils. Red cells do not take up the stain.

Interpretation and significance

(See Table 3.16.)

Table 3.16 NAP

High NAP score	Low NAP score
PRV	CML
Leukaemoid reaction	PNH
Neutrophilia—any cause	AML
Myelofibrosis	
Essential thrombocythaemia	
Hepatic cirrhosis	
Hodgkin's disease	
Aplastic anaemia	
Down's syndrome	
Cushing's disease	

The NAP score is affected by corticosteroids, oestrogens, and pregnancy (\uparrow NAP). In Hodgkin's disease, the NAP score offers no advantage over simpler tests, such as ESR, for assessment of disease activity. Occasionally of value in a patient with aplastic anaemia who is developing PNH—the NAP score is seen to fall (both of these are very rare disorders). NAP score has been replaced in most hospitals by flow cytometry and other methods.

Further reading

Lewis SM, Bain BJ, Bates I (eds.). *Dacie & Lewis Practical Haematology*, 9th edn, Edinburgh: Churchill Livingstone, 2001.

Blood transfusion

Due to space limitation, it is inappropriate to go into major details about the investigations used in transfusion medicine. However, we have provided the more important tests in current use which include:

- Blood group and antibody screen.
- Cross-match (compatibility test).
- DAT.
- Antiplatelet and antineutrophil antibody testing.

Safe transfusion practice

Each year, patients are transfused with the wrong blood. In 2015, seven patients were given ABO-incompatible blood, whilst 280 patients were given incorrect blood components (not just ABO-incompatible) in 2015.⁴ Note that reporting is currently voluntary and very likely underestimates actual incidents. However, with 2.5 million blood components transfused annually, it is a small percentage overall. Pulmonary complications, particularly transfusion-associated circulatory overload (TACO), are a major cause of morbidity and death. However, it is also clear that delay in appropriate transfusion also contributes to mortality.

A common error is clerical and generally involves the cross-matched sample being taken from the wrong patient, and so the compatibility test is performed on the wrong sample. Occasionally, the staff carrying out the transfusion connect the blood up to the wrong patient. In any event, the result varies from no symptoms to shock and possible death.

How to minimize errors

- First, ask yourself, 'Does this patient really need to be transfused with blood or blood products (e.g. fresh frozen plasma (FFP), platelets, etc.)?' For example, a post-operative patient who is asymptomatic with Hb of 9g/dL probably does not require red cell transfusion. Use clinical judgement in helping decide whether or not to proceed with transfusion.
- Before taking the blood sample, check that you are taking blood from the correct patient—ask for his/her name and check the identity bracelet.
- Label the patient's blood bottle at the bedside (i.e. no prelabelling of bottles). Many transfusion laboratories insist on 1, 2, 5, 6, and 7, and either 3 or 4 from:
 1. Surname and forename (correctly spelt)
 2. DoB
 3. Hospital/A&E/new NHS number
 4. First line of address
 5. Sex
 6. Time and date blood taken
 7. Signature of person taking blood

⁴ Serious Hazards of Transfusion (SHOT) Steering Group (2016). *The 2015 annual SHOT report.* ↗ <https://www.shotuk.org/wp-content/uploads/SHOT-2015-Annual-Report-Web-Edition-Final-bookmarked.pdf>; *Annual SHOT report 2015 summary.* ↗ https://www.shotuk.org/wp-content/uploads/SHOT-Summary-Infographic_Final.pdf.

- Ensure details on the form match those on the bottle.
- Complete the request form properly:
 - State what is required (e.g. 2U of packed cells, etc.).
 - Detail any previous transfusions, reactions, antibodies (if known).
 - Let the laboratory know when you want the blood or blood product.

►► Adhesive patient labels are fine for forms but are not suitable for specimen bottles, and they are usually not accepted by transfusion laboratories. Transfusion specimens should be labelled by hand—at the bedside.

The TACO checklist should be used to identify those at risk. Defer transfusion, if safe, pending assessment or treatment of risk factor. Single unit transfusion, followed by a review, is preferable.

The bedside checklist should be used to:

- Confirm +ve patient identification.
- Check identification of the component against the patient's wristband.
- Check the prescription.
- Check the component.
- Check for specific requirements.

If this sounds cumbersome and bureaucratic

Remember many people die annually because they are transfused with the *wrong blood*. In most cases, clerical error is to blame—people have filled out bottles in advance and failed to check patient identity.

Further reading

- Estcourt LJ, Birchall J, Allard S, et al.; British Committee for Standards in Haematology. Guidelines for the use of platelet transfusions. *Br J Haematol* 2017; **176**: 365–94.
- Hunt BJ, Allard S, Keeling D, Norfolk D, Stanworth SJ, Pendry K; British Committee for Standards in Haematology. A practical guideline for the haematological management of major haemorrhage. *Br J Haematol* 2015; **170**: 788–803.
- Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee (JPAC).  <http://www.transfusionguidelines.org.uk/>.
- McClelland B (2001). *Handbook of transfusion medicine*, second edition. London: HMSO.
- NHS Blood and Transplant.  <http://www.blood.co.uk/>.
- Scottish National Blood Transfusion Service.  <http://www.scotblood.co.uk/>.

►► Transfusion reactions

(See Table 3.17.)

Table 3.17 Transfusion reactions

Symptoms	Signs
Patient restless/agitated	Fever
Flushing	Hypotension
Anxiety	Oozing from wounds or venepuncture sites
Chills	Haemoglobinaemia
Nausea and vomiting	Haemoglobinuria
Pain at venepuncture site	
Abdominal, flank, or chest pain	
Diarrhoea	

Rapid fever, chill, rigor, hyper- or hypotension, collapse, flushing, urticaria, or respiratory distress at the start of transfusion indicate transfusion should be stopped and resuscitation initiated (suggests a severe or life-threatening acute transfusion reaction).

If the temperature rises to above 39°C or >2°C from baseline, with other signs/symptoms, consider bacterial contamination and monitor the patient carefully. Investigate as appropriate.

- If an isolated temperature of >38°C and a 1–2°C rise with no symptoms, or rash only, providing the patient is not acutely unwell, continue the infusion. Fever often due to antibodies against WBCs (or to cytokines in platelet packs).

►► Immediate transfusion reaction

Intravascular haemolysis (→ haemoglobinaemia and haemoglobinuria). Usually due to anti-A or anti-B antibodies (in ABO-mismatched transfusion). Symptoms occur in minutes/hours. *May be fatal.*

Immediate transfusion reaction or bacterial contamination of blood

If predominantly extravascular, may only suffer chills/fever 1h after starting transfusion—commonly due to anti-D. ARF is not a feature.

Mechanism

Complement (C3a, C4a, C5a) release into recipient plasma → smooth muscle contraction. May develop DIC or oliguria (10% of cases) due to profound hypotension.

Initial steps in management of acute transfusion reaction

- Stop blood transfusion immediately.
- Replace the giving set; keep the IV access open with 0.9% saline.

- Check the patient identity against the donor unit.
- Insert a urinary catheter and monitor the urine output.
- Give fluids (IV colloids) to maintain urine output $>1.5\text{mL/kg/h}$.
- If urine output $<1.5\text{mL/kg/h}$, insert a CVP line and give a fluid challenge.
- If urine output $<1.5\text{mL/kg/h}$ and CVP adequate, give furosemide 80–120mg.
- If urine output still $<1.5\text{mL/kg/h}$, consult senior medical staff for advice.
- Contact the blood transfusion laboratory before sending back the blood pack and for advice on blood samples required for further investigation (☞ **Urgent investigations below**).

Complications

- Overall mortality ~10%.

Urgent investigations

Your local blood transfusion department will have specific guidelines to help you with the management of an acute reaction. The following guide lists the samples commonly required to establish the cause and severity of a transfusion reaction (see Box 3.1). If you are uncertain about the laboratory procedure or management of a patient who appears to have suffered a severe reaction, you must notify your hospital's haematology medical staff who will provide advice.

►► Delays may threaten the patient's life.

Febrile transfusion reactions

Seen in 0.5–1.0% of blood transfusions. Mainly due to anti-HLA (human leucocyte antigen) antibodies in recipient serum or granulocyte-specific antibodies (e.g. sensitization during pregnancy or previous blood transfusion).

Box 3.1 Investigation of transfusion reaction

1. Check the compatibility label of the blood unit matches with the patient's identity band, forms, and case notes.
2. If mistake found, tell the blood bank urgently—the unit of blood intended for your patient may be transfused to another patient.
3. Take blood for:
 - Haematology:
 - FBC.
 - DAT.
 - Plasma Hb.
 - Repeat cross-match sample.
 - Coagulation screen.
 - Chemistry: U&E.
 - Microbiology: blood cultures.
4. Check urinalysis and monitor urine output.
5. Do ECG and check for evidence of $\uparrow [K^+]$.
6. Arrange repeat coagulation screens and biochemistry 2- to 4-hourly.

Delayed transfusion reaction

Occurs in patients immunized through previous pregnancies or transfusions. Antibody weak (so not detected at pre-transfusion stage). 2° immune response occurs—antibody titre ↑.

Symptoms and signs

- Occur 7–10 days after blood transfusion.
- Fever, anaemia, and jaundice.
- ± haemoglobinuria.

Management

- Discuss with the transfusion laboratory staff.
- Check DAT and repeat compatibility tests.
- Transfuse the patient with freshly cross-matched blood.

Further reading

Kardon EM (2016). *Transfusion reactions in emergency medicine*.  <http://www.emedicine.com/emerg/topic603.htm>.

Bacterial contamination of blood products

Uncommon, but potentially fatal, adverse effect of blood transfusion (affects red cells and blood products, e.g. platelet concentrates). Implicated organisms include Gram -ve bacteria, including *Pseudomonas*, *Yersinia*, and *Flavobacterium*.

Features

Include fever, skin flushing, rigors, abdominal pain, DIC, ARF, shock, and possible cardiac arrest.

Management

As per *Immediate transfusion reaction* (↗ p. 304):

- Stop transfusion.
- Urgent resuscitation.
- IV broad-spectrum antibiotics if bacterial contamination suspected.

Antiglobulin test

The old term is Coombs' test. DAT detects antibodies or complement, or both, on the RBC surface, and the indirect antiglobulin test (IAT) detects the presence of antibodies in serum. A useful investigation when investigating haemolytic anaemia.

- Sample: EDTA.

Interpretation

- Positive DAT in most AIHA.
- Lymphoproliferative disorders, e.g. CLL.
- Drug-induced haemolysis (e.g. methyldopa, levodopa).
- Haemolytic disease of the fetus and newborn (HDFN), e.g. rhesus (Rh) HDN.

Note: as with many tests in medicine, things are never entirely black or white—a +ve DAT does not necessarily imply that haemolysis is actively occurring and a -ve DAT does not exclude haemolysis.

Further reading

Coombs RRA. A new test for the detection of weak and 'incomplete' Rh agglutinins. *Br J Exp Pathol* 1945; 26: 255–66.

Kelton JG. Impaired reticuloendothelial function in patients treated with methyldopa. *N Engl J Med* 1985; 313: 596–600.

Kleihauer test

Uses

To determine whether fetal red cells have entered the maternal circulation and, if so, the volume of such fetal cells.

Background

If an Rh (D) -ve mother has a baby that is Rh (D) +ve, she may develop antibodies (maternal anti-D) against fetal red cells. This may result in fetal red cell destruction termed *rhesus haemolytic disease of the newborn*, a serious haemolytic disorder that is seen less today due to a greater understanding of the underlying mechanism and our ability to prevent it. Sensitization to the fetal red cells occurs when fetal RBCs enter the maternal circulation, e.g. at birth or through obstetric manipulations, e.g. amniocentesis, previous pregnancies, etc.

Fetal RBCs in the mother's circulation can be detected and quantified (in mL) using the Kleihauer test, which exploits the resistance of fetal red cells to acid elution (acid washes adult Hb out of the mother's red cells, but fetal RBCs contain HbF, which is not washed out). The Kleihauer test should be performed on all Rh (D) -ve women who deliver an Rh (D) +ve infant.

Fetal cells appear as darkly staining cells against a background of ghosts (these are the maternal red cells). 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.

Calculating the volume of fetal red blood cells in maternal circulation

Basically, a calculation is made by the laboratory staff, based on the number of fetal RBCs seen in the Kleihauer film. The actual calculation is:

$$1800^* \times \text{ratio of fetal/adult RBCs} \times 4/3 \text{ (correction factor)}$$

For example, if there are 1% of fetal RBCs in maternal circulation:

$$1800 \times 1/100 \times 4/3 = 24\text{mL}$$

(* where 1800 is the maternal red cell volume)

A 4mL bleed (i.e. 4mL of fetal RBCs) requires 500IU of anti-D given IM to the mother, with a further 250IU of anti-D for each additional mL of fetal RBCs.

Do not panic!

The laboratory carrying out the Kleihauer test will tell you the volume of fetal RBCs detected, since they will count the cells and do the calculation for you. If the total is >2mL of fetomaternal haemorrhage, the maternal sample is sent for FMH confirmation by flow cytometry. After this, you will need to calculate the dose of anti-D to give the mother, but if you are unsure, either discuss with the haematology medical staff or contact your local transfusion centre.

Further reading

Chanarin I (ed.). *Laboratory Haematology: An Account of Laboratory Techniques*. Edinburgh: Churchill Livingstone, 1989.

Erythropoietin assay

Epo is the hormone produced largely by the kidney that drives red cell production. The typical anaemia found in renal disease is a result of failure of Epo production. Epo assays are of value in renal medicine and haematology. For example, in the assessment of polycythaemic states, an ↑ Epo level may be appropriate (e.g. in hypoxia where the body is attempting to ↑ O₂ availability to tissues) or inappropriate (e.g. some tumours). The Epo assay is carried out using an RIA method and is not available in all haematology laboratories (may need to be sent to another hospital or laboratory).

- *Normal range:* 35–25mU/mL, steady-state level, no anaemia. May rise to 10,000mU/mL in hypoxia or anaemia.

Causes of ↑ Epo (appropriate)

- Anaemias.
- High altitude.
- Hypoxia:
 - Lung disease.
 - Sleep apnoea syndromes.
- Cyanotic heart disease (e.g. right → left shunts).
- High-affinity Hb.
- Cigarette smoking.
- Methaemoglobinemia.

Causes of ↑ Epo (inappropriate)

- Renal disease:
 - Hypernephroma.
 - Nephroblastoma.
 - Post-renal transplant.
 - Renal cysts.
 - RAS.
- Hepatoma.
- Uterine fibroids.
- Cerebellar haemangioblastoma.
- Phaeochromocytoma.

Other causes of ↑ Epo

- Androgen therapy.
- Cushing's disease.
- Hypertransfusion.
- Neonatal polycythaemia.

Causes of ↓ Epo

- Renal failure.
- Polycythaemia vera.
- RhA and other chronic inflammatory diseases.
- Myeloma and other cancers.

Further reading

Cotes PM, Doré CJ, Yin JA, et al. The use of immunoreactive erythropoietin in the elucidation of polycythemia. *N Engl J Med* 1986; 315: 283–7.

Immunohaematology

Immunohaematology is the study of the effects of the immune system on the blood and its components. This includes red cells, white cells, platelets, and coagulation proteins.

Tests for antiplatelet and antineutrophil antibodies

These tests are usually requested by the haematology department for patients with either thrombocytopenia or neutropenia, respectively. These assays are used to detect the presence of specific antibodies against platelet or neutrophil antigens on the cell surface.

Antibodies may be *alloantibodies* (e.g. antibodies produced by the mother against fetal antigens) or *autoantibodies*, which are antibodies produced by the patient against his/her own antigens (see Table 3.18).

Antiplatelet antibody tests

Generally platelet immunofluorescence tests (PIFTs) or monoclonal antibody immobilization of platelet antigens (MAIPA) are used. These are useful for detecting even weak antibodies or where there are only a few antigenic sites per cell.

Elegant though these tests are, they are actually not useful in clinical practice for the diagnosis of neutropenia or thrombocytopenia where the cause is autoimmune, since these are largely clinical diagnoses. (Platelet-associated IgG or IgM may be high in autoimmune thrombocytopenia. However, it may also be high in non-immune causes of thrombocytopenia.) Where these tests are of value is in the neonatal setting where the neonate has low platelets or neutrophils.

Table 3.18 Disorders with neutrophil-specific allo- and autoantibodies

Disorders with neutrophil-specific alloantibodies

- Neonatal alloimmune neutropenia
- Febrile transfusion reactions (HLA antibodies)
- Transfusion-related acute lung injury (TRALI)

Disorders with neutrophil-specific autoantibodies

- 1° autoimmune neutropenia
- 2°:
 - SLE
 - Evans' syndrome (AIHA + ↓ platelets)
 - Lymphoproliferative disorders (e.g. CLL)
 - Immune dysfunction (e.g. HIV, graft-versus-host disease)

Further reading

Roitt I. *Essential Immunology*, 10th edn. Oxford: Blackwell Science, 2001.

Immunophenotyping

This describes the identification and counting of cell types using powerful MoAbs specific for cell surface proteins.

Uses

(See Table 3.19.)

- Diagnosis and classification of leukaemias and lymphomas.
- Assessment of cellular DNA content and synthetic activity.
- Determination of lymphocyte subsets, e.g. CD4⁺ T cells in HIV infection.
- Assessment of clonality.
- Allows identification of prognostic groups.
- Monitoring of minimal residual disease (MRD, the lowest level of malignancy that can be detected using standard techniques).

Terminology and methodology

Cell surface proteins are denoted according to their cluster differentiation (CD) number. Most cells will express many different proteins, and the pattern of expression allows cellular characterization. MoAbs recognize specific target antigens on cells. Using a panel of different antibodies, an immunophenotypic *profile* of a sample is determined. Immunophenotyping is used in conjunction with standard morphological analysis of blood and marrow cells. The antibodies are labelled with fluorescent markers, and binding to cell proteins is detected by laser. For each analysis, thousands of cells are assessed individually and rapidly. Some antibodies can detect antigens inside cells.

- Sample: heparin.

Table 3.19 Uses of immunophenotyping

Surface immunophenotyping	Leukaemias Lymphomas CD4:CD8 ratios in HIV infection
DNA content of tumours	Ploidy S phase analysis Proliferation markers
TdT measurement	In leukaemias and lymphomas
Bone marrow transplant/stem cell transplantation	
Antiplatelet antibody detection	
Reticulocyte counts and maturation	
Apoptosis	
Detection of small numbers of cells	For example, fetal cells in mother's circulation, microorganisms in blood

Data from Provan D et al. *Oxford Handbook of Clinical Haematology*, 2nd edn, Oxford: Oxford University Press, 2004.

Monoclonal antibodies

These are so-called because they 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. peripheral blood or BM samples are incubated with a panel of MoAbs, e.g. anti-CD4, anti-CD34, which are directly or indirectly bound to a fluorescent marker antibody.
- Sample is passed through a fluorescence-activated cell sorter (FACS) machine.
- FACS instruments assign cells to a graphical plot by virtue of cell size and granularity detected as forward and side light scatter by the laser.
- Allows subpopulations of cells, e.g. mononuclear cells, in blood samples to be selected.
- The reactivity of this cell subpopulation to the MoAb panel can then be determined by fluorescence for each MoAb.
- A typical result for a CD4 T-lymphocyte population is shown: CD3, CD4 +ve; CD8, CD13, CD34, CD19 -ve.

Leukaemia diagnosis: common patterns (profiles)

- AML: CD13+, CD33+, ± CD34, ± CD14 +ve.
- cALL: CD10 and TdT +ve.
- T-ALL: cCD3, 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. non-Hodgkin's lymphoma (NHL) will have surface expression of κ or λ light chains, but not both.
- ▶ Polyclonal B cells from, e.g. a patient with infectious mononucleosis will have both κ and λ expression.

Further reading

Clinical Flow Wiki. <http://wiki.clinicalflow.com/>.

Johansson U, Bloxham D, Couzens S, et al.; British Committee for Standards in Haematology. Guidelines on the use of multicolour flow cytometry in the diagnosis of haematological neoplasms. *Br J Haematol* 2014; **165**: 455–88.

University of Medicine and Dentistry of New Jersey. *Immunophenotyping lymphomas*. <http://pleiad.umdnj.edu/hemepath/immuno/immuno.html>.

Cytogenetics

Uses

- The study of chromosomes.
- Looks at the number of chromosomes in each cell.
- Detects structural abnormalities between chromosome pairs.

Chromosome abnormalities may be constitutional (inherited) or acquired later in life. Cytogenetic analysis of chromosome structure and number has been used for many years for the study of disorders such as Down's syndrome. Acquired chromosomal abnormalities are found in malignancies, especially haematological tumours. The analysis and detection of cytogenetic abnormalities is known as *karyotyping*. Because of the complexity of this subject area, we will concentrate on two main areas where chromosome analysis is of value.

- Prenatal diagnosis of inherited disorders:
 - Detection of common aneuploidies (gain or loss of chromosomes).
 - Detection/exclusion of known familial chromosome abnormalities.
- Detecting acquired chromosome abnormalities for:
 - Diagnosis of leukaemia subtypes, e.g. t(15;17) characteristic of AML M3 subtype.
 - Markers of prognostic information in a variety of diseases such as leukaemias, e.g. t(9;22) in acute leukaemias, *N-myc* amplification in neuroblastoma.
 - Monitoring response to treatment (in CML, the Philadelphia chromosome t(9;22) should disappear if the malignant cells are killed).

Principal indications for cytogenetic analysis are therefore

- Haematological malignancies at diagnosis (assuming the BM is infiltrated).
- Infiltrated solid tumour tissue at diagnosis.
- Patients with equivocal morphology (e.g. type of leukaemia not clear using microscopy and other markers).
- FISH analysis when required in certain treatment protocols, e.g. MRC.
- Confirmation of disease relapse.
- Accelerated phase or blast crisis in CML.

Cytogenetic assays are expensive (around £250 for a leukaemia or lymphoma karyotype), and if there is any doubt as to whether the test is indicated, we would suggest you discuss the case with one of your seniors or the cytogenetics staff. Arranging karyotyping before or during pregnancy is generally carried out by the obstetrician in charge of the woman's care.

(See Table 3.20.)

Table 3.20 Cytogenetic terminology

Constitutional	Present at conception or arising during embryonic life
Acquired	Arise later in fetal life or after birth
Translocation	Exchange of material between chromosomes
Deletion	Loss of part of a chromosome
Duplication	Part of a chromosome is gained
Inversion	Part of a chromosome is rotated through 180°
Diploid	46 chromosomes (somatic cell)
Haploid	23 chromosomes (germinal cell, e.g. egg or sperm)
Trisomy	Extra copy of a chromosome
Monosomy	Loss of a chromosome
Aneuploidy	Loss or gain of certain chromosomes, e.g. monosomy or trisomy

Cytogenetics: prenatal diagnosis

This allows both the detection of genetic diseases associated with specific chromosomal abnormalities, thereby offering the possible prevention of an affected child. With the advent of CVS in the first trimester, karyotyping can be done at an early stage of development (see Figs 3.18 and 3.19). Pre-implantation genetic diagnosis allows abnormalities to be detected even before implantation has occurred.

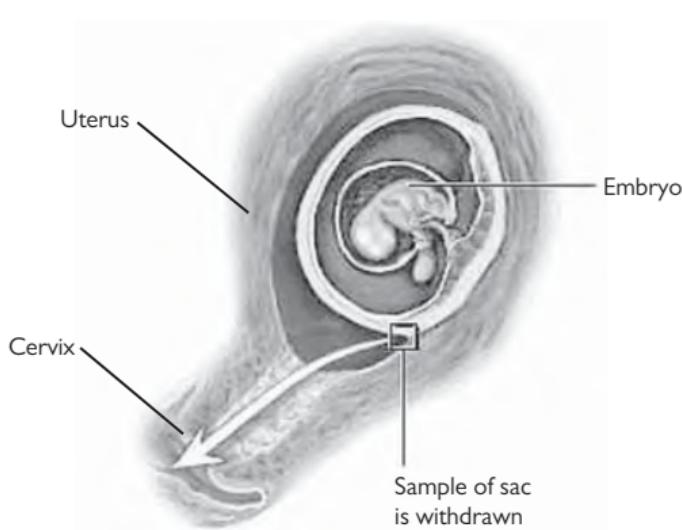


Fig. 3.18 Diagram showing method of chorionic villus sampling.

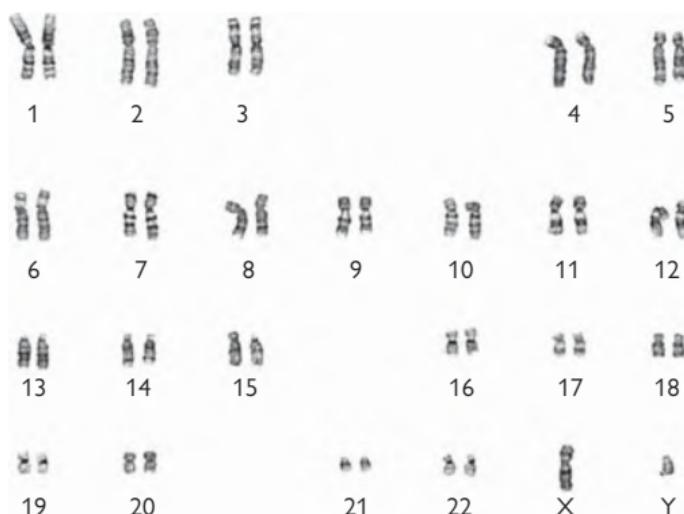


Fig. 3.19 Normal karyotype showing metaphase chromosomes (22 autosomes 1–22, and two sex chromosomes XX or XY, depending on the sex of the patient).

- Sample: amniotic fluid (15–16 weeks' gestation).

Tests available

- AFP level.
- Chromosome analysis.
- Biochemical tests, e.g. acetylcholinesterase.
- Sample: CVS (9–12 weeks' gestation).

Tests available

- DNA analysis.
- Chromosome analysis.
- Biochemistry tests.

Procedure (in brief)

1. Cells are obtained using amniocentesis, CVS, or fetal blood sampling.
2. Cells are cultured in medium.
3. Cell division is arrested at metaphase using, e.g. colchicine.
4. Chromosomes are spread onto slides and stained.
5. Chromosomes are examined directly using light microscopy or with the aid of a computerized image analysis system.

Chromosome anatomy

Note: the banding pattern helps identify individual chromosomes, along with the position of the centromeres (the mitotic spindle attaches to these during cell division), the short (*p*) and long (*q*) arms, and telomeres (chromosome ends). (See Fig. 3.20.)

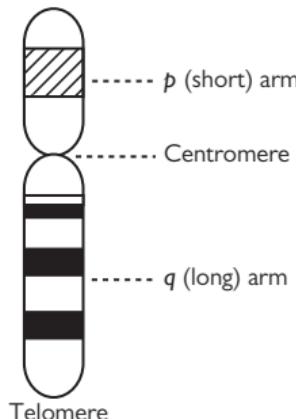


Fig. 3.20 Chromosome anatomy: note short (*p*) arms and long (*q*) arms.

Further reading

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Rooney DE. *Human Cytogenetics*, Vol 2, 2nd edn. Oxford: Oxford University Press, 2001.

University of Washington, Department of Pathology. *Cytogenetics gallery*. <http://www.pathology.washington.edu/galleries/Cytogallery/>.

Cytogenetics: haematological malignancies

Uses

- Aids the diagnosis and classification of haematological malignancy.
- Assessment of clonality.
- Identification of prognostic groups.
- Monitoring response to therapy.
- Determining engraftment and chimerism post-allogeneic transplant.

Terminology

- A normal somatic cell has 46 chromosomes: 22 pairs, and XX or XY.
- Numbered 1–22 in decreasing size order.
- Two arms meet at the centromere—short arm denoted *p*, long arm is *q*.
- Usually only visible during condensation at metaphase.
- Stimulants and cell culture used—colchicine disrupts the spindle apparatus, thereby arresting cells in metaphase.
- Chromosomes are G-banded using Giemsa or Leishman's stain to create characteristic banding patterns along the chromosome. The regions and bands are numbered, e.g. *p*1, *q*3, etc.

Common abnormalities

- Whole chromosome gain: e.g. trisomy 8 (+8).
- Whole chromosome loss: e.g. monosomy 7 (−7).
- Partial gain: e.g. add9q+, or partial loss, e.g. del5q−.
- Translocation: material exchanged with another chromosome; usually reciprocal, e.g. t(9;22)—the Philadelphia translocation.
- Inversion: part of chromosome runs in opposite direction, e.g. inv(16) in M4Eo.
- Many translocations involve breakpoints around known oncogenes, e.g. *bcr*, *ras*, *myc*, *bcl-2*.

(See Table 3.21.)

Molecular cytogenetics

- Molecular revolution is further refining the specific abnormalities in the genesis of haematological malignancies.
- Techniques such as FISH and PCR can detect cryptic abnormalities.
- 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 acute leukaemia, e.g. AML, e.g. PML–RARA in AML M3, t(9;22), t(12;21), and 11q23 rearrangements in paediatric ALLs.

Table 3.21 Karyotypic abnormalities in leukaemia and lymphoma

CML	
t(9;22)	Philadelphia chromosome translocation creates <i>bcr-abl</i> chimeric gene.
AML	
t(8;21)	AML M2, involves <i>AML-ETO</i> gene—has better prognosis
t(15;17)	AML M3 involves <i>PML-RARA</i> gene—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
Myeloproliferative disease	
20q- and +8	Common associations
ALL	
t(9;22)	Philadelphia translocation, poor prognosis
t(4;11)	Poor prognosis
Hyperdiploidy	↑ in total chromosome number—good prognosis
Hypodiploidy	↓ in total chromosome number—bad prognosis
T-ALL	
t(1;14)	Involves <i>tal-1</i> oncogene
B-ALL and Burkitt's lymphoma	
t(8;14)	Involves <i>myc</i> and <i>IgH</i> genes, poor prognosis
CLL	
+12, t(11;14)	
NHL	
t(14;18)	Follicular lymphoma, involves <i>bcl-2</i> oncogene
t(11;14)	Mantle cell lymphocytic lymphoma, involves <i>bcl-1</i> oncogene
t(8;14)	Burkitt's lymphoma, involves <i>myc</i> and <i>IgH</i> genes

Further reading

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 Sandberg AA. *The Chromosomes in Human Cancer and Leukemia*, 2nd edn. New York: Elsevier Science, 1990.

Human leucocyte antigen (tissue) typing

The HLA system or major histocompatibility complex (MHC) is the name given to the highly polymorphic gene cluster region on chromosome 6, which codes for cell surface proteins involved in immune recognition.

Uses

Tissue typing patients (to ensure compatibility between donor and recipient) who are undergoing transplantation to reduce the likelihood of rejection or graft-versus-host disease (GvHD) in the following types of transplant:

- Heart.
- Lung.
- Liver.
- Kidney.
- BM.
- Stem cells.

The gene complex is subdivided into two regions

Class 1

- The A, B, and C loci.
- These proteins are found on most nucleated cells and interact with CD8⁺ T lymphocytes.

Class 2

- Comprises DR, DP, and 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, although there is a small amount of cross-over.
- There is ~25% chance of two siblings being HLA identical.
- There are other histocompatibility loci apart from the HLA system, but these appear less important generally, except during HLA-matched stem cell transplantation 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. There are many problems with the technique and it is too insensitive to detect many polymorphisms. Molecular techniques are increasingly employed, such as single strand polymorphism (SSP). Molecular characterization is detecting enormous class 2 polymorphisms.

Importance of HLA typing

- Matching donor/recipient pairs for renal, cardiac, and marrow stem cell transplantation.
- Degree of matching more critical for stem cell than solid organ transplants.
- Sibling HLA-matched stem cell transplantation is now the treatment of choice for many malignancies.
- Unrelated donor stem cell transplants 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 (CTLp) assays: determine the frequency of cytotoxic T lymphocytes 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.
- Leucodepletion of products by filtration prevents this (the National Blood Service removes the WBCs at source routinely nowadays).
- Diagnosis of refractoriness confirmed by detection of HLA or platelet-specific antibodies in the patient's serum.
- Platelet transfusions matched to recipient HLA type may improve increments.

Further reading

- BSBMT. *Interpreting HLA typing and matching.* <http://bsbmt.org/registry-help-documents/>.
Klein J, Sato A. The HLA system. *N Engl J Med* 2000; **343**: 702–9. <http://content.nejm.org/cgi/content/full/343/10/702?ijkey=oOilwRsUqacag>.
Naik S. The human HLA system. *J Indian Rheumatol Assoc* 2003; **11**: 79–83. <http://medind.nic.in/jaa/t03/i3/jaat03i3p79.pdf>.

Southern blotting

This technique has been around since the mid 1970s. It explained much about the physical structure of genes and was a major advance in the diagnosis of many single gene disorders. The method is simple and elegant, but time-consuming. Not used as much today with the advent of PCR technology. Southern blotting relies on the physical nature of DNA whereby single strands are able to recognize and bind to their complementary sequences (see Fig. 3.21).

- Sample: EDTA sample (heparin can be used, but beware inhibitory effect on PCR amplification; if any chance PCR required, send EDTA).

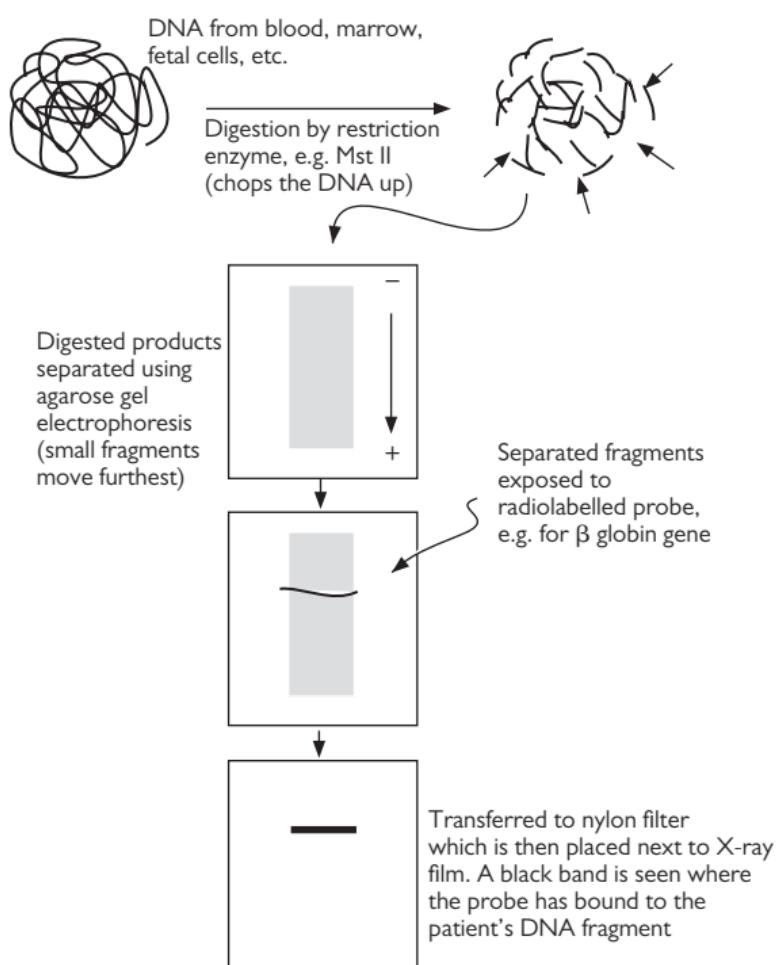


Fig. 3.21 Southern blotting method.

Procedure

1. Genomic (i.e. total) DNA is extracted from WBC in EDTA blood sample.
2. DNA is digested with bacterial restriction endonucleases (enzymes cleave DNA at specific sequences—each enzyme recognizes a different DNA sequence).
3. After digestion of the DNA, the fragments are separated on the basis of size using agarose gel electrophoresis (the smallest fragments travel the farthest).
4. The fragments are transferred to a nylon membrane and fixed permanently to the membrane using ultraviolet (UV) light.
5. Membranes are ‘probed’ using specific (known) gene probes that are radioactively labelled using ^{32}P .
6. The location of specific binding is detected by placing the membrane next to a radiographic film (standard X-ray film).
7. The film is developed using standard techniques, and the autoradiograph generated will show bands corresponding to the position of binding of the labelled probe.
8. Fragment sizes are calculated, and the presence or absence of mutations is worked out by determining whether enzyme cutting sites have been lost through mutation.

Applications

- Historically, many diseases caused by single base changes (loss of restriction enzyme cutting site) have been diagnosed using Southern blotting.
- Globin gene disorders:
 - Sickle-cell anaemia (mutation in β -globin gene).
 - Thalassaemia (mutations or deletions in α - or β -globin genes).
- Clotting disorders:
 - Haemophilia.
- Analysis of Ig or TCR genes to detect clones of cells in suspected leukaemia or lymphoma.
- Detection of chromosomal translocations in leukaemia and lymphoma (e.g. t(9;22) in CML, t(14;18) in follicular lymphoma).

Further reading

Kimball's Biology Pages. *Gel blotting*. <http://www.biology-pages.info/G/GelBlotting.html>.

Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975; 98: 503–17 (Southern’s classic paper and probably the most cited molecular biology paper ever).

Thermo Fisher Scientific. *Southern blotting*. <https://www.thermofisher.com/uk/en/home/life-science/dna-rna-purification-analysis/nucleic-acid-gel-electrophoresis/southern-blotting.html#>.

Polymerase chain reaction amplification of DNA

The ability to use an enzyme to amplify specific DNA sequences has revolutionized modern diagnostic pathology. Whereas Southern blotting might take up to 1 week to produce a result, PCR can do the same thing in 2–3 h! PCR is now in routine use in the analysis of oncogenes, haematological malignancies, general medicine, infectious disease, and many other specialties. Because the system amplifies the starting DNA up to a million-fold, there need only be one cell as starting material; in practice, much more DNA is required, but because of the extreme sensitivity of the technique, PCR has been used in forensic medicine where there may be only a few cells available for analysis (e.g. blood or semen stain).

(See Fig. 3.22.)

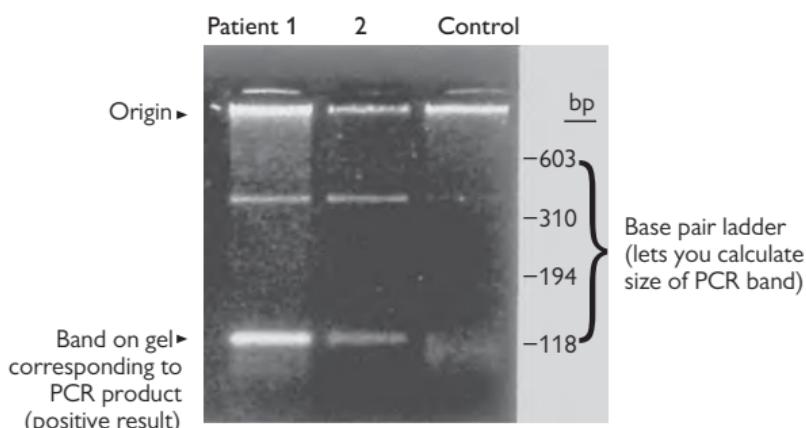


Fig. 3.22 Detection of residual leukaemia using PCR. Patients 1 and 2 have undergone chemotherapy, but as can be seen (arrow), there is still some leukaemia-specific DNA sequence present, i.e. they have minimal residual disease.

Advantages

- Requires very little DNA.
- DNA quality does not matter (can be highly degraded, e.g. with age and still be amplified—DNA from Egyptian mummies has been amplified).
- Rapid results.

Disadvantages

- Expensive, but less so than it used to be.
- DNA sequence of the gene of interest must be known in order to design the short PCR primers (oligos). With the near completion of the Human Genome Project, this is less of a problem now.
- Highly sensitive, and contamination of samples may occur (DNA fragments float through the air constantly; if these drop into the reaction tube, a false +ve result may be obtained).

Procedure (in brief)

(See Fig. 3.23.)

- Two short DNA primers on either side of the gene of interest bind to the fragment of interest.
- The region between the primers is filled in using a heat-stable DNA polymerase (Taq polymerase).
- After a single round of amplification has been performed, the whole process is repeated.
- This takes place 30 times (i.e. through 30 cycles of amplification), leading to a million-fold ↑ in the amount of specific sequence.
- After the 30 cycles are complete, a sample of the PCR reaction is run on agarose gel and bands are visualized.
- Information about the presence or absence of the region or mutation of interest is obtained by assessing the size and number of different PCR products obtained after 30 cycles of amplification.

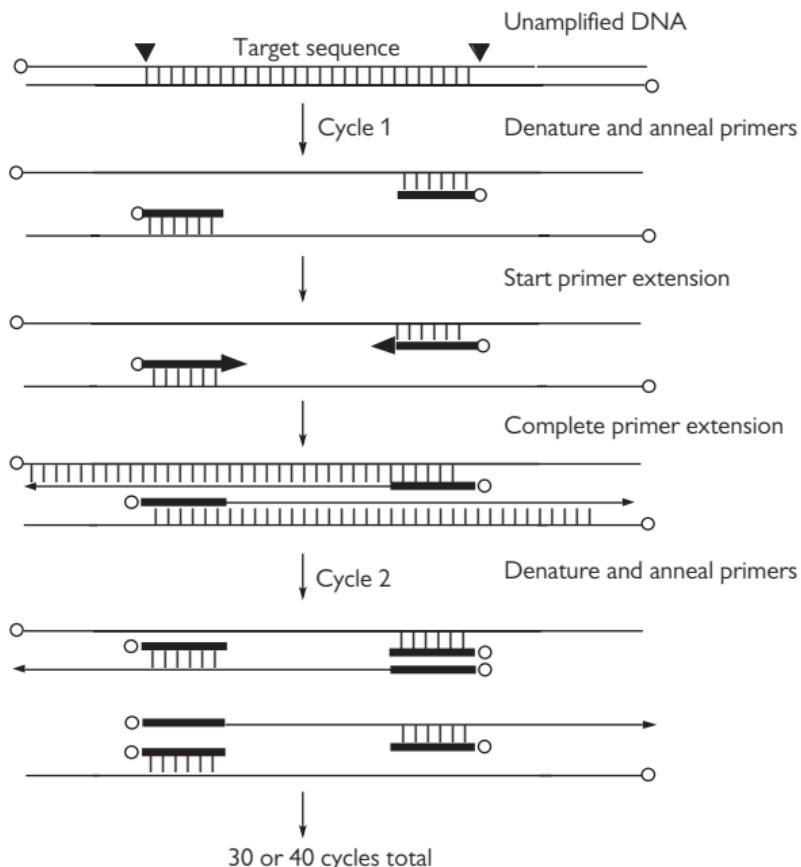


Fig. 3.23 PCR amplification method.

Applications

- PCR is currently used to amplify Ig genes, HIV loci, TB genes, and many other targets that are of use in molecular medicine (cystic fibrosis, haemophilia, thalassaemia, sickle-cell disease, and many others).
- PCR can be used to quantitate messenger ribonucleic acid (mRNA) species in blood samples and tissue samples. Allows gene 'activity' to be measured.

Further reading

Nobelprize.org. *The PCR method—a DNA copying machine.* <http://nobelprize.org/chemistry/educational/pcr/>.

Principles of the PCR. <http://users.ugent.be/~avierstr/principles/pcr.html>.

Saiki RK. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; 239: 487–91 (classic PCR method paper).

In situ hybridization and fluorescence in situ hybridization

Like PCR and other techniques, *in situ* hybridization and FISH are conceptually simple techniques that rely on the ability of a DNA probe to 'find' its counterpart on a chromosome and bind, and if a fluorescent tag is present, it will light up the region of binding (this modification is termed fluorescence *in situ* hybridization, or FISH). These techniques have evolved from standard cytogenetic analysis of metaphase chromosomes in which metaphase chromosomes were prepared on glass slides to which specific labelled probes were applied.

- *Sample:* discuss with your local cytogenetics or haematology laboratory (they will have specialized medium for maintaining cells from blood or marrow, so that they will divide and be suitable for hybridization studies).

***In situ* hybridization**

The location of binding of the probe is detected by visualizing the signal produced after coating microscope slides with photographic emulsion, which generates a black area around the probe which is labelled with ^{32}P .

FISH

A further modification based on the original principles, whereby specific gene probes are hybridized to chromosomes without the need for metaphase preparations (interphase cells can be used). Instead of ^{32}P , the probes are labelled with a fluorescent dye and hybridization may be detected as red, blue, or other coloured dots over the cells (see Fig. 3.24).

Applications of FISH

- Used in the analysis of trisomies (chromosome gains) and monosomies (chromosome losses) associated with leukaemias and lymphomas. The presence of trisomy is detected as three fluorescent dots within the cell, whilst monosity is seen as a single fluorescent dot within the cell.
- FISH has been used widely within paediatric leukaemias, such as ALL, where abnormalities of chromosome number are common.

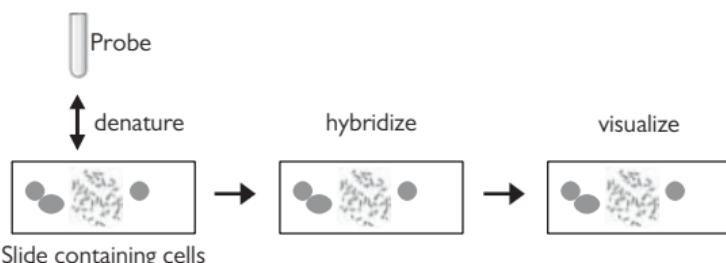


Fig. 3.24 FISH analysis. Metaphase chromosomes are placed on a microscope slide, and the probe (e.g. for the gene of interest) is applied. The chromosome region to which the probe binds will fluoresce—highlighting its exact location in the genome.

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- O'Connor C. Fluorescence *in situ* hybridization (FISH). *Nature Education* 2008; **1**: 171. <https://www.nature.com/scitable/topicpage/Fluorescence-In-Situ-Hybridization-FISH-327>.
- Sinclair PB, Green AR, Grace C, Nacheva EP. Improved sensitivity of BCR-ABL detection: a triple-probe three-color fluorescence *in situ* hybridization system. *Blood* 1997; **90**: 1395–402.
- Vaandrager JW. Direct visualization of dispersed 11q13 chromosomal translocations in mantle cell lymphoma by multicolor DNA fiber fluorescence *in situ* hybridization. *Blood* 1996; **88**: 1177–82.

Specialized haematology assays

The following laboratories provide specialized molecular, biochemical, and cellular investigations for rare haematological disorders. Please contact the laboratory before tests are requested to confirm the specimen(s) required.

Thalassaemia disorders

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Haemoglobin variants, unstable, and altered affinity haemoglobins

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Glycolytic defects, G6PD deficiency, other erythroenzymopathies***Dr Mark Layton***

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Serum immunoglobulins

Units: g/L.

Normal range (adults only):

- IgG: 5.8–15.4g/L.
- IgA: 0.64–2.97g/L.
- IgM (♂): 0.24–1.90g/L.
- IgM (♀): 0.75–2.30g/L.

Principles of assay

The three main classes of Igs are measured by either rate nephelometry or turbidimetry on automated analysers. The principles are similar, dependent on immune complex formation, using antisera specific for the class of antibody. Rarely radial immunodiffusion (RID) may be used; this is slow and less accurate. For automated analysers, coefficients of variation should be in the range of 5–10%. Results are standardized against international standards. In the UK, an external quality assurance (EQA) scheme operates. Laboratories should provide normal ranges, which vary according to age and sex. Unfortunately many laboratories do not adjust ranges for age and sex, which may lead to confusion.

Indications for testing

Measurement of serum immunoglobulin is indicated in the following conditions

- Suspected immunodeficiency (1° or 2°); diagnosis and monitoring.
- Suspected myeloma, plasmacytoma; diagnosis and monitoring.
- Lymphoma.
- Liver disease (PBC, hepatitis, cirrhosis).
- Sarcoidosis; diagnosis.
- Post-BM/stem cell transplantation; monitoring.

Interpretation

Measurement of serum Igs does not provide categorical diagnosis in any disease. Normal serum Igs do NOT exclude immunodeficiency. In all cases, measurement of Igs MUST be accompanied by serum electrophoresis and immunofixation to look for paraproteins (Electrophoresis and immunofixation, pp. 342–3).

Causes of hypogammaglobulinaemia

- X-linked agammaglobulinaemia (XLA) (absent B cells; all Igs low/absent).
- Common variable immunodeficiency (CVID) (reduced T/B cells; low Igs).
- Hyper-IgM syndrome (normal/raised IgM; low/absent IgG, IgA).
- Selective IgA deficiency (absent IgA; normal IgG, IgM).
- Severe combined immunodeficiency (SCID) (mainly children; all Igs low; absent T cells).
- Lymphoma (reduced IgM; IgA normal; IgG normal or low; disease, chemotherapy, or radiotherapy).
- SLE (rare).

- Infections:
 - HIV (rare).
 - Herpesviruses (rare, EBV in X-linked lymphoproliferative disease).
 - Acute bacterial infections.
 - Measles/rubella.
- Drugs (immunosuppressants, e.g. cyclophosphamide, azathioprine, chemotherapy).
- Plasmapheresis.
- Renal loss (IgM normal; IgG and IgA reduced).
- GI loss (IgM normal; IgG and IgA reduced).

Causes of hypergammaglobulinaemia

- *Chronic infection*—all Igs raised:
 - Osteomyelitis.
 - Bacterial endocarditis.
 - TB.
- *Chronic inflammation*:
 - SLE, RA—all Igs elevated.
 - Sjögren's syndrome—raised IgG (all IgG₁).
- *Sarcoidosis*—raised IgG and IgA; IgM usually normal.
- *Liver disease*:
 - PBC—IgM, may be very high (>30g/L) with small monoclonal bands on a polyclonally raised background.
 - Alcohol-related—↑ IgA, polyclonal, β-γ bridging on electrophoresis.
 - Autoimmune hepatitis (↑ IgG, IgA; normal IgM).
- *Hodgkin's disease*—IgE raised (also eosinophilia).
- *Viral infections*:
 - Acute common viral infections—raised IgM, normal IgG and IgA
 - HIV—all Igs raised (IgG very high, but polyclonal).
 - EBV—all raised.

Critical action

ALL patients with recurrent infections* should be reviewed by an immunologist or a paediatric immunologist, according to age, irrespective of age. Any patient with recurrent infections and low serum Igs has an immunological problem until proven otherwise.

Note: (*) recurrent infections can be pragmatically defined as two or more major microbiologically/virologically proven infections, requiring hospitalization, within 1 year. One major infection and recurrent minor infections should also be referred where minor infections are documented infections requiring treatment in the community.

Patients with unusual infections or with illness caused by opportunistic or normally non-pathogenic organisms and patients with infections in unusual sites (without good reason) should all be referred for further investigation.

Immunoglobulin G subclasses

Units: g/L.

Normal range (adults):

- IgG₁: 2.2–10.8g/L.
- IgG₂: 0.5–8.0g/L.
- IgG₃: 0.05–0.9g/L.
- IgG₄: 0.0–2.4g/L.

Principles of test

As for serum Igs, IgG subclasses are normally measured by nephelometry or turbidimetry. RID is still occasionally used.

Indications for testing

There are no absolute indications for testing, as significant immunodeficiency can occur in the presence of normal subclasses, and conversely complete genetic absence of a subclass may be completely asymptomatic. Measurement is usually performed as part of the work-up of patients with recurrent infections. IgG₄ disease has been described recently; this is associated with a wide range of organ-based diseases. IgG₄ levels are significantly raised.

Interpretation

Low levels may be significant in the context of presentation with recurrent infections. Deficiency of IgG₃, which is involved in immunity against viruses and associated with asthma and intractable epilepsy. IgG₂ deficiency may be seen in patients with IgA deficiency and may be associated with poor responses to polysaccharide antigens such as the capsular polysaccharides of bacteria.

Raised IgG₁ with normal or reduced IgG₂, IgG₃, and IgG₄ is seen in Sjögren's syndrome and is a specific pattern, which may occasionally be helpful in diagnosis.

Raised IgG₄ suggests IgG₄ disease.

Evaluation of specific antibody production (antibacterial and antiviral antibodies)

Units: variable: u/L, IU/mL, µg/mL.

Ranges: variable, check with reporting laboratory.

- Pneumococcal antibodies: >20u/L (asplenics >35).
- Tetanus antibodies: >0.1IU/mL (minimum protective level).
- *Haemophilus influenzae* type B: >1.0µg/mL (full protection; asplenics >1.5).

Principles of test

Measurement of antibody production against defined pathogens or antigens purified from pathogens plays an important role in the investigation of suspected immunodeficiency. Most assays are carried out by enzyme-linked immunoassay, but some viral antibodies are still measured by haemagglutination or complement fixation. Pre- and post-immunization samples should be run on the same run for direct comparison, as coefficients of variation for the assays tend to be high—15–25%. An EQA scheme exists. Assays have tended to focus on agents for which there are safe and effective vaccines. *Live vaccines should NEVER be given to any patient in whom immunodeficiency is suspected.*

Antibodies normally run in immunology laboratories include pneumococcal polysaccharides, which may be further differentiated as IgG₁ and IgG₂, *H. influenzae* type B (Hib), and tetanus. Diphtheria antibodies are not run by many laboratories, as the assay performance has been so poor. Meningococcal C polysaccharide antibodies are run by a few specialized laboratories, but correspondence with known clinical status has been poor. Anti-streptolysin O titre (ASOT) may be helpful.

Antibodies to pneumococcal serotypes are available from reference laboratories and may be valuable in assessing response to the conjugated pneumococcal serotype vaccine. There is debate about the protective levels (0.2–0.35).

Viral antibodies may be valuable to natural exposure and immunization antigens such as polio, measles, mumps, rubella, chickenpox, EBV, and hepatitis B (if immunized).

Indications for testing

These assays should be used in the work-up of patients with suspected immunodeficiency or in monitoring change in such patients. Responsiveness to immunization is a helpful marker of immunological recovery post-bone marrow transplant (BMT). Annual monitoring of levels may be valuable in asplenic patients, as such patients lose immunity more rapidly than a eusplenic population.

Interpretation

The interpretation is entirely dependent on the context. Assays for pneumococcal polysaccharides measure a composite of responses to the 23 strains in the Pneumovax® vaccine. This can be misleading as not all strains

represented in the vaccine are equipotent as immunostimulators. This means that a 'normal' response may actually mean a good response to the immunogenic strains, masking failure of response to the less immunogenic strains. For this reason, evaluation of such patients should be carried out by an immunologist with an interest in immunodeficiency. More weight should be placed on change in response to immunization than to actual values.

A 'normal' response to immunization has never been standardized, with publications frequently using different criteria, rendering comparison impossible. The following is a useful working definition: 'a 4-fold rise in titre, which rises to well within the normal range'.

Antibody deficiency in adults

This usually presents with upper and lower respiratory tract infections (*Streptococcus pneumoniae*, *Haemophilus*, *Staphylococcus*, *Klebsiella*), leading to chronic bronchiectasis and sinusitis; GI infections (*Salmonella*, *Giardia*, *Campylobacter*); skin infections (recurrent boils); autoimmune features (ITP, haemolytic anaemia, diabetes, thyroid disease) only in CVID, not XLA. ↑ incidence of lymphoma.

Normal serum IgS DO NOT EXCLUDE antibody deficiency (IgG subclass deficiency, specific failure of antibody production against polysaccharides). Use test immunization with killed or purified component vaccines to test humoral responses.

Asplenia

Patients are at ↑ risk of overwhelming sepsis: *S. pneumoniae*, *H. influenzae*, *Staphylococcus aureus*, *Meningococcus*, *Klebsiella* species, *Capnocytophaga canimorsus* (from dog bites), fulminant malaria, and babesiosis. Risk is life-long. Asplenia may result from trauma, involvement in malignancy, removal for diagnosis (rare these days), coeliac disease, and sickle disease and rarely due to congenital absence. Serum IgS and IgG subclasses are normal, but responses to polysaccharide antigens are often poor, especially in patients with lymphoma. Blood film will show Howell–Jolly bodies. Absence (if not known from records) will be shown by ultrasound (US).

Immunoglobulin D

IgD is rarely measured in clinical practice, as its main function is as a membrane receptor. Elevated levels may be seen in periodic fever syndrome, in hyper-IgD syndrome, due to deficiency of mevalonate kinase, and in IgD-secreting myeloma. Measurement is usually by RID.

Electrophoresis and immunofixation

Units: not applicable to electrophoresis (qualitative). Paraprotein quantitated by scanning densitometry reported in g/L.

Normal range: N/A

Principles of testing

In serum or urinary electrophoresis, the relevant body fluid is applied to an electrolyte-containing agarose gel. A current is applied across the gel and causes the proteins to migrate through the gel on the basis of their charge and, to a lesser extent, their size until they reach a neutral point in the electric field. The proteins are then visualized with a protein-binding stain. If the total protein is known, then the electrophoretic strip can be scanned and the absorption by the stain measured, which will be proportional to the amount of protein in the particular region in the gel. Thus, any monoclonal bands can be directly measured. This is useful for patients with myeloma, as immunochemical methods for measurement of Iggs may be inaccurate in patients with myeloma.

Immunofixation is the technique by which monoclonal Iggs are identified by overlaying the electrophoresed strips with antisera against heavy and light chains. These precipitate with the monoclonal proteins in the gel, and unbound antisera can be washed free prior to staining.

The same techniques can be carried out with urine, although this may require concentration to provide the clearest results.

Indications for testing

Electrophoresis and, if necessary, immunofixation of serum is an integral part of measurement of serum Iggs. ALL requests for serum Iggs must have electrophoresis carried out; failure to do so will lead to important abnormalities being missed. There is no place for carrying out electrophoresis as a stand-alone test.

Interpretation

See Table 4.1 for interpretations of results.

Table 4.1 Interpretation

Report	Interpretation
Reduced albumin	Chronic inflammation, nephritic syndrome
Absent α_1 band	Absent/reduced α_1 -antitrypsin
$\uparrow \alpha_2$ band	Chronic inflammation/infection; also seen in nephrotic syndrome, due to selective retention of α_2 -macroglobulin
$\uparrow \beta$	Seen in pregnancy (raised β -lipoprotein) and iron deficiency (transferrin)
$\beta-\gamma$ bridging	Caused by raised polyclonal IgA, e.g. cirrhosis
$\uparrow \gamma$	Caused by polyclonal \uparrow in IgG: infection/inflammation
Faint band(s) on polyclonal background	Caused by monoclonal escape during polyclonal response to infection/inflammation (does NOT indicate myeloma)
Monoclonal band in γ	Due to myeloma, lymphoma, and MGUS*
Absent/reduced γ	Due to inherited or acquired Ig deficiency

* MGUS, monoclonal gammopathy of uncertain significance—most evolve to myeloma, given time (years).

Monoclonal proteins may polymerize to give >1 band. Some patients will have >1 clone present producing different Igs.

Densitometry cannot be used where the monoclonal protein overlies the β -region, as the figures include non-Ig proteins.

Serum free light chains

Modifications in the techniques for measurement of free, as opposed to bound, light chains are valuable in monitoring light chain-only myelomas and other myelomas that produce excess free light chains, in addition to a whole paraprotein, which would previously have been monitored by measurement of 24h urinary light chain excretion. Urinary measurement can be problematic where there is renal impairment, and as light chains are nephrotoxic, as the disease advances, urinary measurements become less accurate.

'Bence-Jones proteins'; urine electrophoresis and immunofixation

Bence-Jones proteins are urinary free light chains, i.e. unbound to heavy chains. During normal antibody synthesis, a small excess of light chains are produced which are excreted. Hypergammaglobulinaemic states, such as RhA and chronic infection, may therefore be accompanied by excretion of polyclonal free light chains. Monoclonal free light chains are seen in myeloma and may be the only marker in light chain-only myelomas, which do not produce any heavy chains at all.

Testing is carried out as for serum.

Cryoglobulins

Units: usually reported qualitatively, but a ‘cryocrit’ can be measured in a similar way to a manual Hct using capillary tubes.

Normal range: tiny amounts of cryoglobulins may be found in normal individuals.

Principles of test

Cryoglobulins are IgS that precipitate when serum is cooled. The temperature at which this occurs determines whether disease will result. If the blood circulates through a part of the body where the temperature is below the critical temperature, then the protein will precipitate in the capillaries, causing obstruction, vascular damage, and eventually necrosis. The temperature of the hand is ~28°C at ambient room temperature. To check for the presence of cryoglobulins, take blood using a warmed syringe into a warmed bottle and transport to the laboratory at 37°C, using a Thermos™ flask with either pre-warmed sand or water at 37°C. The laboratory will allow the blood to clot at 37°C and then cool the serum. Cryoglobulins will form a precipitate as the temperature drops. The precipitate is then washed and re-dissolved for analysis by electrophoresis and immunofixation.

Cryoglobulins are not the same as cold agglutinins (a feature of *Mycoplasma pneumoniae* infection) (→ Chapter 3).

Indications for testing

All patients with Raynaud’s phenomenon of new onset or with winter onset of purpuric or vasculitic lesions on the extremities should be tested. Chronic hepatitis C infection is often accompanied by type II cryoglobulinaemia and a characteristic syndrome—‘mixed essential cryoglobulinaemia’ = autoimmune phenomena, arthritis, ulceration, glomerulonephritis, neuropathy. C3 normal; C4 reduced. Patients with myeloma, SLE, Sjögren’s syndrome, and RhA are also at risk.

Interpretation

Interpretation of the results of testing cryoglobulins is provided in Table 4.2.

Table 4.2 Interpretation

Type	Nature of cryoprecipitate
Type I	All monoclonal IgS; myeloma, lymphoma
Type II	Monoclonal Ig with RF activity; myeloma, lymphoma, connective tissue diseases, infections (especially HCV; SBE)
Type III	Polyclonal RF: connective tissue diseases, infections

Cryofibrinogen

This is found less commonly than cryoglobulins. It will not be detected unless both EDTA and heparinized blood samples are sent warm to the laboratory. The main association is with occult malignancy (and thrombophlebitis migrans). Also associated with connective tissue disease, pregnancy, OCP use, DM, and cold urticaria.

β_2 -microglobulin

Unit: mg/L.

Normal range: 1–3mg/L.

Principles of test

The test measures free β_2 -microglobulin, which normally forms the light chain of HLA class I molecules but is shed when there is ↑ lymphocyte turnover. It is usually rapidly cleared by the kidneys. Measurement is usually by an automated analyser, nephelometry, or turbidimetry. RID is still used.

Indications for testing

The main indication is as part of routine monitoring of patients with myeloma and HIV. Other biomarkers are now felt to be more useful. Very high levels are seen in renal failure and patients on dialysis, which can cause β_2 -microglobulin amyloid.

Interpretation

Levels elevated in:

- HIV infection (surrogate marker of progression).
- Myeloma (marker of tumour mass).
- Lymphoma.
- CVID (correlation with severity).
- Renal dialysis (depending on type of membrane).

Acute phase proteins (CRP, ESR, SAA)

Units:

- C-reactive protein (CRP): mg/L.
- Erythrocyte sedimentation rate (ESR): mm/h.
- Serum amyloid A (SAA): mg/L.

Normal ranges:

- CRP 0–6mg/L.
- ESR (⌚) Erythrocyte sedimentation rate, p. 252).
- SAA (not measured routinely).

Principles of test

Serum proteins CRP and SAA are amenable to measurement by nephelometry or turbidimetry. Measurement of the ESR is covered in ⌚ Chapter 3.

Indications for testing

Acute and chronic infections, vasculitis, connective tissue disease, arthritis.

Interpretation

Clinicians are usually confused by ESR and CRP—they do not give the same information and should be used together. CRP is like blood glucose, whilst ESR is like HbA_{1c}. CRP rises within hours of onset of inflammation/infection and falls quickly once treatment is instituted. It is therefore useful for rapid diagnosis and monitoring response. The ESR rises slowly, being dependent, in part, on fibrinogen, a long-lived protein, and falls equally slowly (see Fig. 4.1). In active SLE, the ESR is high, but CRP is not elevated. CRP is driven by interleukin (IL)-6 and may be elevated in myeloma. See Table 4.3 for interpretation of results.

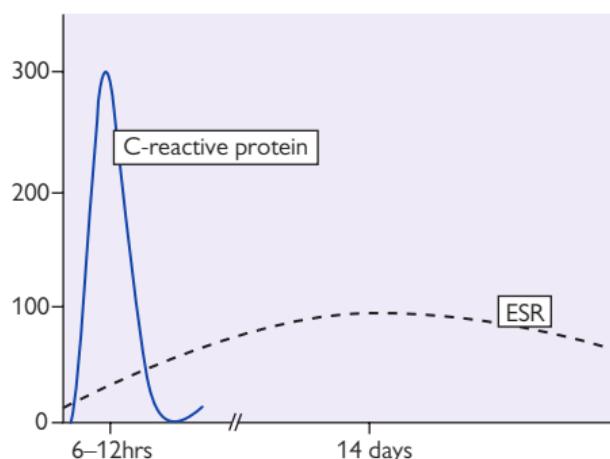


Fig. 4.1 Time course of acute phase response proteins. ESR in acute phase response parallels fibrinogen level.

Table 4.3 Causes of elevated CRP

Level of CRP	Common associations
Little or no change (<4–100mg/L)	Most viral infections Active SLE Systemic sclerosis and CREST Inactive RhA Myeloma Most tumours
Moderate elevation (100–200mg/L)	EBV/CMV infection Bacterial infection Active RhA Polymyalgia rheumatica TA Lymphoma Hypernephroma
Large elevation (>200mg/L)	Severe bacterial sepsis <i>Legionella</i> Active vasculitis (Wegener's, rheumatoid)
Huge elevation (>400mg/L)	Overwhelming sepsis (deep tissue abscess) Fulminant <i>Legionella</i> At this level, death usually ensues!

CREST, calcinosis, Raynaud's syndrome, oesophageal motility dysfunction, sclerodactyly, and telangiectasia.

Levels in very young children may be much lower for a given stimulus. A very small number of patients do not make inflammatory responses that exceed the normal range but seem to run on a lower 'normal' range (10-fold less); ultra-sensitive assays for low-level CRP are available.

Amyloidosis

Amyloid refers to the deposition of altered proteins in tissues in an insoluble form. The precursor protein varies according to the cause and can often be measured specifically. Amyloid is usually confirmed by special stains on histological examination of biopsies. Measurement of serum IgS and electrophoresis, β_2 -microglobulin, and CRP is essential if amyloid is suspected (see Table 4.4).

Table 4.4 Types of amyloid

Amyloid protein	Protein precursor	Clinical syndrome
AL, AH	Light or heavy chain of Ig	Idiopathic, multiple myeloma, γ -heavy chain disease
AA	Serum amyloid A	2°, reactive: inflammatory arthritis, familial Mediterranean fever, hyper-IgD syndrome, TRAPS (periodic fever), Behçet's, Crohn's disease
$\text{A}\beta_2\text{M}$	β_2 -microglobulin	Dialysis amyloid
ACys	Cystatin C	Hereditary cerebral angiopathy with bleeding (Iceland)
ALys, AFibA	Lysozyme, fibrinogen Aa	Non-neuropathic hereditary amyloid with renal disease
AIAPP	Islet amyloid polypeptide	DM type 2; insulinoma
AANF	Atrial natriuretic peptide	Senile cardiac amyloid
ACal	Procalcitonin	Medullary carcinoma of the thyroid
Alns	Porcine insulin	Iatrogenic
ATTR	Transthyretin	Familial amyloid polyneuropathy, senile cardiac amyloid
A β	$\text{A}\beta$ -protein precursor	Alzheimer's disease
AprP	Prion protein	Spongiform encephalopathies

TRAPS, tumour necrosis factor receptor-associated periodic syndrome.

Measurement of serum complement components

Units: g/L.

Normal ranges:

- Complement C3 0.68–1.80g/L.
- Complement C4 0.18–0.60g/L.
- Factor B (rarely measured routinely).
- Other components usually reported as percentage of normal human plasma.

Principles of test

C3, C4, and factor B are usually measured by rate nephelometry or turbidimetry. Other components are measured by RID or simply by double diffusion where presence or absence is the only result of interest. Complement breakdown products are measured by RID or enzyme-linked assay (EIA).

Indications

Valuable in:

- SLE (C3, C4, C3d).
- Suspected complement deficiency (C3, C4, haemolytic complement).
- Suspected anaphylaxis (anaphylotoxins C4a, C5a).
- Suspected hereditary angioedema (C3, C4, C1q, C1 esterase inhibitor, immunochemical AND functional).

Complement deficiency is common (especially C4 and C2 deficiencies); predisposes to recurrent neisserial disease, bacterial infections (C3 deficiency), and immune complex disease (lupus-like). Anyone with >1 episode of systemic neisserial disease has a complement deficiency until proven otherwise.

C1 esterase inhibitor

Units:

- *Immunochemical*: g/L.
- *Functional*: reported as percentage activity, compared to normal fresh plasma.

Normal range:

- *Immunochemical*: 0.18–0.54g/L (paediatric ranges not well defined, but lower than adults).
- *Functional*: 80–120% of normal plasma.

Principles of tests

Immunochemical measurement carried out by RID; functional assay is usually a colorimetric assay.

Indications for testing

Key indication is angioedema occurring WITHOUT urticaria at any age. If urticaria is present, diagnosis is virtually never C1 esterase inhibitor deficiency. C4 is a useful screen; normal C4 during an attack excludes C1 esterase inhibitor deficiency.

Interpretation

C1 esterase inhibitor deficiency causes hereditary angioedema.

Two types:

- *Type I* (common, 80%); absence of immunochemical C1 esterase inhibitor (C1-inh).
- *Type II* (rare, 20%); presence of non-functional C1-inh; immunochemical levels normal or high.

Both are inherited as autosomal dominant. Present with angioedema, NO urticaria; may involve the larynx and gut, usual onset at puberty. C4 absent during acute attacks. Treat with purified C1-inh (FFP may be a substitute but can make attacks worse) or icatibant; maintenance therapy with danazol, stanozolol, or tranexamic acid to ↓ frequency of attacks. Pregnancy/oral contraceptive exacerbate.

A rare type (type III) of hereditary angioedema is also described, thought to be due to gain-of-function mutations in clotting factor XII. Angioedema can also be caused by deficiency of ACE and C4-binding protein.

Rare acquired form due to autoantibody to C1-inh (SLE, lymphoma); C1q levels are reduced, and paraproteins may be present.

Haemolytic complement (lytic complement function tests)

Units: can be reported in arbitrary units, or as a percentage of normal plasma, but better reported as normal reduced or absent.

Normal range: present (80–120% of reference plasma).

Principles of test

Haemolytic complement assays screen for the integrity of the classical and alternate pathways and the terminal lytic sequence, and use either antibody-coated sheep cells (CH100, classical pathway) or guinea pig red cells (APCH100, alternate pathway). Either a gel or liquid assay can be used, but the gel is easier! Both tests must be performed in parallel.

Indications for testing

Any patient in whom deficiency of a complement component is suspected. Testing is also used to monitor the effectiveness of the MoAb eculizumab, used to treat atypical HUS.

Interpretation

Reduced levels of haemolytic activity will be seen during infections and during immune complex diseases such as serum sickness and SLE. Testing for absence of a component needs to be undertaken a minimum of 4–6 weeks after recovery from infection. Absence in both CH100 and APCH100 indicates a deficiency in the terminal lytic sequence C5–C9 (C9 deficiency will give slow lysis). Absence of CH100 indicates a missing component in the classical pathway C1–C4. Absence of APCH100 indicates deficiency in the alternate pathway (factor D, factor B, C3).

Follow-up testing to identify the missing component will be performed by the laboratory automatically (if they are doing their job!).

Critical action

Anyone who has a single episode of neisserial meningitis with an unusual strain or a second episode with a common strain MUST be assumed to have a complement deficiency until proven otherwise. REFER after recovery to the immunologist for investigation.

Factor H and factor I

Deficiency of either of these factors may lead to HUS. Measurement is possible in specialized centres, with follow-up genetic testing. Autoantibodies against these proteins have been described.

C3 nephritic factor

This is an autoantibody which reacts with a neo-antigen in the alternate pathway convertase C3bBb to stabilize the convertase and ↑ complement breakdown. Typically seen in membranoproliferative glomerulonephritis and in lipodystrophy. C3 will typically be extremely low.

Autoimmunity

Autoantibodies are usually divided into organ-specific and organ-non-specific, but clinical testing rarely follows this pattern. They are therefore covered in convenient groups, associated with types of testing.

Rheumatoid factor

Units:

- Titre (particle agglutination assay).
- IU/mL (nephelometry, EIA).

Normal range:

- Titre <1/20: <16 years.
- Titre <1/40: 16–65 years.
- Titre <1/80: >65 years.
- <30IU/mL.

Principles of test

Tests detect autoantibodies binding to human Ig; these can be of any class, but assays commonly recognize IgG and IgM autoantibodies. Suggestions that IgA RF may be helpful have not been widely accepted. Assays use either agglutination of Ig-coated particles (visual assay) or latex particle-enhanced nephelometry.

Indications for testing

The test is only relevant in patients already diagnosed with RhA.

Interpretation

NOT a diagnostic test for RhA! Only +ve in 70–80%. High titre in a patient with known RhA is a risk factor for extra-articular manifestations and poorer prognosis.

RF is also found in:

- Healthy elderly (asymptomatic).
- Chronic bacterial (SBE) and viral infections (HIV, HCV).
- Acute viral infections (transient; especially adenovirus).
- Myeloma (often type II cryoglobulins).
- Lymphoma.
- Connective tissue diseases (SLE, Sjögren's, systemic sclerosis, polymyositis, undifferentiated connective tissue disease (UCTD)).

Antibodies to cyclic citrullinated peptide (anti-CCP) may be more valuable in the diagnosis of RhA, as they are more specific.

Autoantibody screen

Another abused test! Usually used as an immunological fishing expedition. Multiple tissues (rodent), often with human Hep-2 cell line; gives rapid and semi-quantitative results for the following autoantibodies:

- ANAs (see Table 4.5 for patterns).
- Anti-ribosomal antibodies.
- Antimitochondrial antibodies (AMA).
- Anti-smooth muscle antibodies (ASMA).
- Anti-liver–kidney microsomal (LKM) antibodies.
- Anti-gastric parietal cell (GPC) antibody.

Where Hep-2 cells are used, other patterns of nuclear and cytoplasmic fluorescence may be seen. See Fig. 4.2 for examples.

Increasingly, laboratories are using multiplex analysers (modified enzyme-linked or flow cytometry-based testing) which allow high-throughput screening for nuclear and related antibodies.

Table 4.5 Patterns of antinuclear antibodies (ANAs)

Homogeneous	SLE, drug-induced SLE (ds-DNA or histones)
Coarse-speckled	UCTD*, SLE (U1-RNP)
Fine-speckled	SLE, Sjögren's (Ro and La)
Nucleolar	Systemic sclerosis, polymyositis, SLE
Centriole	Commonest in <i>Mycoplasma</i> pneumonia, also scleroderma
Proliferating cell nuclear antigen (PCNA)	SLE—highly specific, but rare
Centromere	'CREST' syndrome, limited scleroderma, Raynaud's, never diffuse scleroderma; may be confused with multinuclear dot pattern, which is seen in mitochondrial antibody-negative PBC
Nuclear matrix	SLE and UCTD
Nuclear mitotic spindle	Non-specific: SLE, RA, CREST, UCTD, and Sjögren's syndrome
Histones	SLE, drug-induced (>90% of patients); other connective tissue diseases (low frequency)

* UCTD, undifferentiated connective tissue disease (previously mixed connective tissue disease).

Note: ribosomal antibodies associated with SLE, especially neuro-lupus (cytoplasmic pattern, not nuclear).

Titre of antibodies does NOT correlate with disease activity.

ANAs may be seen transiently after viral infections, especially in children. Therefore, observe a child where low-titre antibodies occur in the absence of clinical symptoms compatible with juvenile arthritis.

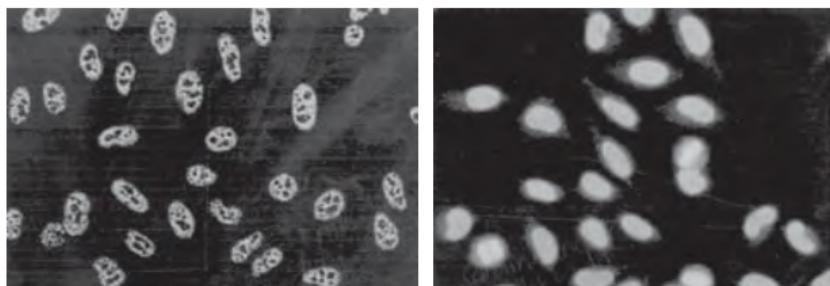


Fig. 4.2 ANA on Hep-2 cells with a speckled pattern (left) and a homogeneous pattern (right).

Principles of test

The traditional method is to overlay suitably diluted serum into frozen sections of rodent liver, kidney, and stomach, and human Hep-2 cells. Bound antibody in the serum is then identified using a fluoresceinated anti-human IgG (or IgM, IgA) as the second stage. Slides are then read manually. Enzyme-based immunoassays are being introduced for screening, including multiplex bead-laser array systems. These can be very specific when purified or recombinant antigens are used but lose out because of their inability to pick up unexpected patterns.

Enzyme-linked assays are used for confirming antigens such histone antibodies and double-stranded (ds-)DNA antibodies.

Indications for testing

The correct use of testing is to identify which specific autoantibody is being sought as part of the differential diagnosis.

Interpretation

Because of the multiple patterns detected in this system, the interpretation for each is covered separately (see Table 4.5).

Double-stranded DNA antibodies

Unit: IU/mL.

Normal range: varies according to assay:

- <30IU/mL: -ve.
- 30–50IU/mL: borderline.
- >50IU/mL: +ve.

Principles of assay

The original and still best assay is the Farr assay, a radioisotope-based assay. EIA tends to be widely used but is less specific, due to frequent contamination with single-stranded DNA. Crithidia assay used only rarely, as quick fluorescent screen (kinetoplast is pure ds-DNA).

Indications for testing

Suspected SLE or autoimmune hepatitis; used to monitor SLE, in conjunction with complement studies.

Interpretation

Sensitive and specific for SLE and autoimmune hepatitis (AIH); +ve result is significant (in correct clinical context).

Antibodies to extractable nuclear antigens (ENA)

Units: reported qualitatively.

Normal range: dependent on antibody, normally –ve.

Principles of test

Usually carried out by enzyme-linked immunoassay. However, counter-current immunoelectrophoresis and western blotting are still widely used, especially for rare antibodies. ELA is much more sensitive than other methods and has led to clinical confusion.

Indications for testing

Should always be carried out in patients with suspected connective tissue disease. Monitoring at yearly intervals should be carried out in diagnosed patients, as the antibody pattern may change with time and this may correlate with changes in the clinical profile.

Interpretation

Reported qualitatively; normally laboratories will carry out a 6-antigen screen: Ro, La, ribonucleoprotein (RNP), Sm, Jo-1, and Scl-70 (see Table 4.6). A wide range of other antibodies are described, some of which may be available through reference laboratories.

Diagnosis and monitoring of SLE

ANAs and ds-DNA antibodies, both +ve; low C3 and C4; raised complement breakdown products in active disease (C3d); normal CRP. ANA-negative lupus often anti-Ro positive. West Indian lupus anti-Sm+. Always check for cardiolipin antibodies and lupus anticoagulant (dRVVT) (→ Antiphospholipid antibodies, p. 362). Ribosomal P antibodies may be a marker for neuropsychiatric lupus.

Monitor with CRP (differential between infection and flare of disease), C3, C4, and ds-DNA antibodies (no value from serial ANAs); rising titre of DNA antibodies often heralds a relapse (actual titre not related to disease activity).

Table 4.6 Antibodies to extractable nuclear antigens

Ro/SS-A	SLE, Sjögren's, neonatal lupus, neonatal congenital complete heart block (cause of ANA-negative lupus, as not picked up by standard ANA screen, which does not include Hep-2 cells). Newer assays will distinguish Ro52 and Ro60
La/SS-B	SLE, Sjögren's, neonatal lupus, neonatal congenital complete heart block (rare)
(U1)-RNP	Mixed (undifferentiated) connective tissue disease (if present alone); SLE (if present with ds-DNA). Other RNPs may be reported
Sm	Highly specific marker for SLE (mainly West Indians; rare in Caucasians)
Jo-1	Polymyositis, dermatomyositis; transferase syndrome (fibrosing lung disease; 65% +ve); many other specificities are known (all recognizing transfer RNA (tRNA)-transferases)
Scl-70	Systemic sclerosis (diffuse scleroderma); only 30% of patients are +ve
Pm-Scl (PM1)	Scleroderma–myositis overlap
Ku, Ki	Rare: SLE, UCTD, Sjögren's syndrome, polymyositis
Mi-2	Rare: steroid-responsive polymyositis
Histones	Found in connective tissue diseases, especially drug-induced lupus

Antiphospholipid antibodies

Principles of testing

Many types of antibodies to phospholipids are recognized. However, routinely available tests are IgG and IgM anticardiolipin antibodies detected by EIA, and the presence of a 'lupus anticoagulant' detected using the dilute Russell viper venom test (dRVVT). BOTH tests must be carried out together, as either may be +ve without the other, but the clinical significance is the same. Antibodies to β_2 -glycoprotein I are also important, as the protein is a key co-factor for pathogenic antibodies. Other specificities are available only as research tools.

Indications for testing

Patients with unexplained venous or arterial thrombosis; recurrent miscarriage (>3); connective tissue disease; early TIAs or stroke (<60 years). Livedo reticularis is a cutaneous marker.

Interpretation

- Prolonged APTT and reduced platelet count ($80\text{--}120 \times 10^9/\text{L}$) are typical features. May get false +ve VDRL (Venereal Disease Research Laboratory).
- Persistent +ve IgM anticardiolipin antibodies as the only marker of syndrome unusual, but clinically significant.
- Hughes' syndrome = antiphospholipid antibodies without evidence of other connective tissue disease or vasculitis. Presents with recurrent miscarriage, thrombosis, or strokes. Fulminant disease causes multi-organ failure.
- Antibodies also found in SLE, but NOT cerebral lupus, Behçet's, and Sneddon's syndrome. Also seen after EBV infection (asymptomatic and disappear).
- Treatment is lifelong warfarinization if symptomatic (heparin in pregnancy); aspirin may be acceptable if asymptomatic (but no controlled studies). No indication for intensive immunosuppression.

Other patterns of autoantibodies identified on 'autoantibody screen'

Antimitochondrial antibodies (AMA) and anti-M2 antibodies

Units: reported as titre (AMA) Anti-M2 reported as +ve or -ve.

Normal range: not usually detectable.

Principles of testing

AMA are identified on fluorescent screen, and previously unknown +ves should be followed up with EIA or blot-based test for anti-M2 antibodies.

Indications for testing

Suspected liver disease, especially with raised ALP (and in ♀); investigation of unexplained pruritus (early feature of PBC).

Interpretation

Ninety-five per cent of PBC patients +ve for AMA (anti-M2, against dihydrolipoamide acyltransferase, E2). The remainder may have antibodies against S100 antigen of the nuclear membrane (Nsp-II pattern; multinuclear dots) or gp210, another nuclear antigen. The type of antibody present has no influence on prognosis or response to therapy. Marked elevation in IgM.

Autoantibodies in autoimmune hepatitis

Units: reported as titres (ASMA, anti-LKM antibodies). Anti-liver cytosol (LC-1) and soluble liver antigen (SLA) reported as +ve or -ve.

Normal range: not usually detectable.

Principles of testing

ASMA and LKM are identified on fluorescent screen, and previously unknown +ves should be followed up with EIA or blot-based test for anti-LC and anti-SLA antibodies. Also do ANCA (→ Vasculitic syndromes, pp. 372–3).

Indications for testing

Suspected autoimmune liver disease (abnormal LFTs, alcohol and viral infections excluded).

Interpretation

Antibodies to HCV or HCV PCR+ = exclusion criteria for AIH.

Autoimmune hepatitis

- Type 1 (AIH-1) is ANA +ve, smooth muscle antibody (SMA) +ve, p-ANCA +ve, and SLA antibody +ve. Typically occurs in adults and has a better prognosis and responds well to therapy.
- Type 2 (AIH-2) is typically LKM-1 and LKM-3 antibody +ve and LC-1 antibody +ve. AIH-2 is seen in children and has a worse prognosis with poor response to therapy.
- In serological studies, 50% of AIH-1 are ANA +ve/SMA +ve, 15% ANA +ve only, and 35% SMA +ve only. However, there are biopsy-proven serologically –ve hepatitis; 8% of AIH-1 are SLA +ve only. 43% of AIH-2 are LC-1 +ve only. Therefore, necessary to do SLA and LC-1 tests. Prognosis is dependent on type and early diagnosis.
- LKM antibodies are also associated with drug-induced hepatitis (especially halothane) and chronic hepatitis C or D.
- Non-actin SMA may be seen in SLE and after viral infections (especially adenovirus).
- Sclerosing cholangitis may be associated with atypical p-ANCA (→ Vasculitic syndromes, pp. 372–3).

Antibodies to gastric parietal cells and intrinsic factor

Units: GPCs may be reported as titre or simply +ve/-ve (as titre of no clinical value); IF antibodies reported as +ve/-ve.

Normal range: GPC antibodies found in healthy normals without evidence of B_{12} deficiency or gastritis—may be at risk of later PA. IF antibodies only found in PA.

Principles of testing

GPC antibodies are detected as part of the 'autoantibody screen'; IF antibodies usually detected by either RIA or EIA, but assays are inconsistent. There is a robust EQA scheme for GPC antibodies, but none currently for IF antibodies.

Indications for testing

GPC antibodies should be checked in all patients with thyroid disease, as there is a close association ('thyrogastric disease'). Also check in patients with unexplained macrocytosis \pm low B_{12} . Positive antibodies identified incidentally should be followed up with a blood count and, if the MCV is high, a B_{12} level. IF antibodies may help in patients with a high MCV and low B_{12} to confirm PA. Diagnosis of PA more difficult now Schilling test withdrawn.

IF antibodies are NOT suitable for screening, as present in only 50% of patients with PA and pre-administration of B_{12} will interfere with detection.

Interpretation

GPC antibodies found in 90% of patients with PA and in 40% of patients with other organ-specific autoimmune diseases; the antigen is the β subunit of gastric H^+/K^+ ATPase. Anti-IF antibodies are of two types: those that block B_{12} binding to IF (70% of patients with PA) and those that block uptake of the B_{12} -IF complex (35% of patients with PA).

Thyroid disease (thyroid peroxidase antibodies)

Units: variable—check with the laboratory.

Normal range: low levels of antibodies may be found in asymptomatic individuals, although higher levels may indicate a predisposition to later development of thyroid disease.

Principles of testing

TPO antibodies are the test of choice. Tg antibodies not monitored now, except as part of monitoring for thyroid carcinoma (interfere with assays for Tg, which is used as a tumour marker). Antibodies to TSH receptor may be stimulating or blocking, but these can only be identified by bioassay or RIA in specialized laboratories.

Indications for testing

Suspected thyroid disease and as reflex testing when thyroid function is abnormal; often now combined with thyroid testing on same analyser. Also check in patients with PA. Use TPO antibodies. Other antibodies are for specialist use only.

Interpretation

TPO (thyroid microsomal) antibodies in 95–100% of Hashimoto's thyroiditis, 70% of Graves' disease; highest titres seen in Hashimoto's. Tg antibodies add little to diagnosis (also found in other endocrinopathies and thyroid carcinoma). Antibodies to TSH receptor (stimulating or blocking) found in 95% of Graves' patients.

Islet cell antibodies (ICA), anti-GAD antibodies, and insulin receptor and insulin antibodies

Units: qualitative (previously measured in JDF units) for ICA; numeric values for other tests.

Normal range: 0.4% of normal population +ve for ICA.

Principles of testing

ICA can be detected by immunofluorescence on pancreatic sections; other antibodies detected by EIA.

Indications for testing

All newly diagnosed early-onset diabetics should be tested; they should also be tested for coeliac disease using endomysial or tTG antibodies. May be used to screen normoglycaemic first-degree relatives for likelihood of developing diabetes. Also advisable to screen children with coeliac disease.

Interpretation

ICA found in 75–86% of type 1 diabetics, but only 10% of type 2 diabetics and 2–5% of first-degree relatives. Levels decline with time and may disappear completely in long-standing type 1 patients. Antigen is glutamic acid decarboxylase (GAD) (similar antibodies cause stiff man (person!) syndrome, although a different epitope on the molecule is recognized); GAD65 and GAD67. Other target antigens for autoantibodies, with high specificity for diabetes, are:

- Insulin/proinsulin (seen at diagnosis in 40% of type 1 diabetics).
- Insulin receptor (associated with acanthosis nigricans and insulin resistance).
- IA-2 (present in 60% of newly diagnosed type 1 DM).
- ZnT8 (zinc transporter) found in 60–80% of type 1 DM.

Monitoring is of no value.

Note: IgE antibodies to porcine or bovine insulin may occur in insulin allergy, due to exogenous ‘foreign’ insulin.

Adrenal antibodies and other endocrine autoantibodies

Units: qualitative.

Normal range: not detectable.

Principles of testing

Usually detected by immunofluorescence on appropriate tissue (adrenal, ovary, testis, parathyroid, pituitary). EIA for 21-hydroxylase, 17-hydroxylase, and P450 side chain cleavage enzymes (targets of adrenal antibodies) in research centres.

Indications for testing

Suspected autoimmune endocrinopathy (adrenal insufficiency, premature ovarian failure, hypoparathyroidism).

Interpretation

Positives indicate autoimmune disease of relevant organ.

Classification of autoimmune polyglandular syndromes

Refer to Table 4.7.

Table 4.7 Classification of autoimmune polyglandular syndromes

Syndrome	Major criteria	Minor criteria
Type I	Candidiasis Adrenal failure Hypoparathyroidism	Gonadal failure Alopecia Malabsorption Chronic hepatitis
Type II (Schmidt's syndrome)	Adrenal failure Thyroid disease IDDM	Gonadal failure Vitiligo Non-endocrine autoimmunity (myasthenia)
Type III	Thyroid disease	Either IDDM Or PA Or Non-endocrine autoimmunity (myasthenia)

IDDM, insulin-dependent (type 1) diabetes mellitus.

Endomysial (EMA) and tissue transglutaminase (tTG) antibodies

Units: qualitative for EMA; numeric for tTG.

Normal range: not usually detected in healthy individuals. +ve IgA EMA or tTG with normal biopsy probably indicates risk of later development of coeliac disease.

Principles of testing

Originally identified as anti-reticulin R1 antibodies on 'autoimmune screen'. Later identified as antigliadin antibodies by modified immunofluorescence or by EIA. EMA detected by immunofluorescence on monkey oesophagus (also umbilical vein, but this is difficult to read); purified recombinant human tTG used for EIA (assays with guinea pig tTG are less sensitive).

Indications for testing

Patients with malabsorption, wheat intolerance, 'irritable bowel'; children with type 1 diabetes (and suggested that they should be monitored every year!). Suspected dermatitis herpetiformis. Unexplained hyposplenism, small bowel lymphoma.

Interpretation

IgA EMA has nearly 100% sensitivity and specificity for coeliac disease. Antigen is tTG. In IgA deficiency (commoner in coeliac), IgG EMA is as good a diagnostic test. Gliadin antibodies (IgG or IgA) are not sensitive and specific and should no longer be used; they are found in a range of bowel diseases and in healthy individuals. Reticulin R1 antibodies may be picked up on routine autoantibody screen; these may also be found in IBD, especially with liver involvement, and non-specific bowel disease (post-infectious, allergic, etc.). Also found in dermatitis herpetiformis (typical rash). Strong association with juvenile type 1 DM.

Antibodies disappear with strict gluten-free diet, over 6–12 months. Persistent positivity of IgA EMA or IgA tTG antibodies in a patient on a gluten-free diet is an indication of incomplete/non-compliance with the diet. Monitoring tTG antibodies is valuable.

Gliadin antibodies and neurological disease

Gluten sensitivity enteropathy is also associated with neurological disease, typically cerebellar ataxia. Reports have suggested that gliadin antibodies are a marker for this syndrome; the lack of specificity of gliadin antibodies means that it is unlikely that these antibodies can be used as an accurate diagnostic test, and specific tests (EMA and tTG) should be used instead.

Autoimmune skin diseases

Units: normally reported qualitatively.

Normal range: antibodies when detected are highly disease-specific.

Principles of testing

Autoantibodies to components of the skin can be detected either by direct immunofluorescence (DIF) on a skin biopsy of affected tissue or indirectly in the patient's serum using monkey oesophagus as a substrate. Saline splitting of the skin may be used to identify the precise location of the antigenic target to differentiate between epidermolysis bullosa acquisita (antigen on the dermal side) and pemphigoid (antigen on the epidermal side).

Indications for testing

Bullous (blistering) skin diseases.

Interpretation

Bullous pemphigoid

Antibodies bind to the dermal–epidermal junction and recognize hemidesmosome antigens (BP 230 (BPAG1) and BP 180 (BPAG2)); detect by DIF on skin biopsies or in serum (only 70% of patients have detectable circulating antibodies in serum). Linear basement membrane deposition of IgG and C3 on DIF.

Herpes gestationis

Antibodies to BP 180 (BPAG2) binding to the dermal–epidermal junction on DIF of biopsies, often –ve for serum antibodies. Linear basement membrane deposition of IgG and C3 on DIF.

Pemphigus

Antibody binds to the cell surface of stratified squamous epithelium and recognizes the desmosomal proteins desmoglein I and III. Eighty to 90% of patients have detectable antibody in serum. Chicken-wire pattern of immunofluorescence in the epidermis. May occur as a paraneoplastic phenomenon in patients with lymphoma, but antigenic specificity is for desmoplakin I and II.

Dermatitis herpetiformis

Granular deposits of IgA at the dermal–epidermal junction in dermal papillae on DIF; EMA and tTG antibodies will be +ve.

Autoantibodies and neurological disease

Principles of testing

Most neurological autoantibodies of interest are rare and are available from reference laboratories. A variety of methods are used; some assays are reported with numeric values.

Indications for testing

These are restricted to very specific neurological syndromes.

Interpretation

- AChRAb associated with MG (90%); reported numerically as high and low +ves to distinguish different clinical phenotypes. May also be found in asymptomatic relatives. Striated muscle antibodies (immunofluorescence on striated muscle section) strongly associated with underlying thymoma.
- Lambert–Eaton myasthenic syndrome (LEMS), occurring with small-cell carcinoma of the lung, associated with autoantibodies to voltage-gated Ca^{2+} channels, α and β subunits. Same tumour also associated with retinal autoantibodies. Antibodies to voltage-gated K^+ channels are associated with neuromyotonia.
- Anti-ganglioside antibodies associated with GBS (GM-1, GD1a) and variants (Miller–Fisher—GQ1b, GT1a), chronic variants (chronic inflammatory demyelinating polyneuropathy), and neuropathy associated with paraproteins.
- Myelin-associated glycoprotein (MAG) antibodies associated with paraproteinaemic neuropathy, especially with Waldenström's macroglobulinaemia.
- Antibodies to myelin basic protein found in MS, but not useful diagnostically.
- Anti-Yo (Purkinje cell antibodies) found in paraneoplastic cerebellar degeneration (gynaecological or breast tumours associated); anti-Hu (anti-neuronal nuclear antibodies (ANNA)) associated with paraneoplastic neuropathies and myopathies (small-cell carcinoma). Anti-Ri (anti-neuronal nuclei) associated with cerebellar ataxia and opsclonus (small-cell carcinoma, gynaecological or breast tumours associated). Other specificities have been defined.
- Anti-neuronal antibodies in the CSF of 74% of patients with cerebral lupus; also associated with anti-ribosomal P antibodies.
- Anti-GAD antibodies found in stiff person syndrome; same antigen to that found in pancreatic islets; diabetes usually occurs in stiff person syndrome. Antibodies appear to inhibit the production of γ -aminobutyric acid (GABA), an inhibitory neurotransmitter. Rasmussen's encephalitis associated with autoantibodies to GluR3 receptor, which cause hyperexcitability of neurones.
- Anti-aquaporin-4 antibodies are associated with neuromyelitis optica (Devic's disease).

Vasculitic syndromes

ANCA

Units: usually expressed as titre (qualitative EIAs are used in some centres).
Normal range: in adults, normal = undetectable.

Principles of testing

Screening is usually carried out by immunofluorescence on ethanol-fixed human neutrophils; rapid EIA screening tests exist for +ve/-ve testing in emergencies. EIA to specific antigens is an essential follow-up. Distinction between antinuclear and perinuclear staining may require the use of Hep-2 cells.

Indications for testing

Suspected vasculitis; acute glomerulonephritis.

Interpretation

- Two main patterns recognized: c-ANCA (mostly Wegener's; 90% of Wegener's ANCA +ve) and p-ANCA (some Wegener's, microscopic polyarteritis, Churg–Strauss syndrome, glomerulonephritis, sclerosing cholangitis, AIH, ulcerative colitis).
- c-ANCA pattern due to antibodies against proteinase-3; p-ANCA pattern due to antibodies against MPO, lactoferrin, cathepsin, and elastase. Follow-up EIA required to identify antigenic specificity: minimum PR3 and MPO ELISA; other antigens available through supra-regional referral laboratories.
- In Wegener's, monitoring titre of ANCA is useful; a rising titre in a patient in clinical remission heralds relapse.
- ANCA may be seen as epiphänomenon in states of chronic neutrophil activation and turnover, e.g. cystic fibrosis.

Anti-GBM disease (Goodpasture's syndrome)

Units: qualitative (quantitation available through reference centres—used only to follow patients post-plasmapheresis or transplant).

Normal range: not detectable.

Principles of testing

Usually carried out by EIA; screening by immunofluorescence is not sensitive. Biopsies will show linear IgG deposition in glomeruli (and alveoli). Antibodies recognize the NC1 region in the α 3 chain of type IV collagen.

Indications for testing

Acute glomerulonephritis, particularly if associated with pulmonary haemorrhage.

Interpretation

Positive antibodies confirm Goodpasture's syndrome. Urgent plasmapheresis is required, and monitoring reduction of antibody with treatment is advisable. Disease may recur in transplanted kidneys, so monitoring of antibody is advised. Some patients may be both ANCA and anti-GBM antibody +ve.

Anti-phospholipase A2 antibodies (anti-PLA-2)

Units: quantitative, through reference laboratories only.

Normal range: not detectable.

Principles of testing

Usually carried out by EIA or western blot. Indirect immunofluorescence also available using a cell line expressing PLA-2.

Indications for testing

Marker antibody for idiopathic membranous nephritis (IMN).

Interpretation

Very high specificity for IMN but may also be found in lupus.

Allergic disease

Total IgE

Unit: kU/L.

Normal range: <100kU/L (>14 years old).

Principles of testing

Previously carried out by RIA, now by EIA.

Indications for testing

There are FEW indications for testing. Screening for atopic disease; investigation of suspected hyper-IgE syndrome (Job's syndrome, a rare immunodeficiency), Churg–Strauss vasculitis. Gating requests for radioallergosorbent tests (RASTs) on the basis of IgE is scientifically unsound (see  Interpretation below).

Interpretation

Significant allergic disease is possible with low levels of total IgE (including anaphylaxis). Only patients with undetectable IgE (<7) are unlikely to have allergic disease. Conversely, levels above the normal range are compatible with no clinical allergic disease. IgE >1000 associated with atopic eczema; IgE >50,000 confirms hyper-IgE syndrome (although patients may have lower levels—diagnosis is clinical). Raised levels are also seen in parasitic infections of the bowel, filariasis, lymphoma (especially Hodgkin's disease), and Churg–Strauss vasculitis.

Skin prick tests

Unit: mm wheal size, compared to histamine and saline controls.

Normal range: no wheal.

Principles of testing

This remains the gold standard for allergy diagnosis. It identifies IgE-mediated reactions (type I) such as inhalant allergy, anaphylaxis, and food allergy. It is dependent on triggering the release of histamine from cutaneous mast cells. Solution of allergen or controls (histamine or saline) placed on the skin and pierced through by a lancet. After 15min, wheal will be visible. Positive is at least 2mm greater than -ve control. Histamine control must be +ve. Not interpretable if the patient is dermatographic (-ve control gives wheal). Can use factory-prepared allergens; also use double-prick technique with fresh foods (prick food, then the patient)—useful where allergens are labile, e.g. fruits.

Indications for testing

Mainstay for diagnosis of all types of allergic disease. Contraindicated when there is significant skin disease, previous severe allergic reactions (use RASTs first), patients on antihistamines (need to be off drug for a week). Other drugs will interfere, e.g. Ca^{2+} channel blockers, tricyclic antidepressants.

Interpretation

Results can only be interpreted in the context of the clinical history. Testing should be tailored to individual patients to answer specific questions. May need to be followed up by open or blinded challenges where -ve results are obtained in patients with good histories.

Good specificity for inhalant allergens and some foods (nuts, fish); results comparable with RAST testing.

Many known families of cross-reactivity between biological families

- Latex allergy associated with food reactions: banana, avocado, kiwi fruit, chestnut, potato, tomato, cannabis, lettuce. Also birch pollen allergy commoner.
- Birch pollen allergy (asthma, rhinitis) with food-related reactions to nuts, apples, plums, cherries, carrots, and potatoes ('oral allergy' or 'pollen-fruit' syndrome).
- Mugwort pollen and celery.
- Ragweed pollen with melon and banana.

Specific IgE: ‘RAST tests’

Units: continuous numeric scale in IU/mL. Also (less frequently now) reported as grades 0–6.

Normal range: grades 0 and 1 indicate insignificant specific IgE. For an overview of grades, see Table 4.8.

Principles of testing

Previously tested by RIA (hence the acronym RAST = radioallergosorbent test); now identified by enzyme-linked or fluorimetric assays.

Indications for testing

‘RAST’ tests are expensive and should be reserved for cases where skin prick testing is not possible: extensive skin disease, patient on antihistamines, severe reactions, small children, dermographic patients. Testing MUST be guided by the history—do not request ‘allergen screen’.

Interpretation

Presence of specific IgE does NOT equate to allergic disease—indicates sensitization only. Must be interpreted in the context of the clinical history. Numerical value does NOT correlate with severity of clinical reactions.

RAST tests are of little value for identifying allergy to fruits and vegetables, as the allergens are labile, and to drugs (unreliable). False +ves possible when total IgE is very high due to non-specific binding (less of a problem with newer assays).

Table 4.8 Grades

0	<0.35
1	0.35–0.70
2	0.70–3.50
3	3.50–17.50
4	17.5–50.0
5	50–100
6	>100

Mast cell tryptase

Unit: µg/L.

Normal range: 2–14 µg/L.

Principles of testing

Measured by EIA. Analyte is stable in clotted blood.

Indications for testing

Valuable test for the investigation of acute? allergic reactions. Released when mast cells degranulate, and stable in serum for up to 24h. Also useful for monitoring patients with mastocytosis.

Interpretation

Raised levels indicate mast cell degranulation and will help distinguish anaphylactic and anaphylactoid reactions from other causes of reactions (vasovagal, hyperventilation, carcinoid, phaeochromocytoma, etc.). Persistent elevated levels may indicate mastocytosis.

Drug allergy testing

Investigation of severe drug allergy is a specialized field and all patients should be referred to an appropriate expert for an opinion, usually a consultant in allergy or clinical immunology in a regional centre. Testing will usually involve skin prick testing, followed by intradermal testing and patch testing and, if necessary, blind challenge.

Patch tests

Unit: scored qualitatively.

Normal range: -ve.

Principles of testing

This test identifies cell-mediated reactions = delayed-type hypersensitivity (type IV reactions). It should not be confused with skin prick testing. Allergens in petrolatum jelly are placed in contact with the skin for 48h under occlusion with aluminium cups. The test result is read at 96h, looking for eczematous change and blistering. Usually carried out by dermatology departments.

Indications for testing

Investigation of contact reactions, e.g. eczema.

Interpretation

Positive results are invariably significant. Common allergens include metals such as nickel and chromium, dyes and chemical in leather, rubber chemicals (accelerators), and cosmetic chemicals. Panels of allergens used, depending on the clinical history.

Cellular function assays

Investigation of cellular function of lymphocytes, neutrophils, macrophages, and natural killer (NK) cells are restricted to specialized regional immunology laboratories. The tests are labour-intensive and difficult to standardize, with the exception of basic lymphocyte markers. EQA schemes are available only for basic lymphocyte markers.

All tests, other than basic lymphocyte markers, should only be requested after discussion with a consultant immunologist. Their role is in the investigation of suspected cellular immunodeficiency, particularly SCID and 1° disorders of neutrophils. In such cases, urgent referral of the patient to an appropriate paediatric or adult immunologist is more appropriate than fiddling around trying to get tests done, as the immunologist will have direct and immediate access to the appropriate tests. Lives have been lost due to delay in transfer, whilst inexperienced clinicians have tried to make diagnoses.

Lymphocyte surface markers

Unit: cells/ μ L.

Normal range: see Table 4.9.

Table 4.9 Normal ranges for lymphocyte surface markers

CD3+ (total T cells)	690–2540
CD19+ (total B cells; CD20 is equivalent)	90–660
CD3+CD4+ (T helper cells)	410–1590
CD3+CD8+ (cytotoxic T cells)	190–1140
CD16+CD56+ (NK cells)	90–590

Principles of testing

Lymphocyte surface markers should be carried out on a single-platform flow cytometer, which will give a direct absolute count, not requiring a total lymphocyte count from a haematology analyser. Absolute counts are the preferred value; percentages are not useful. Fresh samples are required for optimum results. Many other surface markers are available to answer more specific immunological questions, but these will usually be of interest only to clinical immunologists. An EQA scheme operates.

Indications for testing

There are no absolute indications. Investigation of lymphocyte subsets is an important part of the work-up of any patients with suspected 1° or 2° immunodeficiency and of patients with unexpected lymphopenia. Serial measurements are valuable in patients undergoing BMT or stem cell transplantation, those with 1° immunodeficiencies, those on any immunosuppressive therapy, and those with HIV on therapy with highly active antiretroviral therapy (HAART).

Interpretation

Results can only be interpreted in the context of the clinical question. Lymphocyte surface marker analysis CANNOT be used as a surrogate for HIV testing, as many acute viral and bacterial infections, as well as other medical problems, will give rise to a reduction in CD4+ T cells.

Critical action

Baby <6 months with lymphocyte count $<2 \times 10^9/L$ = SCID until proven otherwise: IMMEDIATE referral to a SCID BMT unit (in the UK, Newcastle General Hospital, Newcastle upon Tyne, and Great Ormond Street Hospital for Sick Children, London). Look at the differential white count, not just the total white count!

Lymphocyte function tests

These are highly specialized and should only be carried out on the recommendation of a clinical immunologist.

Indications for testing

There are no absolute indications for testing, but it is usually carried out as part of the specialized work-up of patients with known or suspected SCID and in the monitoring of immunological reconstitution post-BMT.

Interpretation

Is complex and dependent on the precise clinical circumstances.

Neutrophil and macrophage function

This is a specialized test. Samples do not transport well, and results are often abnormal if the patient has an active infection or is on antibiotics. It is preferable to refer patients to a clinical immunologist who will organize testing if appropriate.

Indications for testing

Patients with deep-seated abscesses, recurrent major abscesses (exclude diabetes, staphylococcal carriage, and hidradenitis suppurativa first), major oral ulceration, and unusual fungal or bacterial infections (*Pseudomonas*, *Serratia*, staphylococci, *Aspergillus*). Atypical granulomatous disease, including atypical Crohn's disease. Genetic defects of neutrophil function may present at any age.

Interpretation

Interpretation is complex; defects of oxidative metabolism may indicate chronic granulomatous disease; defects of phagocytosis are recognized. Also MPO deficiency. Neutropenia may be chronic or cyclical. Genetic testing to follow up may be required.

Natural killer cell function

Is rarely required. The main indication is in recurrent severe infection with herpesviruses. Assays are complex and need specialist interpretation. Always discuss cases with a clinical immunologist.

Further reading

Shoedfeld Y, Meroni PL, Gershwin ME (eds.). *Autoantibodies*, 3rd edn. Sand Amsterdam: Elsevier, 2014.
Spickett G. *Oxford Handbook of Clinical Immunology and Allergy*, 3rd edn. Oxford: Oxford University Press, 2013.

Infectious and tropical diseases

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Introduction to infectious diseases

Everything about microscopic life is terribly upsetting ... how can things so small be so important?

(Isaac Asimov—1920–1992)

An old enemy

Infectious diseases have had a huge impact on the human species. Throughout history, mighty armies have been humbled by microbiology. After the introduction of effective antibiotics during the Second World War, there was great optimism that the fight against infectious diseases had been won. In recent years, this hope has been dramatically dashed. Almost all new diseases are infections, and some of the twenty-first century's most pressing problems are pathogens that have only appeared in the 30 years prior to this book being written. Globally, the most important of the newer organisms are HIV and hepatitis C, though old enemies, such as TB, *Pneumococcus*, and malaria, are still killing millions throughout the world. The dreadful epidemic of Ebola virus disease has shaken the world's biosecurity.

New challenges

As we proceed through the twenty-first century, several factors are serving to ↑ the relative importance of infection over other areas of medicine. Infections such as Ebola, zika, and avian/swine influenza are continually emerging and re-emerging. Antimicrobial resistance is increasing, meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE), and carbapenemase-producing *Enterobacteriaceae* (CPE) being the most infamous examples. There are more immunosuppressed patients as a result of ↑ use of chemotherapy agents and organ transplantation. Tourists and other travellers are making their way to ever more remote parts of the world. Medical tourism is a cheap way of getting cosmetic surgery. Migration of populations has always spread disease and this continues today. There are concerns of wilful bioterrorism. All of these factors mean that the infectious differential diagnosis—even in the developed world—grows ever longer.

It is always worth bearing in mind infection in a differential diagnosis is often treatable. Accordingly, it is always better not to miss treatable options over incurable ones. Furthermore, some infectious diseases like Ebola, multidrug-resistant tuberculosis (MDR-TB), severe acute respiratory syndrome (SARS), Middle East respiratory syndrome coronavirus (MERS-CoV), and avian/swine influenza have major public health consequences, including for the treating physician.

A challenge to the clinician

The same infection is often capable of causing a wide variety of clinical pictures. HIV, for example, is a great mimicker. Acquired immune deficiency syndrome (AIDS) is defined by its consequences. This is not so surprising, given the genetic variety of mankind, hence individual responses to a bewildering variety of infecting agents. Some clinical syndromes can be caused by many, quite different pathogens. Good examples include pneumonia, hepatitis, and endocarditis.

Furthermore, some infectious diseases can resemble non-infectious diseases. For example, amoebic colitis can resemble ulcerative colitis; syphilis can present with serious psychiatric symptomatology; a brain abscess or a tuberculoma can resemble a brain tumour; and TB of the vertebral column can resemble metastatic malignancy. Getting it wrong can be catastrophic for the patient.

Other diseases can mimic infections

Non-infectious diseases can resemble infection. Examples include gout of the first MTP joint, rather than cellulitis; cervical lymphadenopathy due to lymphoma, rather than TB; familial Mediterranean fever as a cause of PUO; SLE leading to Libman–Sachs endocarditis; adult Still's disease as a cause of fever and neutrophilia; and inflammatory carcinoma of the ♀ breast resembling a pyogenic breast abscess.

Importance of epidemiological factors

Epidemiology is fundamental to determining which, if any, infecting agents, and therefore investigations, are relevant in a given patient.

Geography

This is very important and needs to be specific.

Some infections are common the world over and include

- Staphylococcal infection.
- *Salmonella* infections.
- Pneumococcal pneumonia.
- Gonorrhoea.
- Thrush (candidiasis).
- TB.
- Influenza.
- EBV.
- HIV (although sub-Saharan Africa is still the worst affected area).
- Hepatitis C.
- Herpes simplex.
- Threadworm (*Enterobius*).
- Syphilis.

Some infectious diseases are commoner in the tropical world, e.g.

- Malaria.
- Diphtheria (although still common in Eastern Europe).
- Rheumatic fever.
- Enteric fever (typhoid and paratyphoid).
- Hepatitis E (although increasingly widespread).
- Poliomyelitis (very restricted now).
- Rabies (although Eastern Europe has significant disease).
- Viral haemorrhagic fever (VHF), including Ebola, Lassa, Marburg.
- Onchocerciasis (river blindness).
- Schistosomiasis.
- Leishmaniasis (although commonly found around the Mediterranean).
- Ascariasis.
- Cutaneous myiasis (e.g. tumbu and bot fly).

Some infectious diseases are common in some parts of the developed world, but not in others, including

- Lyme disease.
- Babesiosis.
- Ehrlichiosis.
- Histoplasmosis.
- Hydatid disease.
- Anisakiasis.

In the USA, only certain areas are endemic for

- Lyme disease.
- Coccidioidomycosis.
- Babesiosis.
- Histoplasmosis.
- Tick-borne diseases.

Travel and vaccination history

This is important for many reasons. Travel exposes patients to new infectious agents to which they have no immunity. Immunization schedules differ throughout the world, and some groups refuse to have their children vaccinated. Vaccination before travel does not necessarily happen as recommended. The clinician must therefore be aware of the distribution of common infections. Great variation in antibiotic resistance patterns can be observed in different parts of the world; this clearly has an impact on the choice of empirical treatment. Finally, travel often has an impact on patterns of sexual and risk-taking behaviour (see Fig. 5.1).

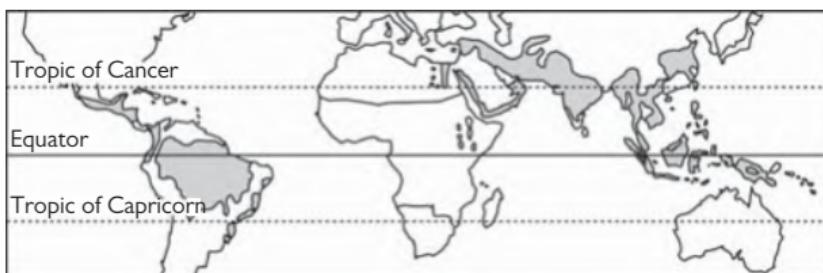


Fig. 5.1 The importance of taking a geographic history. Malaria, which can be life-threatening, is a very common disease in many parts of the world but is not indigenous to most parts of the developed world. Making a diagnosis depends heavily upon the clinician eliciting the clues in the patient's history. Even if s/he has been taking antimalarial drugs, a patient who has been on holiday to Kenya, Thailand, or Brazil may die if the disease is not diagnosed. Clinical suspicion should lead to blood films (on 3 consecutive days) and a platelet count. Bear in mind that the patient may not have been taking adequate prophylaxis, may have been missing tablets, or may not have been absorbing them.

Sexual and drug-taking activity

Searching and personal questions may need to be asked. This is sometimes difficult to do, even with great experience. Patients will/may not admit to high-risk sexual activity or the use of illegal substances and may need to be pressed. Beware of using family members as 'translators'. The clinician must maintain high clinical suspicion at all times, even and especially when the patient does not fit a social stereotype. Bear in mind that any patient may have a 'double life', of which even his/her spouse is unaware. It is dangerous for the clinician to assume that being married equates to sexual fidelity or even heterosexuality.

It may not be immediately obvious that the fever, rash, and hypotension in a woman may be related to her tampon usage (toxic shock syndrome), yet menstruation can be a difficult subject to discuss in some cultural settings. TB of the ♀ genital tract may present as infertility or menorrhagia. 'Lumpy semen' may be indicative of *Schistosoma haematobium* infection, and a 'urinary tract infection' or a septic arthritis in a 19-year-old man may be gonorrhoea.

Social and professional

Pets, hobbies, and jobs may well be important. The patient with pneumonia and a budgerigar could have psittacosis. The tropical fish salesman with a chronic rash on his hand could have *Mycobacterium marinum* infection (aka 'fish tank granuloma'). The jaundiced volunteer cleaning out canals at weekends could have leptospirosis related to contact with rats. The cat owned by the middle-aged lady with recurrent axillary lymphadenopathy may be the key to her problem of cat-scratch disease (*Bartonella henselae*).

Assessing the patient

The recognition of an infectious disease in a patient (or the absence of one) goes far beyond the Petri dish, the microbiology bench, and PCR testing technology. The Andromeda Strain phenomenon (with all due credit to Michael Crichton MD) should be borne in mind. The disease in front of you might be the first ever presentation or the first in a new outbreak! Almost the only significant new human diseases that will appear in the future will be infectious diseases and they will keep appearing till the end of the human species (see Fig. 5.2).

Assessment should include

- A detailed history.
- Full physical examination (including temperature).
- The generation of a differential diagnosis.
- Specimen collection before antimicrobial therapy.
- Laboratory tests.
- Non-invasive procedures (including radiological tests where appropriate).
- Invasive procedures.
- The making of a definitive diagnosis (wherever possible).

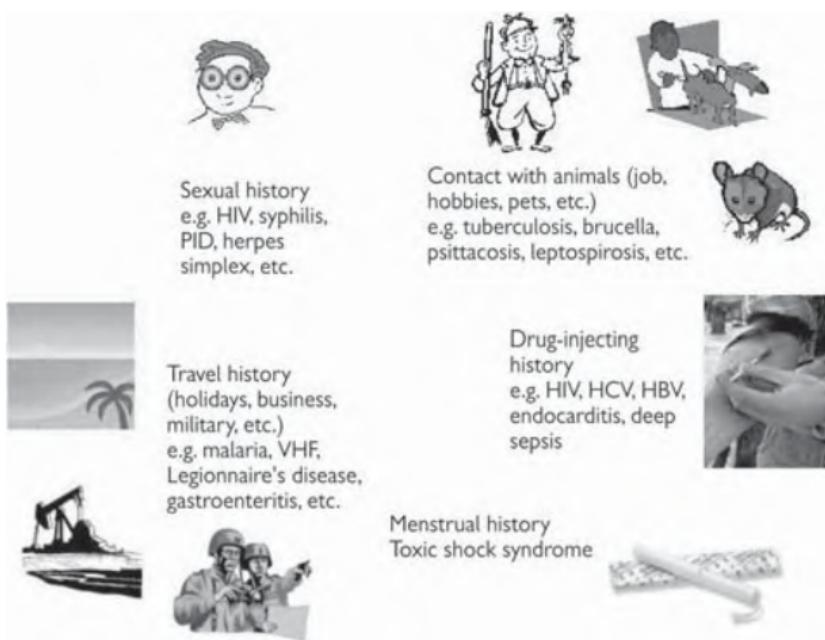


Fig. 5.2 Infection and history taking.

When considering the possibility of an infective process, one should always consider the basic taxonomy

- Bacteria (including primitive forms).
- Mycobacteria.
- Fungi.
- Viruses.
- Protozoa.
- Helminths.
- Prions.
- Myiasis.

Investigations available to the infectious diseases or general physician

Many tests will be performed with a view to making a diagnosis. Investigation of a patient should be rational and evidence-based, wherever possible. Although the interrogative armamentarium of the infectious diseases and tropical medicine physician is enormous—as with any other branch of medicine—the history and examination will point the way. Results will emerge which, whilst not producing a diagnosis as such, will nevertheless require following up. For example, low C5 levels in recurrent meningococcal septicaemia may need immunological assessment. (Remember also that there is a new drug eculizumab which specifically inhibits C5.) IgG deficiency leading to recurrent pneumonia may require regular infusions of γ globulins. A low CD4+ cell count, which is not due to HIV infection, could be a feature of sarcoidosis.

Making a diagnosis alone is not the only issue at stake. Some tests must be done if a patient is going to be treated safely. Examples might include: G6PD levels before administering primaquine for hypnozoite eradication in malaria; TB cultures for antibiotic sensitivity prior to starting empirical therapy; and exclusion of pregnancy before using certain antibiotics such as doxycycline and ciprofloxacin. Other tests relate to the fact that some infectious diseases are dangerous to others, including the doctor! Prime examples of this would be MDR-TB and extensively drug-resistant TB (XDR-TB), avian influenza, SARS, MERS-CoV, and Ebola, all of which are potentially dangerous for the population at large and need to be identified (or at least suspected wherever appropriate) and treated in an isolation unit.

Investigating the infectious diseases/tropical medicine case

Available diagnostic techniques

Direct detection

- **Microscopy:**
 - Direct—e.g. faecal parasites (\pm iodine) or malarial and trypanosomal blood films.
 - Special stains—these include Gram and ZN stains.
 - Electron microscopy (EM)—for viruses and other pathogens.
 - Immunofluorescence—with specific sera.
- Presence of toxin: e.g. *Clostridium difficile*.
- Antigen detection (☞ Serology, pp. 396–9).
- Molecular assays (☞ Molecular diagnostics, pp. 406–7): these include gene probes, amplification assays, e.g. PCR.
- Point-of-care tests: bedside rapid diagnostic tests (RDTs) for malaria, influenza, HIV, and HBV

Culture

- All body fluids and tissues can be cultured. As a general principle, the larger the sample sent, the greater the yield. It is good practice to forewarn the laboratory before sending any unusual samples, especially if there is a risk to laboratory staff, and label accordingly (☞ Biohazards, pp. 402–5) Laboratory preparation and specialist containers may be required. Adequate clinical details should be written on any request forms, including travel history. The choice of culture technique can vary dramatically, depending on the organism that is being sought. There may be only one chance to culture the correct organism, so it should not be wasted.
- Once a culture has grown, identification may be:
 - Through special growth media, culture temperature, or atmosphere.¹
 - By biochemical reactions (e.g. catalase or coagulase), API strip, or matrix-assisted laser desorption/ionization (MALDI).
 - With specific antisera (e.g. latex agglutination or immunofluorescence).
 - Using molecular-based methods (e.g. specific probes, restriction enzyme patterns, DNA sequencing).
 - Using antimicrobial susceptibility testing (e.g. metronidazole sensitivity for anaerobes and vancomycin for Gram +ves).

Serology

- (☞ Serology, pp. 396–9.

¹ Todar's Online Textbook of Bacteriology. ☞ <http://textbookofbacteriology.net>.

Other tests as appropriate

(See appropriate sections.)

- Biochemistry.
- Haematology.
- Immunology.
- Molecular tests.
- Radiology.
- Stool and bowel contents.
- Tissue biopsy and deep aspiration specimens.
- Other tests.

Culture techniques

Microorganisms exist in nature as mixed populations. Diagnosis of an infection means identifying the relevant pathogen. Furthermore, different organisms can cause the same disease (e.g. pneumonia), require very different treatment and management, and have different prognoses. Whilst some specimens (e.g. stool, sputum) contain extremely large numbers of varied organisms, some specimens (e.g. blood, CSF, urine) should be sterile, unless infected or contaminated during their collection.

Microbiological culture assists with the aetiological diagnosis of bacterial, fungal, protozoal, or viral illness by enabling identification and susceptibility testing of the isolated organism(s). Bacterial culture was the first to evolve, but useful data on other pathogenic groups can also be obtained through the use of culture-based methodologies (although options for treatment are currently more limited for viruses and fungi than for bacteria). Furthermore, culture of mycobacteria, viruses, and fungi usually takes longer than most bacterial cultures; therefore, the data obtained are most valuable for late confirmation of the diagnosis or for epidemiological purposes (e.g. for predicting the appropriate constituents for a polyvalent influenza vaccine).

Bacteria

Three major steps are involved in extracting pure cultures from a diverse population of microorganisms and identifying a pathogen. Many of these processes can now be automated (see Fig. 5.3).

1. *An isolation plate is created.* To do this, the mixture must be diluted until the various individual microorganisms have been dispersed far enough apart on an agar surface, so that, after incubation, they will form visible colonies isolated from the colonies of their neighbours. Specialized culture media (such as selective media, differential media, enrichment media, and combination selective and differential media—a great many exist) may be used to supplement mechanical techniques of isolation. Culture can be aerobic or anaerobic. (Note: specimens for the isolation of anaerobic pathogens require special care, as anaerobic bacteria die in the presence of O₂. Such specimens should therefore be transported in a reduced container.) Lastly temperature can be used to further select for pathogenic organisms, e.g. *Campylobacter jejuni* is unusual as it will grow at 41°C.
2. *A pure culture is created.* To achieve this, an isolated colony will be selected out and carefully 'picked off' the isolation plate for transferring to a new sterile medium. Following incubation, all the organisms in the new culture will be descendants of the same organism.
3. *The organism can then be identified through various manoeuvres:*
 - Increasingly, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and other automated systems (see below).

- Other techniques include:
 - The colony appearance and susceptibility to specific antibiotic discs.
 - The microscopic appearance.
 - The staining responses (e.g. Gram +ve vs Gram -ve).
 - The use of a range of biochemical tests designed to uncover characteristics typical of a particular organism, e.g. catalase reaction, sugar fermentation as in the API strip. The presence of the enzyme coagulase is useful for distinguishing *Staphylococcus aureus* from less pathogenic coagulase -ve staphylococci (normal skin commensals).

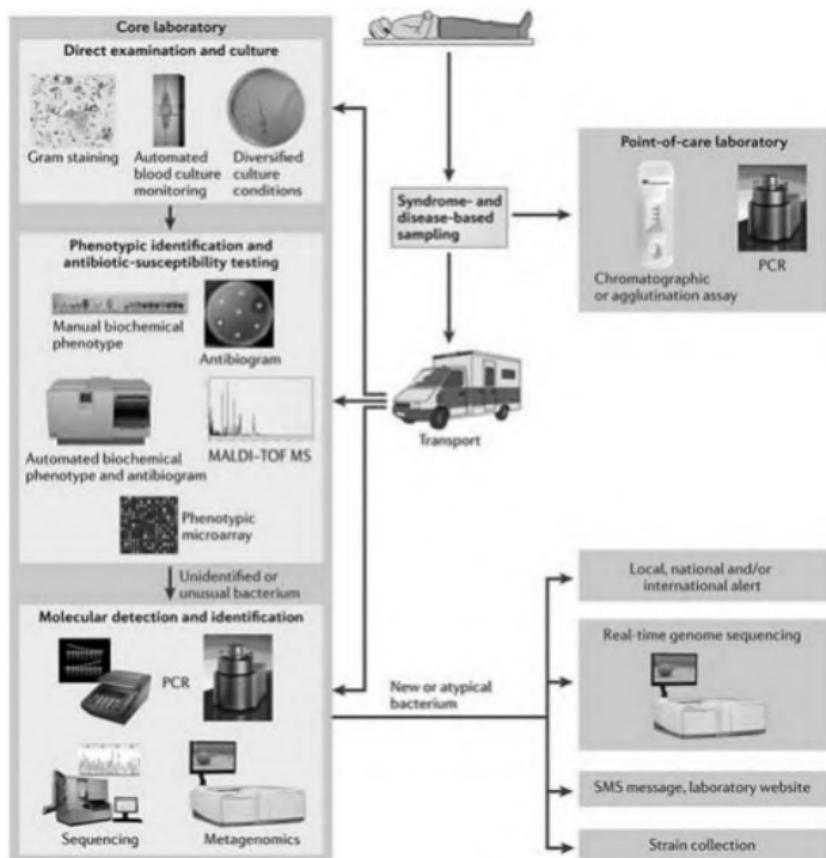


Fig. 5.3 Overview of new methods in the workflow of clinical microbiology laboratories.

Reproduced from Fournier, Pierre-Edouard et al, Modern clinical microbiology: new challenges and solutions, *Nature Reviews Microbiology* 11, (2013) 574–585 doi:10.1038/nrmicro3068, with permission from Macmillan Publishers Ltd.

- Less frequently now is the use of antisera (direct serology) for culture confirmation:
 - Agglutination and latex agglutination tests can be used on colonies to identify *Escherichia coli* 0157, *Streptococcus pneumoniae*, serogroups of *Neisseria meningitidis*, *Shigella*, and *Salmonella*, Lancefield groups of β-haemolytic streptococci, and serotypes of *Haemophilus influenzae*.
 - Detection of specific antigens by direct fluorescent antibody (DFA) staining can be used to identify colonies of *Streptococcus pyogenes*, *Bordetella pertussis*, and the species and serotypes of *Legionella*.

Ideally, specimens for bacterial culture should be taken before antibiotics are administered. This may not always be feasible, but the information yielded may well be less than ideal.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

MALDI-TOF MS is a new automated technique using mass spectrometry, which allows the analysis of biomolecules such as DNA, proteins, peptides, sugars, and large organic molecules, which tend to fragment when ionized. MALDI-TOF MS is a 3-step process. Firstly, the sample is mixed with a matrix material and applied to a metal plate. Secondly, a pulsed laser irradiates the sample, triggering disintegration. Finally, the analyte molecules are ionized in a plume of hot ablated gases, which are then accelerated into a mass spectrometer for analysis. The resultant spectra generated can be used for the identification of microorganisms, when compared to stored database profiles. Species diagnosis by this procedure is much faster, more accurate, and cheaper than other techniques. MALDI/TOF has become the standard method for species identification in large, modern medical microbiological laboratories. It is easy to use, robust, and rapid, allowing accurate bacterial identification of a large variety of species within a few minutes, with only a small amount of culture sample required for the analysis (10⁴–10⁶ CFU). The absence of the need to purify the suspect colonies allows for a much faster turnaround time. There are other new automated systems on the market such as the BD Phoenix identification method (BD Diagnostic Systems, Sparks, MD) which uses modified conventional, fluorogenic, and chromogenic reactions. Similarly, the Biologue system (Biologue, Inc.) combines up to 2000 phenotypic tests. The older Analytical Profile Index (API, bioMérieux) system uses direct inoculation into a strip of various reactants, manually producing a code which is then compared to a database. (See Fig. 5.4.)

Antibiotic sensitivity

Once a bacterium is isolated, it can be cultured in the presence of antibiotic(s) to assess if it is susceptible to that agent or not. The minimum inhibitory concentration (MIC) is the lowest antibiotic concentration at which the microorganism under assessment shows no visible growth *in vitro*. The MIC can provide the clinician with precise information about the infecting bacterium's degree of antibiotic susceptibility and enable him/her to avoid antibiotics to which the organism shows resistance.

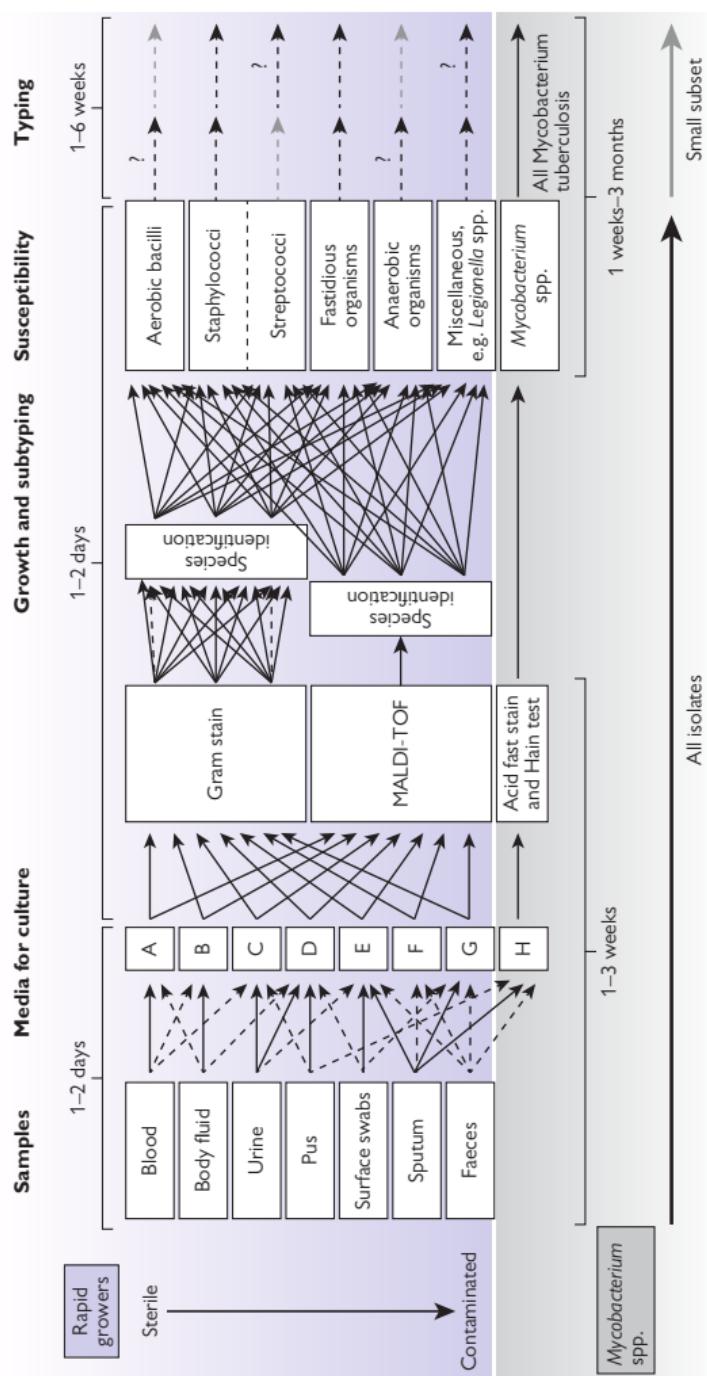


Fig. 5.4 MALDI-TOF MS.

Nature Reviews Genetics 13, 601–612 (September 2012) doi:10.1038/nrg3226 Transforming clinical microbiology with bacterial genome sequencing Xavier Didelot, Rory Bowden, Daniel J. Wilson, Tim E. A. Peto & Derrick W. Crook.

For organisms exhibiting unusual resistance patterns, susceptibility panels using methodologies such as broth microdilution, gradient diffusion, and/or disc diffusion have been created to assist clinicians.

On occasions, these data will need to be linked to testing of blood levels for some antibiotics (e.g. gentamicin, vancomycin, cycloserine). MALDI-TOF and other automated systems can also be used to identify antimicrobial resistance patterns. Nowadays genome sequencing can identify genetic mutations associated with resistance.

Viruses

Viral culture is rarely used now, but it differs significantly from bacterial culture as viruses require a very different type of medium to grow. Molecular techniques, such as PCR and antigen detection, have become more effective, can quantitate the amount of virus present, and have almost completely replaced culture.

The appropriate type of specimen to collect, the best means of transport, and the most appropriate cell culture to use will vary with the particular virus suspected, the specimen site, and the time of the year.

- The choice of specimen is very important. Numerous viruses enter via the mucosa of the upper respiratory tract, yet that virus may compromise multiple or distant tissues and organs.
- Swabs can be used to collect a variety of specimens from the body surfaces for viral detection, e.g. nose, throat, eye, skin, and rectum. A nasopharyngeal aspirate (NPA) may be the more appropriate specimen if influenza is suspected. It is important to collect mucosal cells, as this is where the virus resides. Deeper specimens, such as blood and CSF, will be appropriate for some viruses. Different viruses will need different collection approaches, e.g. heparin, citrate, and EDTA are all acceptable for the detection of CMV by PCR, antigenaemia testing, or culture, but for some other viruses, only citrate should be used if they are to be cultured.
- Unlike many bacterial or fungal pathogens, the time of year is important to keep in mind when making a diagnosis of certain viral diseases. For example, enteroviruses circulate almost exclusively in the summer months and influenza likewise circulates during the winter months.
- Timing is important when collecting specimens for viral detection. They should be collected as early as possible after the onset of symptoms, as once viral shedding ceases, culture will be impossible and serological and molecular techniques may be the only way of diagnosing the viral pathogen.
- Some viruses cannot be cultured, e.g. viral agents of diarrhoea (caliciviruses, astroviruses, and coronaviruses), HCV, and HBV.

Laboratory assays for antiviral susceptibility testing include phenotypic and genotypic assays. Genotypic assays have almost completely replaced phenotypic testing, especially for HIV and HBV. Genotyping of HCV is critical in guiding treatment. Phenotypic assays require growth of the virus *in vitro* and so present a biohazard. In these circumstances, genotypic assays (➔ Molecular diagnostics, pp. 406–7) are now routinely available and very useful.

Fungi

Unlike bacterial and viral diseases, direct microscopy can often be used to diagnose fungal infections (based on distinctive morphological characteristics of the invading fungi, e.g. *Aspergillus* or tinea, and/or the judicious use of special stains such as methylthioninium chloride (methylene blue)). However, histopathological diagnoses should be confirmed by culture, wherever possible. Conversely, although diagnoses are usually made by isolating the causative fungus from bodily samples, the presence of a fungus in a culture from a non-sterile site does not mean that it is pathological (e.g. *Candida* isolation from sputum). Fungal infection can only be definitively established with evidence of tissue invasion histologically. There are also a range of serological tests available for systemic mycoses (→ Serology, pp. 396–9), but few provide definitive diagnoses by themselves. Antigen detection has been promising but generally has poor sensitivity and specificity, even when used in combination. For example, galactomannan and β-D-glucan can be indicative of invasive aspergillosis in combination with PCR for *Aspergillus*, but the context also needs to be considered. Allergic bronchopulmonary aspergillosis (ABPA) patients can have serum samples tested for total IgE and *aspergillus*-specific IgE.

An exception is cryptococcal antigen (CrAg) which is useful in serum and CSF samples for diagnosis of cryptococcal disease, especially meningitis in immunocompromised HIV patients.

Fungal culture techniques are similar to bacterial ones. They are most useful for detecting dimorphic fungi, which manifest both mycelial and yeast forms. This group includes *Candida* species, *Cryptococcus neoformans*, *Blastomyces dermatidis*, *Histoplasma capsulatum*, *Penicillium marneffei*, and *Coccidioides immitis*. MALDI can identify most fungal species.

Protozoa

Protozoa of the genera *Acanthamoeba* and *Naegleria* may cause fatal CNS disease. *Acanthamoeba* species are free-living amoebae associated with keratitis; they may also cause granulomatous encephalitis. Another free-living amoeba *Naegleria fowleri* is able to cause acute fulminant meningoencephalitis and is usually associated with a history of swimming in freshwater lakes or brackish water. In suspected cases, CSF and other suspicious clinical material may be cultured on a non-nutrient agar plate seeded with a 'lawn' of Gram –ve bacteria (such as *E. coli*). Pathogenic amoebae can be identified microscopically.

Worldwide the most important protozoan infection are the plasmodia causing malaria. They can be cultured, but this is rarely of use clinically. The mainstay of malarial identification is direct microscopy, although antigen detection tests are now available.

Immunological tests

Immunological methods are in wide usage to detect many pathogens present in clinical samples. Serology refers to the laboratory usage of antigen–antibody reactions for such diagnostic purposes. Diagnosis is made by detecting antibody or antigen in blood and/or other bodily fluids, or by the identification of pathogens in culture. More recently, interferon γ release assays (IGRA) have been developed to look for evidence of T-lymphocyte reactivity to antigens from pathogens such as *Mycobacterium tuberculosis*.

Both direct and indirect serological tests exist

Indirect serological techniques

Employ antigen–antibody reactions to detect specific antibodies manufactured in response to an antigen or antigens on an infecting pathogen's surface. These antibodies are found circulating in the patient's blood or present in other body fluids.

Direct serological techniques

Employ antibodies to detect specific antigens. Because this technique can be used to identify and type cultured organisms (Culture techniques, pp. 390–5), not only does it have individual clinical value, but it also has important epidemiological applications.

HBV is a good example of an infection where both antigen and antibody profiles are diagnostically, therapeutically, prognostically, and epidemiologically important:

- Antibody detection of a specific antibody, e.g. anti-hepatitis Be antigen (anti-HBe antibody), anti-hepatitis B surface antigen (anti-HBs antibody).
- Antigen detection, e.g. hepatitis B 'e' antigen (HBeAg), hepatitis B surface antigen (HBsAg).
- More recently, pre-core mutants of HBV have emerged that are not picked up by the common antigen tests.

(See Fig. 5.5.)

Antibody tests

A wide variety of methodologies for assessing antibody response are available such as immunofluorescence, agglutination, ELISA, and complement fixation (CF).

Sub-classification of organisms, through serogrouping, can be valuable epidemiologically, e.g. whilst investigating an outbreak of meningococcal (*N. meningitidis*) disease; if the culprit is determined to be type C, vaccination can be utilized to control the outbreak. However, this is being replaced by molecular techniques.

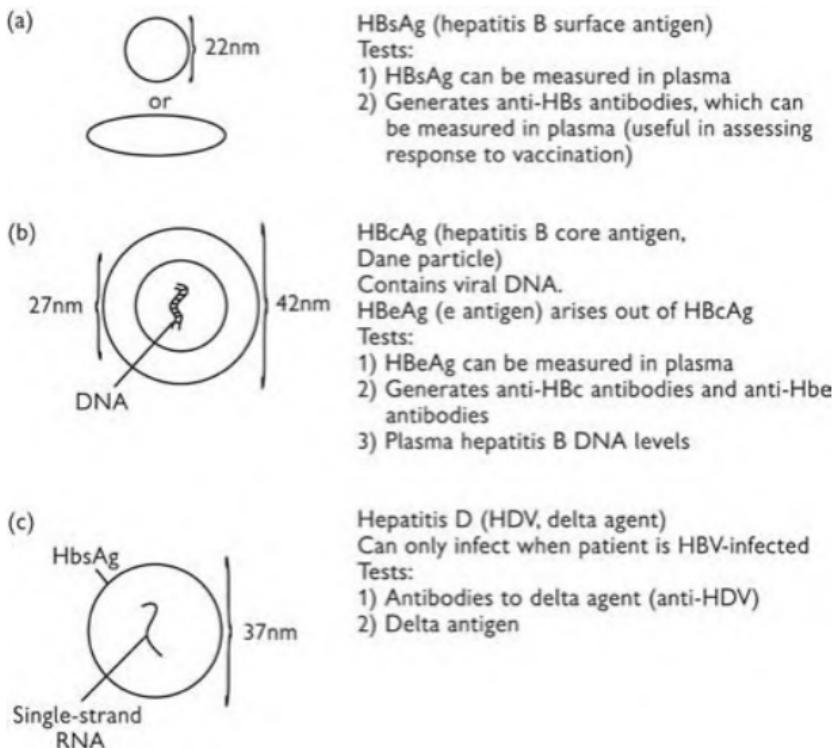


Fig. 5.5 Antibody tests in hepatitis B and D.

General principles

(See Fig. 5.6.)

1. Specific IgM levels indicates a 'new' infection.
2. Specific IgG levels indicates a 'new' or a 'previous' infection, or, in some cases, immunity generated by vaccination.
3. ↑ IgG ('rising titre') when two samples ('paired sera') are taken with an appropriate intervening interval between them indicates a 'new' infection or re-infection. Diagnosis (as indicated by seroconversion) necessitates a diagnostic antibody titre or a 4-fold ↑ in antibody titre.
4. Seroconversion is said to have occurred in situations 1 and 3.

Viral antibody tests

These can be very useful because once viral shedding has ceased, viral culture is of no further value. They include tests for HIV-1, HIV-2, human T-lymphotropic virus (HTLV)-1, HTLV-2, hepatitis A, hepatitis B, hepatitis C, δ agent (hepatitis D), hepatitis E, EBV, CMV, dengue, Ebola virus disease, Lassa fever, respiratory syncytial virus (RSV), mumps, measles, rubella, influenza, parainfluenza, St Louis encephalitis, West Nile virus, yellow fever, SARS, and many more (see Fig. 5.7).

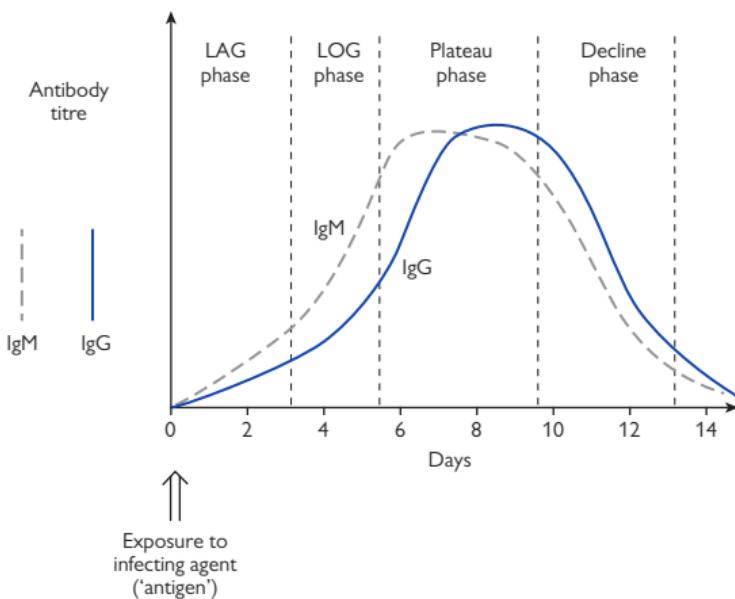


Fig. 5.6 Relative rate of appearance and disappearance of IgM and IgG.

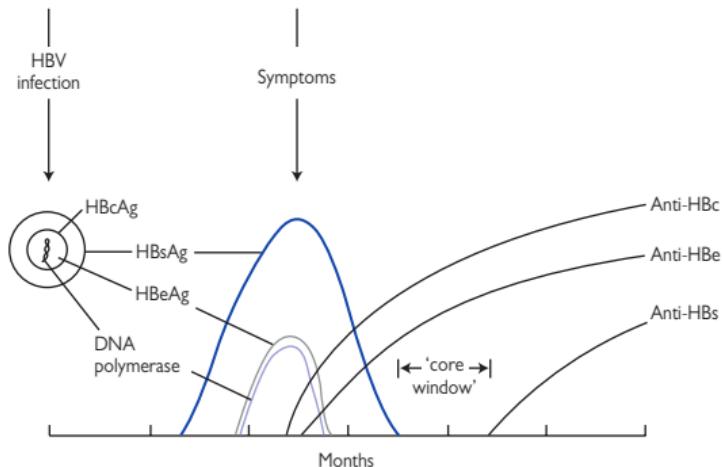


Fig. 5.7 Hepatitis B antigens and antibodies.

Bacterial antibody tests

Less useful, these include anti-streptolysin (ASO) and anti-DNAse B for streptococcal infection.

They can be useful for non-culturable or difficult-to-grow organisms in the correct clinical context, e.g. cat-scratch fever (*B. henselae*), *B. pertussis*, Lyme disease (*Borrelia burgdorferi*), *Brucella* species, *C. jejuni*, *Chlamydia* species, Q fever (*Coxiella burnetti*), *E. coli* 0157, *Francisella tularensis*, *Helicobacter pylori*, *Legionella pneumophila*, *Leptospira interrogans*, *Listeria monocytogenes*, *Mycoplasma pneumoniae*, *N. meningitidis*, *Neisseria gonorrhoeae*, *Rickettsia prowazekii*, *Salmonella* species, *Treponema pallidum* (including *Treponema pallidum* haemagglutination assay (TPHA), rapid plasma reagent (RPR), VDRL, fluorescent treponemal antibody absorption (FTA-ABS), IgM-FTA, IgM-ELISA), *Yersinia enterocolitica*/*Y. pseudotuberculosis* (Widal test).

Protozoal antibody tests

Include tests for amoebiasis, toxoplasmosis, leishmaniasis (kala-azar), African trypanosomiasis (sleeping sickness), American trypanosomiasis (Chagas' disease), babesiosis, and *Toxoplasma gondii*.

Helminthic antibody tests

Include tests for *Echinococcus granulosus* (hydatid disease), *Echinococcus multilocularis* (alveolar echinococcosis), *Microsporidium* species, schistosomiasis (bilharzia), strongyloidiasis, filariasis, onchocerciasis, *Trichinella spiralis*, *Toxocara canis*, *Taenia solium* (cysticercosis or pork tapeworm), paragonimiasis (Chinese lung fluke), and gnathostomiasis.

Fungal antibody tests

See above, but include tests for *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus versicolor*, *B. dermatidis*, *Candida albicans*, *C. immitis*, *C. neoformans*, *H. capsulatum*, Mukorazeen.

Note: in the case of CF antibody assays for antibodies to coccidioidomycosis, these are specific and do not require proof of rising levels. They can provide indispensable confirmatory evidence for a diagnosis of coccidioidomycosis as well as an indication of the relative risk of extrapulmonary dissemination. In a case of chronic meningitis, a +ve CF for anti-coccidioidal antibodies in the CSF often provides the only definite diagnostic indication of the need for aggressive antifungal therapy.

Further reading

British Infection Association. *Infection resources*. <https://www.britishinfection.org/guidelines-resources/infection-resources/>.

Public Health England (2009). *Public health laboratories: general clinical laboratory tests*. <https://www.gov.uk/guidance/public-health-laboratories-general-clinical-laboratory-tests>.

Antigen tests

Antigen measurement is achieved through techniques such as CF and immunodiffusion. A variety of bodily fluids can yield diagnostically useful antigens, including saliva, serum, urine, CSF, and fresh stool. The choice depends upon the clinical context. Again many of these tests have been replaced by PCR or, in the case of HIV, combined antigen detection with antibody responses to improve sensitivity.

Viral antigen tests

Include mumps, CMV, influenza, HIV, hepatitis B and C, RSV, parainfluenza viruses, adenovirus, rotavirus, and varicella-zoster virus.

Bacterial antigen tests

Include *L. pneumophila* (serotype 1) and *B. burgdorferi* (in urine), β-haemolytic streptococci, *Pneumococcus*, *C. difficile*, *H. influenzae*, *N. meningitidis*, *H. pylori*, and *C. jejuni*.

Helminthic antigen tests

Filariasis.

Protozoal antigen tests

Include malaria, giardiasis, *Trypanosoma cruzi* (Chagas' disease), *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*). Malaria antigen tests form the basis for the so-called RDTs which are now the mainstay of diagnosis for malaria in both endemic and non-endemic areas. Two main forms are available; histidine-rich protein 2 (HRP-2) is specific for *Plasmodium falciparum*, whilst Pf LDH can distinguish between *P. falciparum* and *P. vivax*. They are rapid and almost as sensitive as PCR and critically do not need highly trained staff to carry out. They are not so reliable for the other species *P. ovale*, *P. malariae*, and *P. knowlesi*.

Fungal antigen tests

Include *C. neoformans* (CrAg), *H. capsulatum*, and mannoprotein antigen in *C. albicans*.²

² Falci DR, Stadnik CMB, Pasqualotto AC. A review of diagnostic methods for invasive fungal diseases: challenges and perspectives. *Infect Dis Ther* 2017; 6: 213–23.

Collection of specimens

Principles of good specimen collection

- Good-quality specimen and clinical information produce the most valuable data.
- Optimal time of collection, e.g. take bacterial specimens before administering antibiotics.
- Collect the optimal type of specimen wherever possible, e.g. pus is preferable to a 'pus swab'.
- Acquire expertise in specimen collection—ensure minimal contamination by normal flora (e.g. MSU, use of a tongue depressor for throat swab collection).
- Freshness of specimens—rapid transport to the laboratory is essential (especially for anaerobic organisms and for 'hot stools' for parasite diagnosis).
- Collect the appropriate number of specimens at the appropriate intervals, e.g. paired antisera should be taken at least 1–6 weeks apart if a diagnostic rising titre is to be demonstrated.
- Be aware of biological hazards. It is critical to categorize samples with potential pathogens in them and label appropriately.
 - *Biohazard level 1*: bacteria and viruses, including *Bacillus subtilis*, *E. coli*, and varicella, as well as some cell cultures and non-infectious bacteria. Precautions against the biohazardous materials are minimal, most likely involving gloves and some sort of facial protection.
 - *Biohazard level 2*: bacteria and viruses that cause only mild disease to humans or are difficult to contract via aerosol in a laboratory setting such as hepatitis A, B, and C, some influenza A strains, Lyme disease, *Salmonella*, mumps, measles, scrapie, dengue fever, and HIV. Routine diagnostic work with clinical specimens can be done safely at Biosafety level 2 (BSL-2), using BSL-2 practices and procedures. Research work (including co-cultivation, virus replication studies, or manipulations involving concentrated virus) can be done in a BSL-2 (P2) facility, using BSL-3 practices and procedures.
 - *Biohazard level 3*: bacteria and viruses that can cause severe or fatal disease in humans, but for which vaccines or other treatments exist such as anthrax, West Nile virus, Venezuelan equine encephalitis, SARS virus, MERS-Co, hantaviruses, TB, typhus, Rift Valley fever, Rocky Mountain spotted fever, yellow fever, and malaria. The parasites *P. falciparum*, which causes malaria, and *T. cruzi*, which causes trypanosomiasis, also come under this level.
 - *Biohazard level 4*: viruses and bacteria that cause severe to fatal disease in humans and for which vaccines or other treatments are not available such as Bolivian and Argentine haemorrhagic fevers, Marburg virus, Ebola virus, Lassa fever virus, Crimean–Congo haemorrhagic fever, and other haemorrhagic diseases. Variola virus (smallpox) is an agent that is worked with at BSL-4, despite the existence of a vaccine, as it has been eradicated. When dealing with biological hazards at this level, the use of a +ve pressure personnel suit, with segregated air supply, is mandatory. The entrance and exit

of a Level 4 biolab will contain multiple showers, a vacuum room, a UV light room, an autonomous detection system, and other safety precautions designed to destroy all traces of the biohazard. Multiple airlocks are employed and are electronically secured to prevent both doors from opening at the same time. All air and water services going to and coming from a BSL-4 (P4) laboratory will undergo similar decontamination procedures to eliminate the possibility of an accidental release.

Surface specimens include

- **Anal/anorectal:** e.g. gonococcus (*N. gonorrhoeae*).
- **Cervical swab:** e.g. HSV, gonococcus, human papillomavirus (HPV).
- **Ear swab:** e.g. otitis externa, otitis media, bacterial and fungal infections.
- **Foreign bodies:** almost always infected if causing trouble! (Includes iatrogenic foreign bodies such as arthroplasties, cardiac valves, pacemakers, ventriculo-peritoneal shunts, etc.) Foreign bodies in the ear, nose, or vagina can lead to prolonged (and often unpleasant) discharges.
- **Genital ulcers:** dark ground microscopy for syphilis organisms. Also chancroid, *Entamoeba histolytica*.
- **Indwelling catheters:** include urinary catheters, IV cannulae, Portacaths, etc. If a catheter is thought to be the source of an infection, cultures should be performed, and if the catheter or cannula is removed, this can be sent for culture. Urinary catheters are always colonized by bacteria. Intravascular foreign bodies such as central venous catheters and prosthetic heart valves are often affected by bacteria which are normally non-virulent such as coagulase –ve staphylococci.
- **Laryngeal swab:** can be useful for TB.
- **Nasal, pharyngeal, gingival, and throat:** e.g. meningococcus, *S. aureus* carriage, streptococcal infections, pertussis, adenovirus. NPAs are useful for diagnosing upper respiratory tract infections (URTIs) such as influenza and RSV through DIF tests and, more commonly now, PCR. In lepromatous leprosy, a swab from the anterior nares may reveal acid-fast bacilli indicative of this infection.
- **Ophthalmic:** e.g. bacterial conjunctivitis, adenovirus, rabies (from corneal impressions.³ For trachoma, PCR, direct fluorescein-labelled monoclonal antibody (DFA), and EIA of conjunctival smears are useful.⁴
- **Skin:**
 - **Abscess**—culture for bacteria and other unusual organisms.
 - **Dermal scrapings, nail clippings**—fungal infections (tinea—includes pedis, capitis, cruris, versicolor forms).
 - **Petechial rash scrapings**—meningococcus (occasionally gonococcus).
- **Throat:** e.g. *C. albicans*, diphtheria, gonococcus, croup organisms.
- **Urethral:** e.g. *Chlamydia*, gonococcus.
- **Vagina (high vaginal swab):** e.g. *S. aureus* in toxic shock syndrome (including toxin testing), *Gardnerella*, gonococcus.

³ Zaidman GW, Billingsley A. Corneal impression test for the diagnosis of acute rabies encephalitis. *Ophthalmology* 1998; 105: 249–51.

⁴ Rostami S (2016). Trachoma. <http://www.emedicine.com/OPH/topic118.htm>.

Normally sterile fluids include

- **Amniotic fluid:** bacterial infection can cause premature delivery, and rDNA was detected by PCR (➔ Molecular diagnostics, pp. 406–7) in samples from 15 (94%) of 16 patients with +ve amniotic fluid cultures.⁵ Hydrops fetalis can be caused by congenital infections (e.g. CMV, parvovirus B19, toxoplasmosis, syphilis, and Chagas' disease), and making a diagnosis may involve analysis of amniotic fluid with cultures, PCR, etc.
- **Ascites:** consider TB (consider laparoscopy for biopsying peritoneal lesions for culture as well as histology; ➔ Gastrointestinal tract investigations, pp. 412–13).
- **Blood:** multiple samplings at separate times from separate body sites may need to be taken, e.g. in endocarditis. For some organisms and pathologies, an extended period of culture may be needed.
- **CSF:** possibilities include, e.g. meningococcus, pneumococcus, *L. monocytogenes*, TB, fungi (e.g. *C. neoformans*), and viruses (➔ Tissue biopsy and deep aspiration specimens, pp. 418–21).
- **Ejaculate (semen):** if the semen contains a high number of leucocytes, this may be an indication of either infection or inflammation. WBCs are considered significant if >1 million found in each mL of ejaculate. Sexually transmitted diseases (STDs), e.g. gonorrhoea, or urea, plasma, and prostate infections come into the differential diagnosis. *S. haematobium* (bilharzia) may cause haemospermia and be found in ejaculate.⁶ Acute mumps orchitis can be associated with loss of spermatozoa.
- **Ocular fluids (intra-):** include aqueous humour and vitreous humour. Bacterial, fungal, and parasitic problems can affect the interior of the eye.
- **Pericardial fluid:** the commonest organisms will include staphylococci, streptococci, pneumococci, *H. influenzae*, meningococci, and TB.
- **Pleural fluid:** numerous pathologies, including underlying bacterial pneumonia, tuberculous pleurisy, parasitic infections (such as strongyloidiasis), and fungal diseases (such as histoplasmosis). Biopsy of the pleura under direct visualization by video-assisted thoracoscopy (VATS) may give a better diagnostic yield.
- **Synovial fluid (joint aspirate):** bacterial infections can be very destructive and the options are legion. Staphylococcal disease is the commonest, but TB must always be borne in mind. Viral arthritides are usually self-limiting, and treatment is supportive.
- **Urine:** standard culture and sensitivity, e.g. MSU specimen, catheter specimen of urine (CSU)—useful for diagnosing cystitis, pyelonephritis, prostatitis, etc. (prostatic massage may be helpful for improving diagnosis of prostatic infections); 'early morning urine' (EMU) for TB, and a terminal specimen for *S. haematobium* (bilharzia) best collected around midday.

5 Hitti J, Riley DE, Krohn MA, et al. Broad-spectrum bacterial rDNA polymerase chain reaction assay for detecting amniotic fluid infection among women in premature labor. *Clin Infect Dis* 1997; 24: 1228–32.

6 Torresi J, Yung A. Usefulness of semen microscopy in the diagnosis of a difficult case of *Schistosoma haematobium* infection in a returned traveler. *J Travel Med* 1997; 4: 46–7.

Normally infected fluids include

- **Pus:** e.g. abscess contents, wound swab/aspirates, drainage swabs. Usually bacterial (consider both aerobic and anaerobic), but amoebic and hydatid options need to be considered when the lesion is in the liver.
- **Saliva:** normally contains a wide range of commensal flora. Cannulation of a parotid gland duct may yield a specific pathogen that is causing a problem in that gland.
- **Sputum:** includes tracheal aspirate, induced sputum (obtained with physiotherapy assistance), and bronchoalveolar lavage (BAL), which may be needed in sicker patients unable to produce sputum or in conditions where copious sputum production may not be a feature (such as *P. jiroveci* pneumonia (PCP) in HIV infection). C&S assists with identifying a vast range of organisms, including and especially TB (always ally sputum culture to direct microscopy).
- **Stool:** vast range of uses (➡ Gastrointestinal tract investigations, pp. 412–13). Includes direct microscopy for parasites, ova, and cysts, i.e. giardia and ascariasis, culture for *Salmonella*, *Campylobacter*, *Shigella*, *E. coli* 0157, typhoid and paratyphoid, *Plesiomonas shigelloides*, and enteroviruses, and antigen detection of rotavirus. ‘Hot stools’ (from patient to the microbiology bench in <1h) may be helpful for amoebae, strongyloides larvae, etc.

Further reading

Health and Safety Executive. *Biological agents: managing the risks in laboratories and healthcare premises*.
🔗 <http://www.hse.gov.uk/biosafety/biologagents.pdf>.

Molecular diagnostics

These tests are now the mainstay of mainstream clinical practice. HIV viral load and antiretroviral drug resistance are considered routine tests, whilst examination of the CSF for JC virus DNA by PCR is the method of choice for the diagnosis of progressive multifocal leukoencephalopathy (PML). Genetic testing for the rifampicin resistance mutation in *RPO* provides a rapid indication of MDR-TB, e.g. Cepheid GeneExpert. (See Fig. 5.8 for the immunological profile of HIV disease.)

The areas of greatest value include

- Detection and quantification of viruses to monitor and guide therapy, e.g. HCV, HIV, HBV, CMV.
- Detection of slow-growing organisms, e.g. TB, atypical mycobacteria.
- Diagnosis of pathogens, which are potentially too dangerous for the laboratory staff to handle, e.g. VHF, smallpox, avian/swine flu.
- Detection of organisms killed by antibiotics prior to culture samples being taken, e.g. meningococcal sepsis.
- Detection of organisms that cannot be cultured, e.g. HCV.
- Detection of unusual diseases, e.g. helminthic diseases, fungi.
- Detection of toxins elaborated in small quantities by bacteria, e.g. toxic shock syndrome toxins.
- Detection of mutations manifesting resistance to antimicrobial agents (genotypic resistance testing), e.g. HIV, CMV, TB.
- Elucidation of pathogens that are as yet ‘undiscovered’, e.g. 16s ribosome testing.

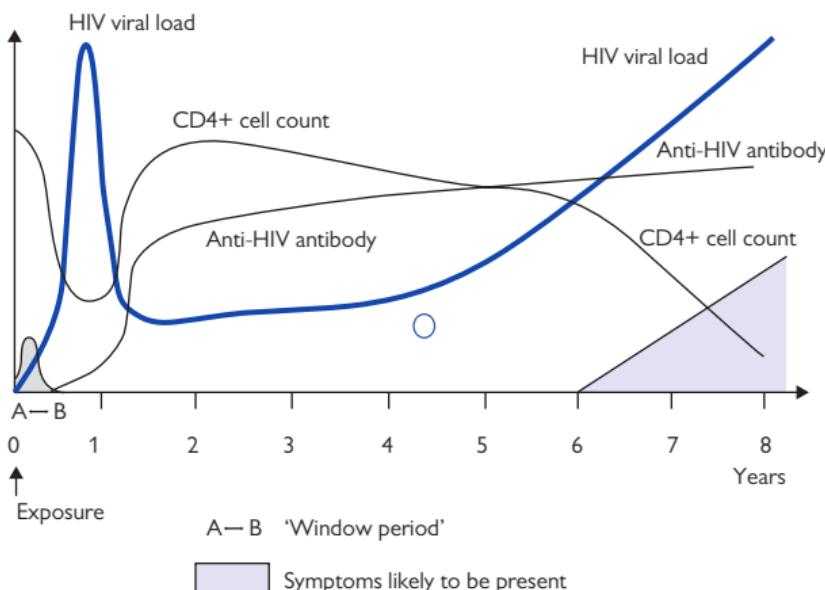


Fig. 5.8 The immunological profile of HIV disease.

Available molecular techniques include

- PCR: this test uses probes to look for the presence of the genes of infecting organisms (→ Polymerase chain reaction amplification of DNA, pp. 324–6).

There are numerous PCR tests available, and it is particularly valuable for hepatitis C (including for genotyping), HIV, and TB. A universal eubacterial PCR (for genus and species identification of prokaryotes) and a universal fungal PCR (genus and species identification of fungi) are available. HBV DNA quantification is accomplished through PCR.

Choosing an appropriate sample for the application of PCR testing is important (e.g. biopsy of possible Kaposi's sarcoma lesion and Kaposi's sarcoma-associated herpesvirus (KSHV) (human herpesvirus (HHV)-8); BAL fluid and PCP; small bowel biopsy and Whipple's disease; CSF and meningococcal disease, HSV, or *M. tuberculosis*). Note: fluid samples for PCR usually have a higher yield when not spun in a centrifuge.

- *LCR (ligase chain reaction)*: works through specific probe amplification through the use of DNA ligase. Greatest value in *Chlamydia* infection.
- *Transcription-mediated amplification (TMA)*: uses an isothermal amplification system. Amplified telomerase products are rRNA, detected using a non-isotopic hybridization protection (HPA) system. Identification of HCV, TB, gonococcus, and *Chlamydia* are among its potential uses.
- *Branched-chain DNA (bDNA)*: a signal amplification methodology able to quantify HIV RNA levels.
- *Nucleic acid sequence-based amplification (NASBA)*: a quantitative test for HIV RNA; also of value with CMV.
- *Hybridization with nucleic acid probes*: detects specific ribosomal RNA and is most widely used for culture confirmation of an organism (e.g. fungi, mycobacteria).
- *Sequencing*: organisms are identified by direct sequencing of amplified gene fragments. Has been applied to TB, *H. pylori*, enteroviruses, and HIV (for assessing drug resistance). Whole genome sequencing (next-generation sequencing) is moving forward rapidly but brings with it the problems of bioinformatics and 'big data'.
- *Restriction fragment length polymorphisms (RFLPs)*: restriction enzymes are used to cut up DNA into pieces, and the fragments are then subjected to gel electrophoresis (such as Southern blotting; → Southern blotting, pp. 322–3). The patterns produced can be used to identify organisms.

Further reading

Shanson DC. *Microbiology and Clinical Practice*, 3rd edn. Oxford: Butterworth-Heinemann, 1999.

Haematology

Many infectious diseases manifest haematological changes that are diagnostically valuable.

Blood film

Blood smear examination provides general data on the size and appearance of cells, as well as data on particular cell segments, whilst pathogens may be seen, e.g. malaria, African trypanosomiasis, Chagas' disease, babesiosis, borrellosis, bartonellosis, ehrlichiosis, filaria (time of day the blood is taken may be significant in this condition), haemolysis, and evidence of hypoplasia. Thick and thin blood films should be considered, especially where malaria is concerned; at least three blood films, each taken 24h apart, should be performed. Blood films are also useful in assessing if a patient has DIC.

Bone marrow examination

Culture (for, e.g. TB, brucellosis, *Mycobacterium avium intracellulare*, typhoid/paratyphoid, CMV), microscopy (for, e.g. leishmaniasis), and establishing cell line integrity (e.g. WBC abnormalities). An aspirate is generally useful for culture purposes and for establishing what cells are present in the marrow, but a trephine is needed if structural information is needed (e.g. to establish if granulomata suggestive of TB are present).

Coagulation studies, fibrin degradation products, D-dimers

Useful where DIC is suspected. DIC is a common association of severe sepsis (especially meningococcal disease). Coagulation abnormalities are also present in conditions such as VHF, *P. falciparum* malaria, rickettsial diseases, etc. D-dimers may assist with the diagnosis of thrombosis but are generally raised in infection.

Cold agglutinins

A haemagglutination-based test. Can be caused by *M. pneumoniae* (most commonly), influenza A, influenza B, parainfluenza, and adenoviruses.

Differential white cell count in peripheral blood. Useful associations include:

- Eosinophilia and parasite infection.
- Neutrophilia and bacterial sepsis.
- Neutropenia and atypical pneumonias.
- Atypical lymphocytes and EBV.
- Neutropenia and pyrexia.

Erythrocyte sedimentation rate

Together with CRP (Biochemical tests, pp. 416–17), the rate of erythrocyte sedimentation is sensitive to the extent of a body's response to a lesion or disease. The ESR is important for pointing to the possible existence of an organic disease, but a normal result does not exclude the presence of disease. A ↑ ESR points to the need for additional investigations and, if ↓, is very useful in monitoring the course of a disease. Procalcitonin (PCT) may eventually prove to have even greater value in a similar role.⁷

⁷ Meisner M, Tschaikowsky K, Palmaers T, Schmidt J. Comparison of procalcitonin (PCT) and C-reactive protein (CRP) plasma concentrations at different SOFA scores during the course of sepsis and MODS. *Crit Care* 1999; 3: 45–50. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=29013>.

Ferritin levels

↓ in iron deficiency, e.g. associated with hookworm infestation of the bowel (*Ancylostoma duodenale*, *Necator americanus*) or *H. pylori*-associated gastritis. Ferritin levels are often extremely high in Still's disease, an important non-infective cause of pyrexia and neutrophilia. It is also markedly raised in haemophagocytic lymphohistiocytosis syndrome (HLH). Serum iron and TIBC may be helpful in a fuller evaluation (→ Biochemical tests, pp. 416–17).

Glucose-6-phosphate dehydrogenase

Useful in therapy of benign malarias, e.g. *P. vivax* and *P. ovale* (a deficiency will cause severe haemolysis when primaquine is used to kill the hypnozoite phase to prevent relapse).

Hb concentration and red cell parameters (especially mean cell volume)

Useful in, e.g. anaemia of chronic infection, haemolysis, iron deficiency (microcytosis) associated with hookworm infestation of the bowel or *H. pylori*-associated gastritis, macrocytosis due to vitamin B₁₂ deficiency with *Diphyllobothrium latum* (fish tapeworm) infestation.

Hb electrophoresis

Can detect haematological conditions such as thalassaemia, sickle-cell disease, etc. These can predispose to certain infections via hyposplenism such as salmonellosis and melioidosis.

Haemolysis screen (including reticulocyte count)

May be abnormal in, e.g. malaria, DIC, EBV, VHF, *E. coli* 0157 gastroenteritis due to HUS, rickettsial infections, dengue, and gas gangrene. Hp levels can be useful (→ Biochemical tests, pp. 416–17), since they are generally ↓/ absent in haemolysis.

Monospot test (Paul Bunnell test)

Diagnostic of EBV infection.

Sickling test

Uncovers sickle-cell disease, known to be associated with *Salmonella* osteomyelitis, chronic leg ulcers, etc.

Thrombocytopenia

Characteristic in some conditions, e.g. HIV disease, *P. falciparum* malaria, and dengue.

Vitamin B₁₂ level

↓ in *D. latum* infestation, TB of the terminal ileum, etc.

Radiology

Plain X-rays

- Chest: the potential diagnoses are legion, including pneumonia, TB, pleural effusion/empyema, bronchiectasis, PCP, tropical eosinophilia and other parasite-related diseases (e.g. paragonimiasis), occupational risks for infections (e.g. silicosis and TB), and post-varicella calcification. Also useful to exclude non-infectious causes of fever such as cancer or sarcoidosis.
- Plain abdominal X-ray: e.g. bowel dilatation and perforation, calcification of adrenal glands and lymph nodes (e.g. TB, histoplasmosis), 'babies head' sign of schistosomal bladder calcification.
- Dental radiography (*orthopantomogram*): occult dental sepsis.
- Elsewhere: e.g. limbs for osteomyelitis, skeletal muscles for calcified cysticercosis lesions, joints for Charcot changes (such as in syphilis).

More sophisticated imaging

Endoscopic retrograde cholangiopancreatography

Using contrast medium and radiographs to define the anatomy of the biliary tree and pancreatic duct. Useful for HIV-associated biliary tree disease (including porta hepatis nodal lymphoma), parasites (e.g. *Clonorchis sinensis*, *Ascaris lumbricoides*), and pancreatic disease such as TB.

Intravenous urogram

Defines renal anatomy. Renal infection, such as pyelonephritis, renal calculi, malignancy, or anatomical abnormalities (including congenital) leading to recurrent infections. Computed tomography intravenous pyelography (CT-IVP) is generally considered superior now.

Magnetic resonance imaging

Including with contrast enhancement using gadolinium.

- Cranial: vCJD (exhibits bilateral pulvinar high signal), encephalitis, rabies, sagittal sinus thrombosis, PMLE. MRI is also more sensitive than CT when looking for infective SOLs such as tuberculoma, especially in and around the cerebellum.
- Bones: essential for the diagnosis of osteomyelitis.
- Spinal cord: essential for diagnosis of spinal infections and myelitis.
- Elsewhere in the body: defining solid lesions, fluid-filled lesions, etc.
- Magnetic resonance cholangiopancreatography (MRCP): non-invasively defines the hepatopancreatic biliary tree anatomy.
- New techniques: e.g. as cardiac MRI imaging, show great promise in the diagnosis of valvular disease.

Computed tomography

Including with contrast enhancement.

- Cranial: e.g. brain abscess, paranasal sinus disease, middle ear disease, orbital sepsis, cysticercosis, mastoid air cells.
- Chest: e.g. cardiac lesions (possibly with associated endocarditis risk), mediastinum (e.g. lymphadenopathy, including retrosternal), lung lesions such as bronchiectasis, lung abscess, other non-infectious pathologies.

- **Abdomen:** delineates intra-abdominal abscesses and abnormalities in retroperitoneal and mesenteric lymph nodes, defects in the spleen, liver, kidneys, adrenals, pancreas, and pelvis.
- **CT-IVP:** powerful tool for defining the anatomy of the urinary tract.
- **Multislice CTPA:** e.g. for defining PEs as a cause of PUO.
- **CT-guided biopsy:** to specifically pick out an area for sampling, e.g. liver lesion, lymph node, mediastinal mass.
- **CT colonoscopy:** helpful in diagnosing colonic disease, especially in older patients unable to undergo invasive testing (e.g. when looking for an associated colonic tumour in a patient with *Streptococcus bovis* endocarditis).

Ultrasound

- **Abdomen:** evidence of pancreatic, liver, renal, and biliary tree/gall bladder abnormalities (e.g. abscess, hepatic cyst, presence or absence of spleen, ascites, gallstones, etc.).
- **Thoracic:** pleural effusion, empyema (can assist with drainage).
- **Echocardiography:** to help exclude cardiac vegetations of endocarditis, TB pericarditis (with effusion), myocarditis. Note: both TTE and TOE approaches are available, each yielding data of differing value in different situations.
- **Doppler studies of blood vessels:** to exclude DVT such as in the legs.
- **Guided biopsy:** to specifically pick out an area for sampling, e.g. liver lesion, lymph node.
- **Drainage:** to specifically pick out an area for draining, e.g. liver abscess, pleural effusion.

Radionuclide scanning

- Has limited value but can localize area of inflammation for biopsy.
- **Indium (¹¹¹In)-labelled granulocyte scan:** may help localize many infectious or inflammatory processes (i.e. deep sepsis).
- **^{99m}Technetium bone scan:** bone and joint sepsis.
- **V/Q scan:** to exclude PE as a cause of PUO, to delineate consolidation, abscess, bronchiectasis, etc.

Positron emission tomography-computed tomography (PET-CT)

Enormous potential for locating localized infective processes, especially in the brain. Increasingly used in oncology and can be helpful in PUO.

These techniques are all described in detail in  Chapter 13.

Gastrointestinal tract investigations

Biopsy-based

- *Duodenal biopsy* (Crosby capsule and endoscopic methods ± EM): e.g. Whipple's disease, giardiasis, cryptosporidium, strongyloidiasis.
- *Gastric biopsy*: *H. pylori*.
- *Laparoscopy*: useful to exclude TB and other infections in the presence of ascites (biopsies should be sent for histology and C&S; peritoneal biopsies have a higher yield than ascitic fluid).
- *Liver biopsy*: Tissue biopsy and deep aspiration specimens, pp. 418–21.
- *Oesophageal biopsy*: e.g. candidiasis, CMV (e.g. in advanced HIV disease).
- *Sigmoidoscopy and bowel biopsy*: e.g. amoebiasis, pseudomembranous colitis (*C. difficile* infections), exclusion of microscopic colitis and IBD.

Gastrointestinal contents-based

- These are rarely performed now, as endoscopic sampling is standard.
- *Baermann concentration technique*: the method of choice for the detection of *Strongyloides stercoralis*.
- *Capsule endoscopy*: involving the swallowing of a pill-sized capsule containing digital video recording equipment, which broadcasts to a receiver outside of the body. Enormous potential for diagnosing pathology within the GIT, including small bowel infective processes such as TB.⁸
- *Duodenal aspirate*: e.g. giardiasis, cryptosporidium, strongyloidiasis.
- *Enterotest (string test)*: e.g. giardiasis, cryptosporidium, strongyloidiasis.
- *Hot stools*: little evidence that they need to be hot! (Culture techniques, pp. 390–5.)
- *Stool C&S* (Culture techniques, pp. 390–5).
- *Stool microscopy*: for ova, cysts, parasites (e.g. amoebae, helminths such as *A. lumbricoides*).
- *Stool EM*: good for viruses, e.g. rotavirus and norovirus, but rarely done.
- *Stool chromatography*: *C. difficile* toxin, a specific *C. difficile* toxin A and B EIA and PCR are used.
- *Sellotape® (adhesive) strip test*: for the threadworm *Enterobius vermicularis*. To perform this test, roll some clear adhesive tape around four fingers of a hand, sticky side out, whilst an assistant spreads the buttocks. In good lighting, identify the involved perianal area, and apply the tape 1–2 times to the affected perianal area. Place the tape on a slide with the clean side downwards, trim the tape, label the slide, and send to the laboratory.
- *Toxin tests*: most widely used for *C. difficile* toxins A and/or B; the definite diagnosis of botulism food poisoning is the examination of faeces for *Clostridium botulinum* and toxin (EMG is also helpful). In wound botulism (e.g. in drug injectors), the organism may grow in material taken directly from the wound(s); also used for *E. coli* 0157.

⁸ Lee WK, Van Tonder F, Tartaglia CJ, et al. CT appearances of abdominal tuberculosis. *Clin Radiol* 2012; 67: 596–604.

Gastrointestinal tract function

- *D*-xylose absorption test: for malabsorption syndromes such as Whipple's disease and tropical sprue.
- ^{13}C breath test: for detection of *H. pylori*.⁹
- Faecal elastase: for diagnosis of pancreatic exocrine insufficiency in conditions such as HIV
- Vitamin levels: diagnosis of fat-soluble vitamin malabsorption in pancreatic or small bowel disease or of B_{12} levels in terminal ileal problems such as with TB.

⁹ Di Rienzo TA, D'Angelo G, Ojetta V, et al. ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection. *Eur Rev Med Pharmacol Sci* 2013; 17 Suppl 2: 51–8.

Immunology

Cutaneous hypersensitivity tests are discussed elsewhere (➔ Other tests, pp. 422–3).

- Complement (especially ‘terminal’ complements C5–C9): deficiencies can lead to recurrent meningococcal sepsis, pneumococcal disease, etc.
- Cytokine studies: currently experimental, but levels of interferon can sometimes be helpful.
- Differential white cell count (➔ Haematology, pp. 408–9): neutropenia and lymphopenia are associated with bacterial sepsis.
- IgS: deficiencies lead to recurrent infections (some cases may be hereditary, and family history is important). Levels may also be higher—IgM tends to be markedly ↑ in brucellosis, malaria, trypanosomiasis, and toxoplasmosis.
- Splenic dysfunction: indicated by a history of surgical removal (not always clear!) or a condition associated with hyposplenism (e.g. coeliac disease/dermatitis herpetiformis, sickle-cell disease), an abnormal blood film, and an absent spleen on abdominal imaging. This state may be associated with recurrent meningococcal infection and life-threatening pneumococcal sepsis. Once diagnosed, the patient will need appropriate vaccinations and advised to always carry a warning card and/or wear a MedicAlert® bracelet or similar.
- T-cell subsets: the absolute CD4+ (T4) cell count and the CD4+/CD8+ (T4/T8) cell ratio is of value. HIV disease, TB, and sarcoidosis are associated with reduced CD4+ cell levels, HIV with a reversed CD4+/CD8+ cell ratio.
- Human pharmacogenomics:
 - HLA typing—HLA B5701 is associated with hypersensitivity (sometimes fatal) to the antiretroviral drug abacavir, and so patients should be tested before prescribing this drug.
 - IL-28R polymorphisms—these can be associated with treatment failure in hepatitis C infection.

Biochemical tests

A number of biochemical tests are useful in the diagnosis and assessment of a range of infectious illnesses.

- **AFP:** ↑ in hepatocellular carcinoma (associated with HCV and HBV). Note: much higher AFP than in other causes of hepatocellular damage.
- **ABGs:** assessment of sepsis, assessment of pneumonia.
- **Ca-125:** ↑ false +ve in peritoneal TB.
- **CRP:** together with the ESR, a valuable method for monitoring infections. CRP is an acute phase reactant, ↑ in bacterial infections and ↓ in viral infections. PCT may be of value.
- **Creatinine phosphokinase (CPK) level:** ↑ in *L. pneumophila* infection.¹⁰ Also ↑ with zidovudine (AZT) usage in HIV disease.
- **Glucose metabolism:** DM is a common association of infection, particularly TB. Consider performing a fasting glucose level, an OGTT, or checking HbA_{1c} levels.
- **Hp levels:** part of the haemolysis screen (Haematology, pp. 408–9). Iron levels (serum iron), TIBC: iron ↓ (TIBC ↑) in iron deficiency, e.g. associated with hookworm infestation of the bowel (*A. duodenale*, *N. americanus*) or *H. pylori*-associated gastritis. Serum ferritin may be helpful.
- **Lactate levels:** may be ↑ in HIV-associated mitochondrial toxicity syndrome. Also high in severe sepsis syndrome.
- **Lipid abnormalities** (cholesterol, TGs): HIV drug toxicity.
- **LFTs:** abnormalities are present in many conditions, e.g. hepatitis, leptospirosis, yellow fever, antimicrobial drug toxicity (e.g. in TB).
 - **ALP:** in the serum of healthy adults, ALP mostly originates from the liver. Biliary obstruction, often associated with sepsis, leads to an ↑ in serum concentration of ALP.
 - **Bilirubin:** determination of bilirubin levels (conjugated and unconjugated) is important in the differential diagnosis of jaundice.
 - **γGT:** ↑ in serum concentration of γGT is the most sensitive indicator of liver damage.
- **Pancreatic amylase level:** ↑ with pancreatitis in, e.g. mumps (consider also salivary amylase), toxicity with antiretroviral drugs (e.g. didanosine or DDI).
- **Pleural fluid analysis:** analysis for LDH levels is useful (as well as albumin, total protein, and amylase). An exudate, which implies infection in the differential diagnosis, is defined by at least one of the following criteria: pleural fluid/serum total protein ratio >0.5, pleural fluid/serum LDH ratio >0.6, or pleural fluid LDH > two-thirds the upper limits of normal of serum LDH.
- **Pregnancy test:** some infections, e.g. varicella, genital herpes (simplex), and TB, are often more serious in pregnancy. The use of some antibiotics, e.g. ciprofloxacin and tetracyclines, is relatively contraindicated in pregnancy. There is the potential to prevent vertical transmission of HIV if diagnosed prior to delivery.

- *PCT*: shows promise for detecting and following 'inflammation induced by microbial infections'.¹⁰
- *Serum Na⁺ levels*: hyponatraemia associated with legionnaires' disease¹¹ but may also be related to intracranial sepsis or hypocortisolaemia.
- *Synacthen® test*: TB and histoplasmosis can damage the adrenal glands, leading to an Addisonian state (hypocortisolaemia).
- *Vitamin D levels*: if deficient, may predispose to TB and may lead to difficulties with treating TB infections due to hypocalcaemia (consider checking levels in patients with darkly pigmented skins, especially those with a culture of wearing clothing over most of their body surface). Deficiency may be exacerbated by some anti-infective drugs, notably rifampicin and antiretrovirals.

¹⁰ Meisner M, Tschaikowsky K, Palmaers T, Schmidt J. Comparison of procalcitonin (PCT) and C-reactive protein (CRP) plasma concentrations at different SOFA scores during the course of sepsis and MODS. *Crit Care* 1999; 3: 45–50. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=29013>.

¹¹ Kociuba KR, Buist M, Munro R, Lee A, Cleland B. Legionnaires' disease outbreak in south western Sydney, 1992. Clinical aspects. *Med J Aust* 1994; 160: 274–7.

Tissue biopsy and deep aspiration specimens

Whatever part of the anatomy from which they are taken, biopsy specimens should be evaluated both histopathologically (with specialized stains used, wherever appropriate) and by culture for bacteria, mycobacteria, fungi, viruses, and prions (using specialized culture techniques, where appropriate).

Bone marrow biopsy

(Haematology, pp. 408–9)

Important for TB, brucellosis, *Mycobacterium avium intracellulare* typhoid/paratyphoid, leishmaniasis.

Cerebrospinal fluid

Whilst the main objective of an LP is usually to obtain fluid for microscopy and C&S, there are other useful tests that can be performed. The opening pressure should be between 10 and 20cmH₂O—infective and other processes may alter this. Whilst the usual approach to obtaining CSF is through an LP, if the pressure is high, a cisternal puncture can be performed instead (normally the advice and help of a neurosurgeon would need to be sought). In neonates, foramenal puncture is a possibility. A CT scan is usually performed beforehand to assess the risk of ‘coning’, although it is by no means a guarantee.

Along with the Gram staining process and microscopy, other tests to consider include a complete blood cell count and differential measurement of glucose and protein levels, ZN staining for TB, and bacterial, mycobacterial, viral, and fungal cultures. On occasions, other tests that might be considered include:

- Wet mount (for amoebae such as *Acanthamoeba*; (Culture techniques, pp. 390–5)).
- PCR for HSV, herpes varicella-zoster, enteroviruses, TB, JC virus, and cryptococcosis (Molecular diagnostics, pp. 406–7)).
- Antibodies to specific pathogens (e.g. arboviruses; (Serology, pp. 396–9)).
- India ink capsule stain (for cryptococcosis).
- CrAg (Serology, pp. 396–9).
- VDRL, etc. for syphilis (Serology, pp. 396–9).
- 14-3-3 protein—specific protein marker present in the CSF of patients with vCJD.
- Xanthochromia to help exclude SAH also seen in leptospirosis.
- Cytology to help exclude carcinomatous meningitis.
- Assessing comparative CSF protein–cellular levels if GBS (recognized association of infections such as *Campylobacter gastroenteritis*) is being considered.

Liver biopsy

Indications are numerous and include assessment of viral hepatitis (especially HBV and HCV, including possible cirrhosis and/or hepatocellular carcinoma), assessment of PUO (including TB and lymphoma), and

determining if the patient has a medication-induced liver disease. Only on <1% of occasions does the liver biopsy overestimate the amount of hepatic damage. Fibroscan is increasingly used in conjunction with blood tests to non-invasively score liver damage, but it is not as accurate as the biopsy.

The biopsy is commonly preceded by an USS examination of the liver to determine the best and safest biopsy site. Usually, the biopsy is conducted under ultrasonic guidance to avoid major blood vessels. Coagulation status should be optimized at the time of biopsying, including by the administration of clotting factors.

The risks of the traditional liver biopsy (not performed under ultrasound guidance) include

- Pain (1 in 5 patients).
- Haemorrhage (1 in 500 patients).
- Bleeding requiring transfusion or surgery (1 in 1000 patients).
- Pneumothorax and/or puncture of the gall bladder, kidney, or bowel (1 in 1000 patients).
- Death (1 in 5000 patients).

Equivalent figures are not available for USS-guided liver biopsy, but the technique is well established. Liver biopsy material should be subjected to microbiological culture, as well as to histological assessment.

Lymph node sampling

The likely pathologies depend upon whether or not lymphadenopathy is regional or generalized, and upon the site.

- **Biopsy:** for histology and culture, especially for TB, for tropical infections such as chancroid, and for other relevant infections such as cat-scratch fever (*B. henselae*). If regional, the differential diagnosis varies with the site; if intra-abdominal, for example, TB, *Y. enterocolitica*, and adenovirus should be considered.
- **FNA:** useful as full biopsy for culture purposes, but no structural information available (similar to the aspirate vs trephine issue in BM sampling), therefore difficult to exclude lymphoma from differential.

Respiratory samples

Sputum tests

- **Microscopy:** can perform direct microscopy (e.g. for *Aspergillus* species, eggs of paragonimiasis), Gram stain, ZN, PCP (silver staining needed).
- **Induced sputum:** e.g. for TB, PCP, *Aspergillus*.
- **Tracheal aspirate:** used in ill individuals. May produce similar material.

Bronchoscopy

- **BAL:** useful for TB and other mycobacteria, PCP, fungi, melioidosis, resistant bacteria (e.g. *Pseudomonas*), RSV, and paragonimiasis.
- **Lung biopsy:** useful for TB and other mycobacteria, PCP (needs silver staining, immunofluorescence test (IFT), or PCR), fungi, melioidosis, resistant bacteria (e.g. *Pseudomonas*), RSV, and paragonimiasis. Also for exclusion of non-infective causes of non-resolving pneumonia.

Open lung biopsy

When not feasible to obtain intrathoracic tissue by less invasive means.

Pleural disease: effusion, empyema, biopsy

Consider, e.g. TB, pneumococcal sepsis, underlying neoplasm (and rarer conditions like strongyloidiasis). Biochemical analysis of pleural fluid can help (➔ Biochemical tests, pp. 416–17). An empyema will have white cell count, protein, pH, LDH changes compatible with an exudate, and possibly organisms visible and/or culturable within the fluid. A pleural biopsy can be obtained blindly with an Abraham's needle, but in recent times pleuroscopy and VATS has developed into a better option.¹²

Skin biopsy**Biopsy and hair sampling**

- Useful for, e.g. TB, Kaposi's sarcoma (caused by HHV-8 and associated with HIV), onchocerciasis, the aetiology of warts (common viral warts vs molluscum contagiosum—the distinction can be important in view of the therapeutic options and the potential for malignant change in some sites such as the ♀ cervix).
- The identification of pathogenic arthropod parasites, e.g. myiasis (the invasion and feeding on living tissues of humans or animals by dipterous larvae such as that of the tumbu fly), scabies, lice, ticks, and chigger fleas, depends on the offending agent being seen and correctly recognized or the appropriate specimen (e.g. excision biopsy) being taken and examined histologically.

Skin snips

- *Filarial infestations*: examination of skin snips will identify microfilariae of *Onchocerca volvulus* and *Mansonella streptocerca*. Skin snips can be obtained using a corneal–scleral punch, or more simply a scalpel and needle. The sample must be allowed to incubate for 30min to 2h in saline or culture medium, and then examined microscopically for microfilariae that would have migrated from the tissue to the liquid phase of the specimen. In onchocerciasis, nodulectomy is also of value, as is examination of the eye with a slit lamp.
- *Leprosy*: acid-fast bacilli are present in the skin.

Other tissues and collections are numerous and include

- *Bone infection/abscess/osteomyelitis*: consider, e.g. pyogenic sepsis, TB, atypical mycobacteria, sickle-cell disease, ectopic ova of schistosomiasis. History is important, e.g. with a history of fight trauma to a hand, anaerobic bony infection may be more likely.
- *Brain lesions and abscesses*: biopsy and drainage useful for, e.g. TB, herpes simplex, rabies, cysticercosis, encephalitis, vCJD, JC virus, and toxoplasmosis (in HIV infection).
- *Cervix*: HPV, HSV, *N. gonorrhoeae*.
- *Joint infections*: aspirate synovial fluid and consider, e.g. pyogenic sepsis, TB. An acute attack of gout (diagnosed through identifying the birefringent crystals of sodium urate) can mimic an acute infective

¹² Corcoran JP, Wrightson JM, Belcher E, DeCamp MM, Feller-Kopman D, Rahman NM. Pleural infection: past, present, and future directions. *Lancet Respir Med* 2015; 3: 563–77.

arthritis (including a systemic inflammatory response with neutrophilia) and should be excluded.¹³

- **Liver abscess:** consider *Streptococcus milleri*, hydatid disease, amoebic dysentery, necrotic hepatocellular carcinoma in hepatitis C or hepatitis B, obstruction of the biliary tree by *A. lumbricoides* or liver flukes such as *C. sinensis*.
- **Muscle biopsy:**
 - *Cardiac*—may point towards myocarditis or Chagas' disease.
 - *Skeletal*—may be used to identify parasites, including, e.g. trichinosis, cysticercosis.
- **Nerve biopsy:** peripheral nerve biopsy (e.g. posterior auricular nerve) may reveal tuberculoid leprosy.
- **Ocular:**
 - *Vitreous humour*—e.g. intraocular infections, including fungal, HSV, herpes varicella-zoster, pyogenic bacterial.
 - *Cornea*—e.g. rabies, CJD.
 - *Retina*—e.g. herpes varicella-zoster, toxocariasis, CMV.
- **Paranasal sinus aspirates:** e.g. bacteria, fungal (such as mucormycosis).
- **Pericardial biopsy:** particularly important for establishing a diagnosis in chronic pericarditis, e.g. TB, fungal.
- **Peritoneal infection:** via laparoscopic tissue sampling and ascites sampling (→ Gastrointestinal tract investigations, pp. 412–13).
- **Splenic aspiration:** useful in the diagnosis of visceral leishmaniasis (kala-azar) by microscopic examination and culture and demonstration of the organism.¹⁴
- **Tonsillar biopsy:** of particular value for diagnosing vCJD: also consider MRI scanning (→ Radiology, pp. 410–11), EEG, and 14-3-3 protein in the CSF.¹⁵

13 Singh N, Vogelgesang SA. Monoarticular arthritis. *Med Clin North Am* 2017; **101**: 607–13.

14 Sakkas H, Gartzonika C, Levidiotou S. Laboratory diagnosis of human visceral leishmaniasis. *J Vector Borne Dis* 2016; **53**: 8–16.

15 Collins S, Boyd A, Fletcher A, Gonzales MF, McLean CA, Masters CL. Recent advances in the pre-mortem diagnosis of Creutzfeldt-Jakob disease. *J Clin Neurosci* 2000; **7**: 195–202.

Other tests

Antibiotic plasma concentration monitoring

Some drugs are toxic if the plasma levels rise too high and their use is futile if the levels are too low. Monitoring serum drug levels ensures that plasma drug levels remain within the therapeutic range. Antimicrobial drugs that may require this approach include gentamicin, vancomycin, teicoplanin, kanamycin, amikacin, tobramycin, chloramphenicol, streptomycin, cycloserine, amphotericin, flucytosine, voriconazole, and itraconazole.

Cardiac

- **ECG:** serial ECGs can be of value in Lyme disease, rheumatic fever, pericarditis, myocarditis, and toxic shock syndrome. Also of value in conditions where the cardiac conduction mechanism has been damaged, e.g. Chagas' disease (American trypanosomiasis) and with a valve root abscess in severe infective endocarditis. In cholera and enteric fever (typhoid and paratyphoid), the cardiac rate will often be slower than one might anticipate for the degree of fever.
- **Echocardiography:** TTE and TOE for the diagnosis of endocarditis. TOE especially for prosthetic valves.

Dermatological tests

- **TB skin tests:** measure delayed hypersensitivity. The Mantoux test usually involves the intradermal injection of 10 tuberculin units of purified protein derivative (PPD), and the response is quantified. The reaction is read at 48–72h. Most useful epidemiologically, their individual clinical value being relatively limited. Multiple puncture techniques (the Heaf and Tine tests) are likely to be more convenient for large group studies. Interpretation of these tests is more difficult in patients inoculated with the bacillus Calmette Guérin (BCG) vaccine, and IGRA (e.g. QuantiFERON[®]) are generally more helpful as they distinguish between T-cell responses to BCG and TB.
- **Histoplasmin test:** a +ve intradermal skin reaction to histoplasmin (the histoplasmin test) may be the only sign of past infection with *H. capsulatum*. Main value is epidemiological. A similar skin test exists for *C. immitis*.
- **Mazzotti (DEC) test:** for filariasis. Rarely used now, this test relied on the intense pruritic response induced by microfilariae after treatment with the antifilarial agent diethylcarbamazine* (DEC). Used in a minute quantity, it can nevertheless be associated with side effects, ranging from mild discomfort, fever, headaches, and intolerable pruritus to tachypnoea, tachycardia, and even pulmonary oedema.

Note: (*) diethylcarbamazine is not licensed for use as a filaricide.

- **Skin testing for antibiotic allergy:** this can be performed in the same way as for other allergens by patch testing or skin prick testing.

Narcotics and anabolic steroids screen

If +ve, these may point towards occult drug use and a concomitant risk of blood-borne viruses (e.g. HIV, hepatitis C, hepatitis B). vCJD has been transmitted through anabolic steroid injecting. The antiretroviral efavirenz interferes with these tests producing a false +ve.

Neurological

- *EEG*: may help with making a diagnosis of encephalitis (e.g. in patients with HSV encephalitis, the EEG may exhibit focal unilateral or bilateral periodic discharges localized in the temporal lobes), of brain abscess, or of cerebral cysticercosis. It may also be of value in vCJD.
- *LP*: material for C&S can be obtained, but much additional information is also gathered, e.g. the opening pressure is usually elevated in infections (→ Tissue biopsy and deep aspiration specimens, pp. 418–21).
- *EMG*: offers rapid bedside confirmation of the clinical diagnosis of botulism. It shows a pattern of brief, small, abundant motor unit potentials. In GBS (associated with *Campylobacter gastroenteritis*), EMG may be helpful in excluding 1° muscle disease.
- *NCS*: helpful with diagnosing neuropathies (e.g. HIV, leprosy, GBS).

Ophthalmology

- *Slit lamp examination*: can help with the diagnosis of infective and parasitic ocular problems, e.g. uveitis (syphilis, Reiter's syndrome), *O. volvulus* larvae, toxocariasis, toxoplasmosis, candidiasis.
- *Colour vision testing (Ishihara)*: used to assess toxicity associated with ethambutol in treatment of TB.
- *Direct and indirect ophthalmoscopy*: essential for the diagnosis of CMV retinitis in all HIV +ve patients with a CD4 cell count <100.

Pulmonary

- *Pulmonary function tests*: bronchial hyper-reactivity can be assessed for (often provoked by infection, e.g. ABPA) and interstitial lung disease checked for (which can include, e.g. TB, fungal infections, etc.).

Clinical investigation in action: pyrexia of uncertain origin

Common problem in hospital medicine, with huge potential differential diagnosis.

PUO is best defined as a body temperature of 38.3°C centrally (rectally) for 3 weeks or longer without the cause being discovered, despite extensive investigation for at least 1 week.

Assessment should include

Observation of fever pattern

Some conditions, such as VHF, typhoid, and malaria, may exhibit characteristic fever patterns.

Complete and repeated detailed history, with emphasis on the recognized differential diagnosis, including

- Travel history.
- Antimalarial usage.
- Vaccination history.
- Past use of medical services in foreign parts may be especially important (e.g. blood transfusions, splenectomy post-trauma, needlestick assaults).
- Drug-using history (including illicit drugs and especially injecting).
- Exposure to certain agents and/or animals (e.g. pet ownership, occupational risk of animal contact such as veterinary medicine, nursing, farming, meat packing).
- Hobbies (e.g. caving is linked to histoplasmosis and canal fishing to leptospirosis).
- Sexual history (and risk taking).
- Menstrual history.

Complete and repeated physical examination, including re-evaluation of previous findings, e.g.

- Check the skin, eyes, nail beds, lymph nodes, heart, and abdomen.
- A new sign, e.g. cardiac murmur, may have developed over time.
- The judicious use of repeated tests is also critical, depending upon the context.
- Laboratory and radiological tests, taking into account new data, e.g. blood cultures, blood films, autoantibody screen, radiological findings.
- Non-invasive procedures, taking into account new data, e.g. genito-urinary assessment such as high vaginal swab.
- Invasive procedures, e.g. liver biopsy, BM biopsy, laparoscopy, Waldeyer's ring assessment by an otolaryngologist.

Common groups of causes of a PUO in an adult are

- Infections.
- Connective tissue diseases.
- Occult neoplasms (especially leukaemia, lymphoma, and renal carcinoma).

A list of relevant pathologies might include

HIV, TB, endocarditis, osteomyelitis, malaria, syphilis, zoonoses (e.g. brucellosis, Lyme disease, tularaemia), viral hepatitis (especially hepatitis C and B), typhoid/paratyphoid, pelvic inflammatory disease, chronic meningococcaemia, dental sepsis, tumours such as lymphoma, renal carcinoma, liver metastases, familial Mediterranean fever, multiple PEs, drugs, rheumatological, e.g. Still's disease, TA, SLE, granulomatosis with polyangiitis (GPA, previously known as Wegener's granulomatosis), vasculitis, atrial myxoma, factitious fever, Munchausen's syndrome, Munchausen's syndrome by proxy.

With improved non-invasive and microbiological techniques, most cases of PUO are found not to be caused by infections, but rather by other systemic diseases such as sarcoidosis, SLE, and TA. However, there are also infectious diseases capable of causing prolonged fever that should always be considered and factored into the assessment because they are often treatable and/or transmissible to others and will have serious consequences if missed. A definitive diagnosis is not made in around 25% of patients; however, they tend not to come to any harm when observed over a long period.

Endocarditis

Endocarditis is a deep-seated infection that behaves like a deep-seated abscess. Indeed, an abscess can form adjacent to an infected cardiac valve or shunt. The diagnosis involves thoughtful clinical assessment, including whether or not there is a history of injecting drug use, and requires multiple blood cultures and cardiac assessment. The Duke criteria form the basis of the diagnosis.¹⁶ Assess clinically for likelihood, e.g. background of injecting drug use, congenital heart disease, prosthetic valves, rheumatic fever, scarlet fever. Endocarditis may manifest changing cardiac murmurs over a period of time, as well as a number of additional signs.

- Establish diagnosis: echocardiography (especially TOE)—to look at valves, cardiac chambers, shunts, etc.
- Establish aetiology:
 - Blood cultures (multiple)—consider culturing for unusual organisms such as fungi, HACEK organisms (*Haemophilus* species, e.g. *H. parainfluenzae*, *H. aphrophilus*, and *H. paraphrophilus*, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella* species), *L. monocytogenes*, etc.
 - Serology—Q fever (*Coxiella burnetii*) phase I and II, *C. albicans*.
- Assess clinical status:
 - ECG—tachycardia, conduction abnormalities.
 - CXR—cardiac size, PEs with right-sided endocarditis.
 - U&E—to assess renal compromise, if any.
 - Haematology—WBC.
 - Inflammatory markers—ESR, CRP.
 - Proteinuria—to assess renal compromise, if any.
 - Blood-borne virus status—HIV, hepatitis C, hepatitis B if there is a history of drug injecting.

¹⁶ MedCalc. Duke criteria for infective endocarditis. <http://www.medcalc.com/endocarditis.html>.

- Assistance with therapy:
 - Antibiotic sensitivity testing.
 - Serum antibiotic levels (e.g. gentamicin, vancomycin).
- Prevention: dental assessment—for prevention in the future. Endocarditis warning card. MedicAlert® bracelet.

Tuberculosis

Consider pulmonary vs extrapulmonary disease and other epidemiological parameters. (Pleural disease is by definition extrapulmonary.)

- Establish diagnosis/aetiology:
 - Radiological evidence.
 - Bodily fluids (e.g. sputum, EMUs, gastric washings, CSF) and biopsies—always consider performing induced sputum, even with a normal CXR; histology may show caseating granulomata (see Fig. 5.9).
 - PCR testing.
 - Mantoux test.
 - IGAs such as QuantiFERON®.
 - Ca-125 levels—abdominal TB in women.
- Assess clinical status:
 - Inflammatory markers—ESR, CRP.
 - T-cell subsets—low CD4+ cell count characteristic.
 - Body weight.
 - HIV testing—now essential in all patients.
 - Glucose metabolism—may be an association (fasting glucose, OGTT, HbA_{1c}).

CASEOUS GRANULOMA

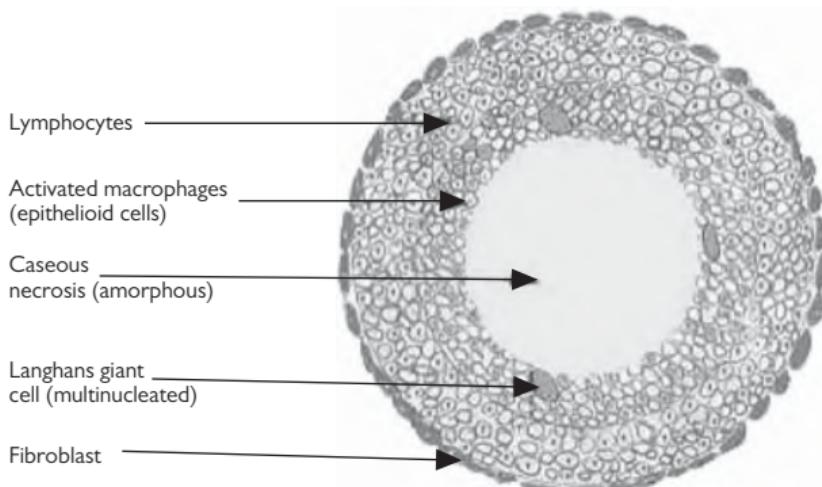


Fig. 5.9 Caseating granulomata are the principal histological feature of TB, together with acid-fast bacilli (detected using the ZN stain). In any tissue affected by TB, caseating granulomata may be present and are accordingly of immense assistance diagnostically.

- Assistance with therapy:
 - Antibiotic sensitivity testing.
 - Serum antibiotic levels (e.g. cycloserine).
 - LFTs.
 - Skin testing.
 - Calcium and vitamin D levels.
 - Gene probes (for rifampicin resistance).
- Prevention: notify cases to public health authorities. Contact tracing.
- TB and biopsies: when a biopsy is being obtained of any organ or tissue, the possibility of extrapulmonary TB should be borne in mind. If histology is performed, caseating granulomata may be seen, and appropriate staining for acid-fast bacilli (such as the ZN stain) may reveal the presence of TB organisms. However, because of the ↑ risk of MDR-TB and XDR-TB, material should always be sent to the microbiology laboratory and appropriate cultures for TB should be set up, both for diagnostic and for drug sensitivity purposes. Molecular techniques, including gene probes and PCR, are now essential parts of the diagnostic armoury for TB. In many instances, having a biopsy taken is an unpleasant experience for a patient, and remembering to perform a TB culture at the outset may prevent the patient from having to undergo an unpleasant procedure more than once.

Malaria (fever in the returning traveller)

Always consider malaria in the febrile individual returning from overseas. A detailed geographical history and malaria prophylaxis history is essential. Always consider the possibility of a coexistent second diagnosis (especially in *P. falciparum* infestation) such as *Salmonella* septicaemia (the so-called 'algid malaria').

- Establish diagnosis/aetiology:
 - Thick and thin blood films—perform three (each 24h apart).
 - Antigen tests—such as ParaSightF® and OptiMAL®.
 - Blood sugar—hypoglycaemia is common and needs immediate treatment.
 - Platelet count—thrombocytopenia suggestive of *P. falciparum*.
 - Haematology—WBC.
 - Inflammatory markers—ESR, CRP.
- Assess clinical status:
 - Blood cultures—to exclude 2° infection.
 - Haemoglobinopathy—to assess for sickle-cell disease.
 - Assess the very ill patient thoroughly for markers of severity (includes LFTs, blood film for haemolysis, coagulation status, CXR, ECG, ABGs, glucose levels, lactate levels, conscious level, etc.). Note that severe *P. falciparum* malaria can present as a diarrhoeal illness.
- Assistance with therapy:
 - G6PD levels.
 - Tests of hearing—deafness can occur with quinine.
- Prevention: avoid blood donation for 18 months.

Jaundice (acute)

Jaundice can be pre- or post-hepatic, or a combination of both. Epidemiological factors are important (drug injecting, travel, unsafe food, unsafe sex, job, hobbies, vaccination history, alcohol, prescribed medications, herbal remedies, etc.). The patient may have an acute exacerbation of a chronic disease, e.g. hepatitis C. Always remember Courvoisier's law (a distended gall bladder in a patient with obstructive jaundice means cancer) and Charcot's triad (the characteristic presentation of acute cholangitis, with biliary colic, jaundice, and spiking fevers with rigors). Haemolysis may lead to jaundice without liver disease being present.

- Establish diagnosis:

- *LFTs*—conjugated and unconjugated bilirubin levels.
- *Urinalysis*.
- *Stool examination*—colour, flushability.
- *Haemolysis screen*—blood film, coagulation studies, antiglobulin test, etc.
- *Hepatobiliary US*—serial scans can assess hepatobiliary status sequentially.
- *AFP levels*—may suggest hepatocellular carcinoma associated with hepatitis C and hepatitis B infection.

- Establish aetiology:

- *Serology*—e.g. hepatitis A through to E, EBV, CMV, toxoplasmosis, leptospirosis, hantavirus, yellow fever.
- *Blood culture*.
- *Stools for ova, cysts, and parasites*—e.g. *C. sinensis*, ascariasis).
- *Monospot for EBV*.
- *Hepatobiliary US*—obstruction by malignancy or parasites, liver parenchyma status, gallstones.
- *ERCP/MRCP*—may diagnose parasitic invasion of the biliary tree, CMV/cryptosporidial disease, porta hepatis lymphadenopathy associated with HIV, etc.
- *Paracetamol levels*.

- Assistance with therapy:

- *HIV testing*—if appropriate; co-infection with HIV, HCV, and HBV an ↑ problem worldwide.
- *Ethanol assessment*—γGT levels, ↑ MCV.
- *Molecular tests*—PCR testing for HCV, circulating DNA levels in HBV.
- *Antigens*—hepatitis B.

- Prevention:

- Notify cases to public health authorities; safe sex education; safe drug-injecting education possible once viral diagnosis of HCV, HBV, and/or HIV established.
- Assess family, sexual partners, etc. for possible infection (HIV, HBV, HCV) and/or need to vaccinate (HBV).
- Vaccination strategies: HBV, HAV as appropriate.

Diarrhoea

Diarrhoea can be acute vs chronic, or acute on chronic. For example, a gastroenteritis illness may uncover pre-existing IBD (such as Crohn's disease) or malabsorption (such as coeliac disease or pancreatic insufficiency). Drugs such as opiates can lead to 'overflow' diarrhoea. Also bear in mind that where there is one bowel pathogen, another one might be present. Antibiotic resistance is common among some bowel pathogens. Diarrhoea can appear infective but, for example, might be endocrine in origin (e.g. carcinoid syndrome, Zollinger–Ellison syndrome, medullary carcinoma of the thyroid), whilst the possibility of bowel cancer must always be borne in mind. Note that the presence of *S. bovis* in blood cultures may be indicative of the presence of bowel cancer until proven otherwise. Irritable bowel disease is being increasingly diagnosed. Malaria can present as diarrhoea.

- Establish diagnosis:
 - Examine stools.
 - Keep a stool chart on the ward.
- Establish aetiology:
 - Stool C&S.
 - Stool microscopy for ova, cysts, and parasites.
 - Faecal fat and elastase for pancreatic insufficiency.

Pneumonic illness

Pneumonia is multi-aetiological. If recurrent, this throws up certain diagnostic possibilities that must be considered. Many epidemiological considerations are important such as travel history, occupation, pet keeping, hobbies, sexual activity, etc. Osler's triad of rigors, pleuritis, and rust-coloured sputum is said to be characteristic of pneumococcal pneumonia.

- Establish diagnosis/aetiology:
 - CXR (or CT chest).
 - Serology—atypical pneumonia organisms (*L. pneumophila*, *M. pneumoniae*, *C. burnetii*, *Chlamydia psittaci*), hantavirus, RSV, influenza.
 - Sputum—including induced sputum, bronchoscopy, and BAL: microscopy and culture.
 - Blood cultures.
 - Serum Na⁺ level—↓ in *Legionella*.
 - Antigen—pneumococcal (blood), *Legionella* (urine).
 - NPA for viral culture—RSV, influenza.
 - Cryoglobulins—e.g. *M. pneumoniae*.
 - Molecular—various PCR tests for viruses and *Mycoplasma*.
 - HIV test—essential in risk groups.
- Assess clinical status.
- (CURB65 score):¹⁷
 - ABGs.
 - Confusion, Urea, Respiratory rate, Blood pressure.
 - US of the chest—if effusion developing (drain if necessary), check pH of fluid.
 - Pulmonary function tests if appropriate.

17 ↗ <http://www.aafp.org/fpm/20060400/CURB65.pdf>.

- Assistance with therapy:
 - Antibiotic sensitivity testing.
 - If recurrent: consider TB testing (see earlier), HIV testing, Ig levels (to check for deficiency), assessing for hyposplenism, checking terminal complement levels (C5–C9).
- Prevention:
 - Notify appropriate cases to public health authorities (e.g. *Legionella*, TB); isolate as necessary.
 - Vaccination strategies: influenza, *Pneumococcus*, Hib.
 - Stop smoking, if relevant.

Meningitic illness (headache and photophobia)

Meningitis can be extremely serious, particularly bacterial, mycobacterial, fungal, and protozoal forms, but viral meningitis is generally less serious. Meningitic infection is often mimicked by much less serious infections such as UTI (especially in women), throat infections (ASO, Monospot), atypical pneumonias, and sinusitis (especially ethmoidal, sphenoidal). A similar picture can also be generated by SAH. Meningococcal infection can be life-threatening without ever causing meningitis. If bacterial meningitis is recurrent, certain diagnostic possibilities must be considered. Brain abscess (consider injecting drug use, congenital heart disease, immunodeficiency, etc.) and, under certain circumstances, encephalitis can present in a similar fashion to meningitic illnesses. Where the patient has a marked petechial rash and a history of travel to Africa, Ukraine, or South America, even VHF (particularly the Congo–Crimean variety) comes into the picture (see Fig. 5.10).

- Establish diagnosis/aetiology:
 - LP/cisternal puncture/foramenal puncture (in neonates)—for CSF pressure, microscopy, bacterial and mycobacterial culture (including special cultures, e.g. for *Listeria*), viral culture, biochemistry (e.g. protein, glucose), differential cell count, viral PCR, xanthochromia, India ink stain, CrAg testing.
 - CT scan of head—sometimes necessary to exclude an SOL prior to performing an LP; CT is little better than a clinical assessment in the exclusion of raised ICP. When ↑ ICP is found, cisternal puncture and foramenal puncture is possible in skilled hands.
 - CXR and assessment for atypical pneumonia, if appropriate.
 - NPA (see earlier).
 - Petechial rash sampling—aspire material from a fresh purpuric lesion using a small-needle insulin syringe, Gram stain, and culture.
 - Molecular—meningococcal PCR (blood and CSF), pneumococcal PCR (blood and CSF).
 - Serology—urine and blood for CrAg; blood for pneumococcal antigen; urine and saliva for mumps antigen; ASO, antibodies to mumps, EBV, *Cryptococcus*, *N. meningitidis*.
 - Nasopharyngeal swab for meningococcus.
 - Stool for enteroviral culture.
 - Monospot test for EBV.

1. A detailed travel history and a high index of suspicion are essential in making the diagnosis of VHF. A recent travel history to an area where one of these viruses are known to be particularly prevalent is suggestive, particularly Africa (e.g. Uganda and Ebola, Nigeria and Lassa) and South America — the incubation period ranges from 3 to 21 days, depending upon the variety. Many VHF cases presenting together may suggest a bioterrorism attack.

2. VHF are severe febrile illnesses that can be complicated by a haemorrhagic tendency, petechiae, hypotension (and even shock), flushing of the face and chest, and oedema. Constitutional symptoms such as headaches, myalgia, vomiting and diarrhoea may occur. Some VHF manifest particular features not shared by the others.



5. Almost always, the true diagnosis will not be a VHF, but they must be considered where appropriate. Strict adherence to isolation and infection control precautions has prevented secondary transmission in almost all cases.

3. Once suspected, VHF are category 4 pathogens, so precautions for healthcare workers must be instituted and the case notified to the proper authorities. Isolation measures and barrier nursing procedures are indicated (Marburg, Ebola, Lassa and Congo-Crimean HF viruses may be particularly prone to aerosol nosocomial spread), usually in a special infectious diseases/tropical medicine unit staffed with clinicians with expertise in the field. Intensive supportive care may be required.

4. Diagnosis requires clinical expertise in infectious diseases/tropical medicine. The differential diagnosis includes malaria, yellow fever, dengue, typhoid/paratyphoid fever, non-typhoidal salmonellae, typhus and other rickettsial diseases, leptospirosis, shigella dysentery, relapsing fever (borreliosis), fulminant hepatitis and meningo coccal disease. Antigen tests, antibody detection and viral culture are all available for most of the VHF. The patient should be fully investigated and treated accordingly.

Fig. 5.10 Investigating a possible case of VHF.

- Assess clinical status:
 - *CT/MRI scan of head*—assess for raised ICP; exclude SAH (xanthochromia), sagittal vein thrombosis; exclude skull fracture, especially of cribriform plate or middle ear (this can lead to recurrent pneumococcal meningitis—if there is a nasal drip, test fluid for glucose to exclude presence of CSF as CSF contains glucose).
 - *Differential WBC in blood*.
 - *Inflammatory markers*—ESR, CRP.
 - *Coagulation screen and platelet count*: for meningococcal sepsis.
 - *ABGs*—to assess acid–base balance in severe cases.

- *Synacthen® test* (Short Synacthen® test, p. 225)—adrenal failure in severe meningococcal sepsis (Waterhouse–Friderichsen syndrome).
- *HIV test*—suggested by some pathologies and may be the overall underlying problem.
- *TB assessment*—may be the underlying pathology.
- *Assistance with therapy:*
 - Antibiotic sensitivity testing.
 - Serum antimicrobial levels, e.g. amphotericin, flucytosine.
- *Prevention:*
 - Notify relevant cases to public health authorities; isolate as necessary.
 - *Vaccination strategies*—meningococcus, pneumococcus, influenza, Hib.
 - *History of skull fracture*—may need neurosurgery, etc.

Urethritis (with or without haematuria)

Pain on micturition can simply represent a UTI or there may be an STD, such as gonorrhoea, present. The patient's sexual and travel history is important. Renal calculi can produce clinical pictures resembling an infection, as can dermatological conditions such as Stevens–Johnson syndrome. UTIs are commoner during pregnancy. Prostatitis can be a problem in older men.

- *Establish diagnosis/aetiology:*
 - *Urine collection (MSU)*—culture (bacterial infections), microscopy (parasites, etc., such as schistosomiasis—use terminal specimen), molecular techniques (LCR for *Chlamydia*).
 - *STDs and pelvic inflammatory disease*—perform high vaginal swab and urethral swabs; screen for gonococcus (includes throat and anal swabs).
 - *Calicular disease*—exclude with urine microscopy, radiology, etc.
 - *Prostatitis*—prostatic massage, CrAg.
 - *TB*—can present like any other UTI.
 - *Reiter's syndrome*—slit lamp examination of the eye, urine, and stool culture/LCR for *Chlamydia*.
- *Assess clinical status:*
 - *Biochemistry*—exclude renal failure (urea, creatinine, etc.).
 - *Markers of inflammation*—CRP, ESR.
 - *White cell count*.
 - Check all other mucosal surfaces of the body (mouth, conjunctivae, nose, etc.) to help exclude Stevens–Johnson syndrome.
- *Assistance with therapy:*
 - *Pregnancy test*.
 - *PSA* to exclude prostatic carcinoma (recurrent UTIs in older men).
 - *Radiology of renal tract*—US, IV pyelography (IVP) (to exclude underlying renal tract anatomical problems, TB involvement, calculi, etc.).
- *Prevention:*
 - *History of unsafe sex, recent new sexual partner, drug injecting*—consider VDRL, HIV, viral hepatitis testing.
 - *TB*—notify, contact trace, etc.
 - *Calculi*—exclude hypercalcaemia, hyperuricaemia, etc.

Red, painful swollen lower leg

One of the most difficult things in medicine is to distinguish effectively between a distal DVT and cellulitis—and a combination of both! Less commonly, a ruptured Baker's cyst of the knee can present in almost the same way. The key is in the history. Sometimes the problem is in the tissues, and sometimes in the joints (even gout and pseudogout can look like cellulitis) or the bone (osteomyelitis). Ulceration may be present on the legs. Venous and arterial insufficiency may complicate the picture—infected legs in older people can be very difficult to treat with antibiotics alone. Recent long-haul air travel may point more towards thrombosis, but swollen legs with compromised veins can easily get infected. Although rare, syphilis, yaws, and *Mycobacterium ulcerans* can cause leg ulcers that are potentially amenable to treatment. Pyoderma gangrenosum can resemble infection of the leg but is associated with non-infectious systemic diseases. Check for eschar from tick bite.

- Establish diagnosis/aetiology:

- Exclude DVT with Doppler US (and possible embolic disease on occasions).
- Swabs: from ulcers, between the toes usually not helpful.
- Blood cultures.
- Rarely VDRL.
- Joint assessment: urate levels for gout; assess (if relevant) for pseudogout, rheumatological screen, synovial fluid analysis (if relevant), Lyme disease titres (depends on the travel history, etc.).
- Leg ulcers in the young: consider sickle-cell disease, hereditary spherocytosis.

- Assess clinical status:

- White cell count.
- Inflammatory markers—ESR, CRP.
- Assess blood vessel integrity—e.g. compression US for venous problems, lower limb arteriography.

- Assistance with therapy:

- Exclude diabetes, HbA_{1c}.
- X-ray, bone scanning, MRI—is osteomyelitis present?

- Prevention:

- Treat diabetes, if present.
- Treat other underlying conditions, if present.
- Patient advised to take care in future (e.g. DVT avoidance whilst travelling).

Vesicular rash

Many vesicular rashes are infective; many are not. In particular, the distribution of the rash should be carefully assessed, and joint assessment and management with a dermatologist are often valuable. If atopic, eczema herpetiformis comes into the picture. Staphylococcal impetigo can cause vesiculation. If there is a relevant travel history, rickettsial pox and monkey pox come into the picture. Erythema multiforme, which often has an infective basis but can also be produced by medications, can produce a vesiculating rash (so check the mouth, eyes, and genitalia, and determine the

medication history). Non-infective blistering conditions include dermatitis herpetiformis (coeliac disease), pompholyx, and pemphigus.

- Establish diagnosis/aetiology:
 - Vesicular fluid—PCR, EM, IFT, culture, etc.
 - Serology—HSV, herpes varicella-zoster, rickettsial pox, Coxsackie virus, ASO titre.
- Assess clinical status:
 - CXR—chickenpox (if compromised, ABGs will be needed).
 - EEG—if cerebral symptoms present (e.g. cerebellar encephalitis can occur with herpes varicella-zoster).
 - Monkey pox, smallpox, rickettsial pox—the patient will be ill and will require full assessment, even possibly intensive care.
- Assistance with therapy:
 - Pregnancy test: herpes varicella-zoster a bigger problem in pregnancy.
- Prevention:
 - Avoid precipitants with erythema multiforme.
 - Manage atopy optimally.

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Cardiology

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Cardiac catheterization

Principle

Cardiac catheterization is an invasive procedure during which catheters are placed within the cardiac chambers, coronary arteries, and great vessels to provide information on cardiac anatomy, pressures, disease states, function, and O₂ saturations.

Indications

- Diagnosis of suspected coronary artery disease (after appropriate non-invasive assessment or if results are equivocal).
- Assessment of coronary artery disease burden and suitability for intervention, e.g. percutaneous coronary intervention (PCI), coronary artery bypass surgery, or cardiac transplantation.
- Measurement of intracardiac pressures in patients with valvular heart disease (largely superseded by non-invasive imaging).
- Detailed measurement of left and right ventricular cardiac output and pulmonary hypertension in patients considered for cardiac transplantation or with suspected congenital heart disease.
- Evaluation of O₂ saturations in cardiac chambers to identify cardiac shunting.
- Myocardial biopsy in patients with cardiomyopathy of unknown cause.
- Monitoring of cardiac transplantation success/rejection.

Contraindications

- Pregnancy is an absolute contraindication to coronary angiography.
- Relative contraindications include:
 - Severe peripheral vascular disease.
 - Aortic aneurysm.
 - Renal failure.
 - Unstable cardiac failure or arrhythmias.
 - Haemodynamic instability.
 - Sensitivity to contrast agents.

Patient preparation

The patient is fasted for 4h prior to the procedure. IV access is required, together with haemodynamic and ECG monitoring. Sedation may be used according to patient request or operator direction. It is important that the patient understands the risks of the procedure and gives informed written consent. The contrast agents used can provoke renal failure in susceptible patients. Metformin should be withdrawn for 48h prior to the procedure. In patients with renal impairment, pre-treatment with acetylcysteine is advised and a less nephrotoxic contrast agent may be considered. Contrast agent volume should be minimized.

Procedure

1. The patient lies flat on a catheter laboratory table and appropriate monitoring is applied.
2. LAN is used and an aseptic technique employed. For a left heart procedure, arterial access is required. The radial artery is the most commonly employed, but the femoral artery provides an alternative route (unless significant peripheral vascular disease is present).
3. The chosen artery is cannulated using the Seldinger technique, and an access sheath inserted.
4. Fluoroscopic screening is used to monitor the passage of a guidewire and catheter to the heart.
5. Catheters are pre-shaped according to the structure being examined. Typically, a Judkins left 4 catheter is used for the left coronary artery, Judkins right 4 catheter for the right coronary artery, and an angled pigtail catheter is placed in the LV and/or aorta for examination of these structures.
6. A contrast agent is injected for image acquisition. Images of the coronary arteries are acquired in multiple planes in order to optimally identify coronary artery stenoses. Continuous pressure transducers can be used for dynamic left heart pressure monitoring, e.g. LV and aorta.
7. Evaluation of the right heart necessitates venous access. The commonest access point is the femoral vein, but the central veins, e.g. subclavian or internal jugular vein, can also be used.
8. A right heart or Swan-Ganz catheter is passed towards the right heart. Right-sided pressures, e.g. vena cavae, right atrium, right ventricle (RV), pulmonary artery (PmA), and pulmonary capillary wedge pressure (indirect left atrial (LAt) pressure), can be measured.
9. Blood samples obtained from these sites can be analysed for O₂ saturation and used to identify the presence and site of cardiac shunts, e.g. atrial or ventricular septal defects. Cardiac output studies can also be performed.

Risks

Cardiac catheterization is an invasive technique, and so there are inherent risks to the procedure (1:200 patients). Full cardiopulmonary resuscitation facilities should be immediately available.

These risks include

- Trauma to arterial/venous access sites, including haematoma, occlusion, aneurysm, pseudo-aneurysm, nerve damage.
- Aortic/coronary artery dissection.
- CVA (embolus).
- MI (embolus/dissection).
- Arrhythmias.
- Pulmonary oedema (left main stem stenosis).
- Renal failure.
- Vasovagal response to arterial access/sheath removal.

Additionally, the patient is exposed to ionizing radiation, and so screening/image acquisition times should be reduced as much as possible. Serial studies should be avoided.

Possible results

Results from a left heart study can provide information on

- Coronary artery anatomy and distribution of disease, to aid decisions regarding the need for revascularization (see Fig. 6.1).
- Left ventricular size and function.
- Left heart pressures.
- Aortic and mitral valve integrity.
- Aortic size and disease.
- Congenital abnormalities.

A right-sided study can provide information on

- Right ventricular size and function.
- Tricuspid and pulmonary valve integrity.
- PmA anatomy.
- Right heart pressures.
- Congenital abnormalities, e.g. shunts.
- Myocardial histopathology where biopsy is taken.

Advantages over other tests

Invasive coronary angiography has high resolution for the evaluation of coronary artery disease and is the current gold standard imaging technique for this application. However, CT coronary angiography is rapidly becoming an accurate alternative means of depicting coronary artery atheroma, particularly in the proximal vessels. Advances in echocardiography and cardiovascular MR (CMR) have reduced the need for right heart catheterization, but the latter is still the only means with which to obtain direct histopathological information from myocardial structures.

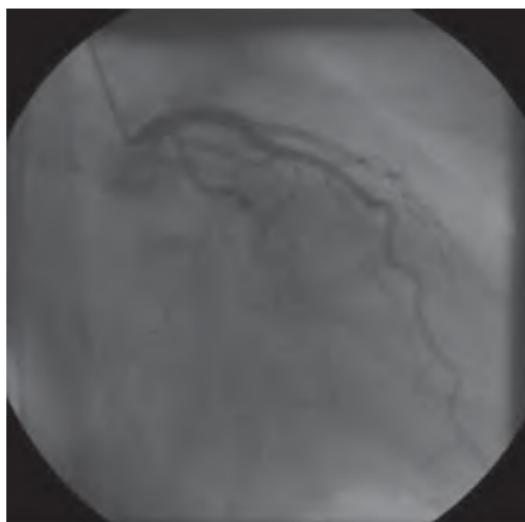


Fig. 6.1 Coronary angiogram showing a normal left coronary artery.

Pitfalls

The procedure is invasive and the risks involved are not insignificant. Whilst coronary angiography is the gold standard for the demonstration of anatomical coronary arterial lesions, it provides little information regarding their physiological significance on the myocardium. The information should therefore be used in conjunction with imaging techniques that can assess the adequacy of myocardial perfusion, e.g. stress nuclear imaging, stress echocardiography, or CMR.

Further reading

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Fihn SD, Blankenship JC, Alexander KP, et al. 2014 ACC/AHA/AATS/PCNA/SCAI/STS Focused update of the guideline for the diagnosis and management of patients with stable ischemic heart disease. *J Am Coll Cardiol* 2014; **64**: 1929–49.

Cardiac markers of myocardial necrosis

Principle

When cardiac muscle is damaged, certain substances, e.g. troponins I (TnI) and T (TnT), myoglobin, and creatine kinase, are released into the bloodstream and can be measured by biochemical assays. Such assays can be helpful in making the diagnosis of ACS or myocardial contusion. Myoglobin and creatine kinase (CK and CK-MB) are relatively non-specific cardiac markers of myocardial necrosis (CK is also released by skeletal muscle) that are elevated within 2–4 h of myocyte damage. Troponins (TnT and TnI) are cardiac muscle proteins that are released into the peripheral circulation more slowly and are highly specific for myocardial injury. High-sensitivity troponin assays (hsTnT and hsTnI) detect troponins at much lower concentrations than the standard troponin assays available previously, allowing more rapid diagnosis in ACS. Using a high-sensitivity assay, an initial elevation of circulating troponin levels is detectable within 3 h of the event, peaking at around 24 h (see Fig. 6.2).

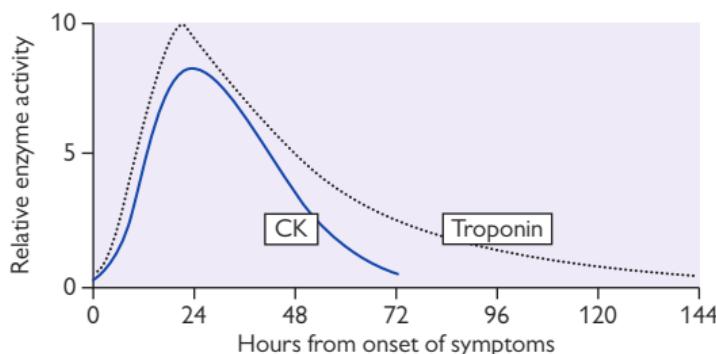


Fig. 6.2 Changes in levels of cardiac markers following acute MI.

Patients presenting with unstable ischaemic cardiac pain are initially labelled as having an ACS and are categorized on the basis of their initial 12-lead ECG into ST-segment elevation ACS (which requires urgent reperfusion via primary PCI or thrombolysis) or non-ST-segment elevation ACS (which requires appropriate antiplatelet and antithrombotic therapy, followed by coronary revascularization as appropriate). MI is confirmed later if there is a rise in markers of myocardial necrosis, most commonly troponins. When these markers are raised, those presenting with an ST-segment elevation ACS are classified as ST-elevation myocardial infarction (STEMI) and those with non-ST-elevation ACS are classified as non-ST-elevation MI (NSTEMI).

Indications

Cardiac markers of myocardial necrosis are useful

- In conjunction with the clinical history and 12-lead ECG to diagnose and categorize suspected ACS.
- To stratify risk in ACS.
- To ascertain the extent of any myocardial injury following coronary revascularization procedures.
- To identify myocardial contusion following thoracic trauma.

Procedure

A 10mL venous blood sample is sufficient. For hsTnT or hsTnI testing, this sample is ideally taken 3–6h after the patient's most severe symptoms. Repeat sampling is necessary to demonstrate a rise in cardiac markers.

Risks

In patients who have had thrombolysis for acute MI, bleeding or haematoma formation may occur spontaneously at venepuncture sites.

Possible results

Within the normal population, 99% of individuals will have a hsTnT <14ng/L. In the appropriate clinical setting of chest pain typical of acute MI ± ECG changes:

- An elevated troponin (hsTnT >30ng/L) is consistent with a diagnosis of MI.
- Acute MI (STEMI and NSTEMI) can be confidently excluded if a high-sensitivity troponin result is within normal limits 6h after the onset of symptoms.

Pitfalls

CK may be elevated in skeletal muscle injury, as well as with MI. Direct estimation of CK-MB, the isoenzyme that is more specific for cardiac muscle, may be helpful. Troponins are more specific for cardiac injury but may also be elevated in cardiac failure, arrhythmias, renal failure, or PE, and so the clinical context should always be taken into account. Timing of blood samples is critical since values may be normal if blood is taken too soon after symptom onset. Troponins may remain elevated for up to 14 days following a cardiac event, and so the diagnosis of re-infarction using troponins alone may be unreliable.

Further reading

Shah ASV, Anand A, Sandoval Y, et al. High-sensitivity cardiac troponin I at presentation in patients with suspected acute coronary syndrome: a cohort study. *Lancet* 2015; **386**: 2481–8.

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Cardiac volumetric imaging: magnetic resonance and computed tomography

Principle

Cardiac volumetric imaging is now established as a powerful tool to visualize the anatomy, size, and function of cardiac structures, i.e. valves, chambers, myocardium, pericardium, and great vessels. Advanced technology MR imagers and multislice CT scanners are increasingly available to clinicians. These techniques provide information regarding the aetiology and severity of most congenital and acquired cardiac abnormalities.

Indications

The indications for CMR and CT are shown in Table 6.1. CMR is especially useful in patients with IHD where a comprehensive assessment of left ventricular function, reversible myocardial ischaemia, and myocardial viability can be made. Cardiac CT is of particular value in screening for coronary artery disease and for assessment of the great vessels and coronary arteries.

Contraindications

- Presence of any ferrous metal, e.g. intracranial clips, intra-ocular foreign bodies, shrapnel (MRI).
- Temporary or permanent pacing systems (MRI), unless MRI-conditional.

Table 6.1 Indications for CMR and CT

Condition	MRI	CT
Congenital heart disease	+++	++
Anatomy, size, or mass of cardiac chambers (left atrium, LV, right atrium, RV)	++++	+++
Global and regional left ventricular function	++++	+++
Screening (cardiomyopathy, including arrhythmogenic right heart)	++++	+
Valvular heart disease (aortic, mitral, tricuspid, and pulmonary)	+++	+
Prosthetic heart valves	+++	+
Cardiac masses	+++	++
Pericardial disease	+++	++
Aortic disease	+++	++++
Coronary artery anatomy and patency	++	++++
Screening for coronary artery disease	+	++++
Myocardial perfusion	+++	-
Stress study in IHD	++++	-
Myocardial viability	++++	+

- Internal cardioverter–defibrillator (ICD) systems (MRI), unless MRI-conditional.
- Clinically dehiscing Starr–Edwards valve prostheses manufactured between 1960 and 1964 (MRI).
- Severe claustrophobia (more of an issue with MRI).
- Allergy to non-ionic contrast agents (CT).
- Haemodynamic instability.
- Pregnancy is a relative contraindication to MRI, but an absolute contraindication to CT.

Patient preparation

A patient questionnaire is performed to exclude contraindications. Patients should be relaxed and have the procedure explained, so that they are able to co-operate effectively. Breath-holding techniques should be practised prior to the scan to achieve optimal image quality. Height and weight are required for indexing cardiac measurements. Where stressors are to be used, a 12-lead ECG should be obtained. IV access is required for contrast agent administration.

Procedure

The patient is positioned on the imaging couch. Electrodes are attached to allow image acquisition to be gated to the ECG. For CMR, a cardiac coil is selected and earplugs supplied. Images are then acquired in order to evaluate the clinical problem.

The following MRI sequences are commonly performed

T1-weighted images

These are used for anatomical assessment and contribute, with optional contrast agent enhancement, towards tissue characterization.

Cine sequences

Cine sequences are used for anatomical assessment and particularly for cardiac function. Repetitive short-axis slices from the cardiac base to the apex are summated to calculate left ventricular function. This is an extremely accurate method, since it avoids geometrical assumptions created by regional wall abnormalities. It is therefore a gold standard method for the calculation of left ventricular ejection fraction (EF), mass, and other cardiac volumes. The technique can be used in conjunction with pharmaceutical stress (e.g. dobutamine) in a manner analogous to stress echocardiography to identify regional reversible myocardial ischaemia.

First-pass contrast agent imaging

Myocardial perfusion is assessed by imaging the first pass of a T1-shortening contrast agent (gadolinium). The contrast agent passes through the right heart and lungs to the left heart. It is carried into the myocardium by the coronary circulation, giving rise to a rapid ↑ in myocardial signal intensity. Myocardial areas with reduced blood flow have slower and reduced signal change. The effect is enhanced by use of pharmaceutical coronary artery vasodilators, e.g. adenosine, and can be used for the identification of MI and reversible ischaemia. In contrast to nuclear perfusion imaging, high

spatial resolution allows detection of subendocardial, as well as transmural, ischaemia.

Delayed contrast agent imaging

Gadolinium is an interstitial contrast agent, and it accumulates within 10min after administration in tissue where extracellular membranes are damaged. This is a powerful tool to delineate between myocardial scar tissue (\uparrow signal intensity) and viable or hibernating myocardium (no change in signal intensity on delayed imaging).

Velocity-encoded imaging

Velocities can be encoded into grey scale to measure the motion of cardiac structures and the flow within great vessels. This has similar applications to echocardiographic Doppler imaging and can be used to quantify valvular disease, e.g. stenosis or regurgitant volumes, and congenital heart disease, e.g. cardiac shunts.

Magnetic resonance angiography

A volume (3D) image acquisition is performed after a bolus of gadolinium reaches an area of interest within the great vessels. This technique is useful for evaluating aortic disease, congenital heart disease, and the presence of RAS.

Cardiac computed tomography

An infusion of iodinated contrast agent is given, and a volume image acquisition is attained during a 15–20s breath-hold. Where coronary arteries are the area of interest, a coronary artery calcification score is performed initially, and these data can then be used to guide further imaging. A β -blocker may be required to lower the heart rate to optimize the image resolution. Images are then post-processed to reconstruct them to attain clinical information according to scan indication.

Risks

If appropriate screening is carried out to exclude patients with contraindications, then MRI carries minimal risk. Patients who are haemodynamically unstable, e.g. acute aortic dissection, should undergo an alternative form of imaging. CT exposes the patient to ionizing radiation, and so this should limit its application.

Possible results

Figures 6.3–6.9 show the types of images that can be acquired by CMR imaging.

Advantages over other tests

Volumetric imaging is non-invasive and facilitates excellent temporal and spatial definition of soft tissues. It overcomes difficulties of echocardiography where acoustic window availability limits the scan. CMR uses no ionizing radiation and so is ideal for serial imaging. A very comprehensive examination can be performed and allows dynamic assessment of the heart and great vessels at a single visit. Multislice CT provides very accurate information regarding coronary artery disease and is a non-invasive alternative to traditional coronary angiography.

Pitfalls

CMR image quality may be impaired by artefact in some patients, especially if metal is present, e.g. non-ferrous surgical clips, spinal rods, or prosthetic valves. Rapid heart rates degrade the image quality in multislice CT.



Fig. 6.3 Examples of volumetric imaging techniques—MRI black blood sequence illustrating aortic coarctation bypass.

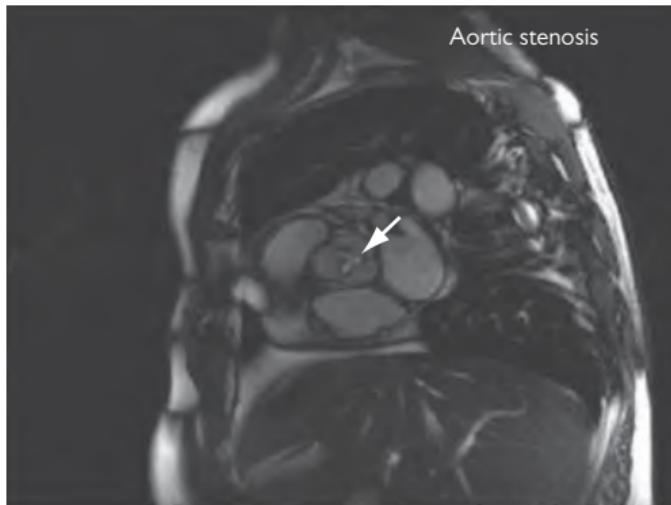


Fig. 6.4 MRI cine sequence of aortic stenosis.

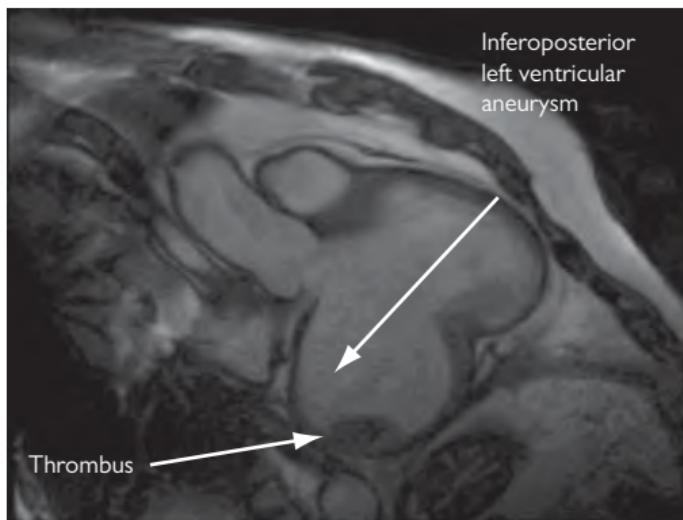


Fig. 6.5 MRI cine sequence demonstrating huge inferoposterior left ventricular aneurysm containing a thrombus.



Fig. 6.6 MRI delayed enhancement study delineating subendocardial scar tissue in the anteroseptal wall following MI.

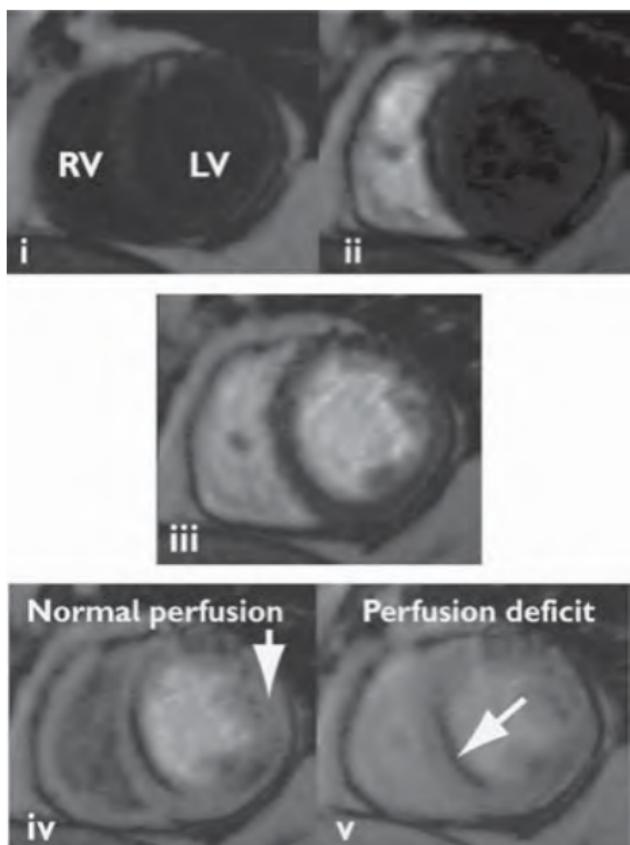


Fig. 6.7 MRI first-pass perfusion: (i) prior to contrast injection; (ii) contrast agent appears in the RV and (iii) LV; (iv, arrow) contrast opacifies normally perfused myocardium, (v, arrow) but identifies an inferoseptal subendocardial perfusion deficit.

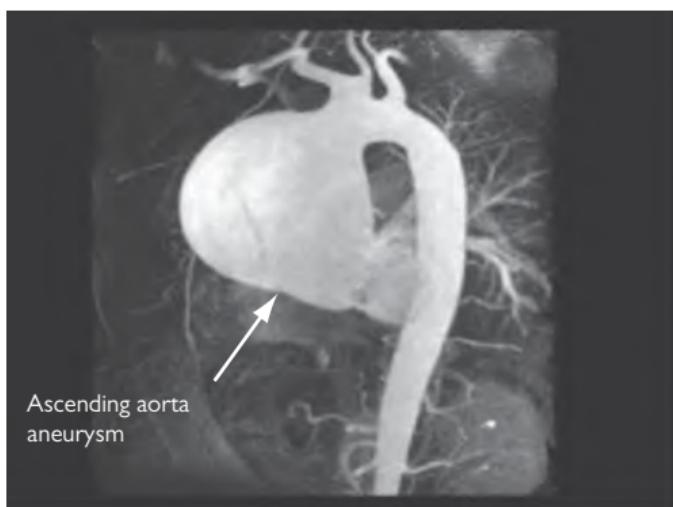


Fig. 6.8 Magnetic resonance angiogram (MRA) of a severe ascending aortic aneurysm.



Fig. 6.9 CT of type II aortic dissection and associated thrombus.

Further reading

- Myerson SG, Francis J, Neubauer S. *Cardiovascular Magnetic Resonance*. Oxford: Oxford University Press, 2010.
Nicol E, Stirrup J, Kelion AD, Padley SPG. *Cardiovascular Computed Tomography*. Oxford: Oxford University Press, 2011.

Echocardiography

Principle

Echocardiography is the use of US to visualize the anatomy, size, and function of cardiac structures, i.e. valves, chambers, myocardium, pericardium, and great vessels. The technique provides information regarding the aetiology and severity of most congenital and acquired cardiac abnormalities.

Imaging modalities

Several imaging modalities are available, including 2-dimensional (2D), 3D, motion-mode (M-mode), Doppler, and contrast agent enhancement (see Figs 6.10–6.15).

2D imaging

2D echocardiography allows real-time visualization of cardiac anatomy, abnormalities of cardiac structures, and their motion. Image quality is enhanced by use of harmonic imaging and contrast opacification. With some systems, 3D imaging is also possible.

3D imaging

3D echocardiography is now widely available and used in both TTE and TOE. The modality is particularly useful for the assessment of left ventricular size and function, of congenital heart disease (morphology and function), and in the guidance of interventional procedures (e.g. percutaneous atrial septal defect closure).

M-mode imaging

M-mode imaging samples movement of cardiac structures along a single scan line, creating a graph of the motion of sampled structures against time. It is useful for accurate timing of cardiac events and measurement of cardiac dimensions.

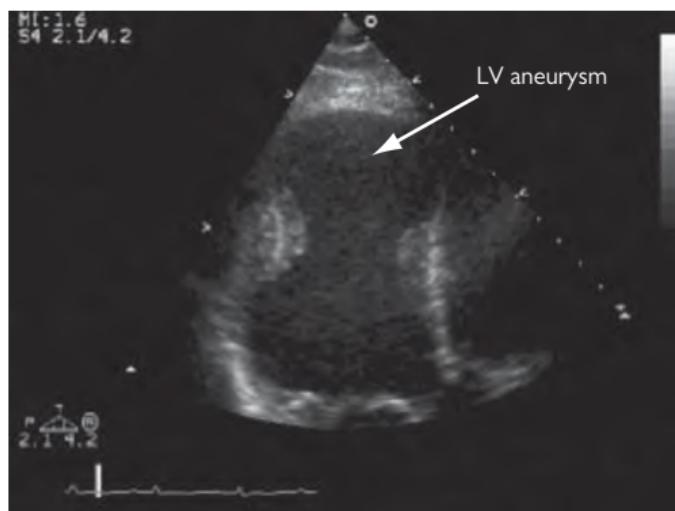


Fig. 6.10 2D TTE image of the LV demonstrating an apical aneurysm.

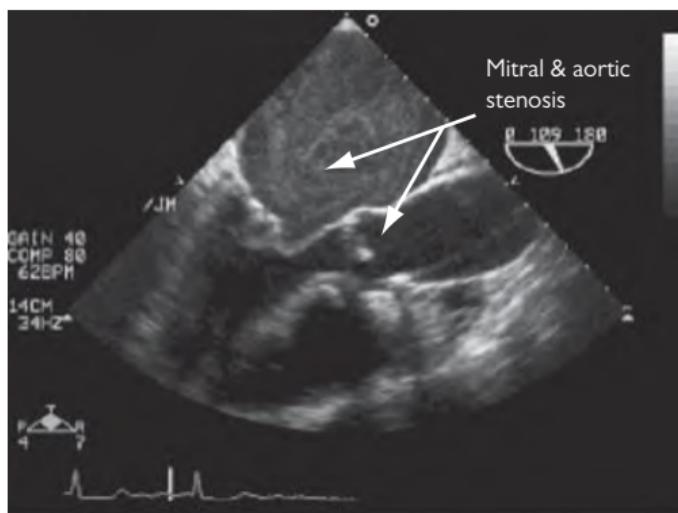


Fig. 6.11 2D TOE image of rheumatic heart disease (mitral and aortic stenosis).

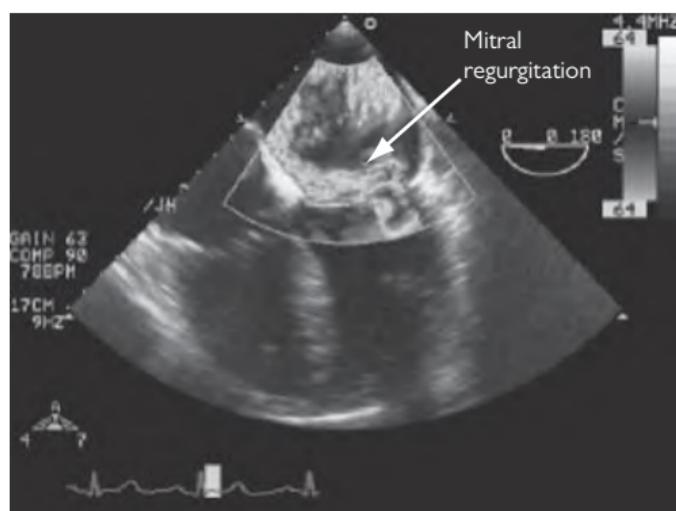


Fig. 6.12 Colour Doppler of eccentric jet of severe mitral regurgitation 2° to mitral valve prolapse. (☞ Colour plate 1.)

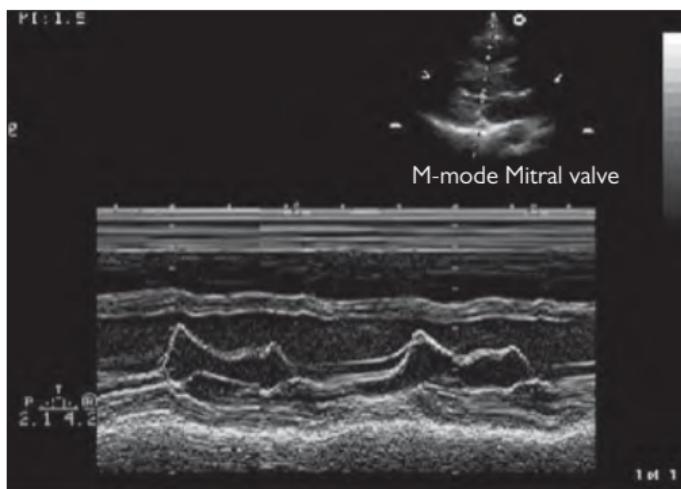


Fig. 6.13 Normal M-mode through mitral valve leaflets.

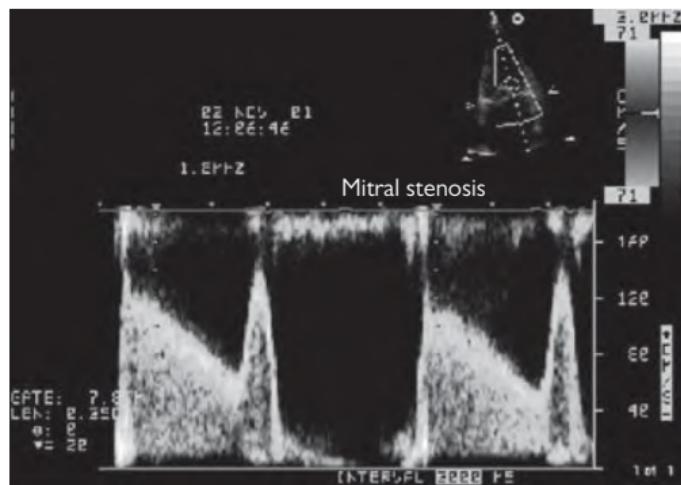


Fig. 6.14 Laminar pulsed-wave Doppler flow in a patient with mitral stenosis.
(Colour plate 2.)

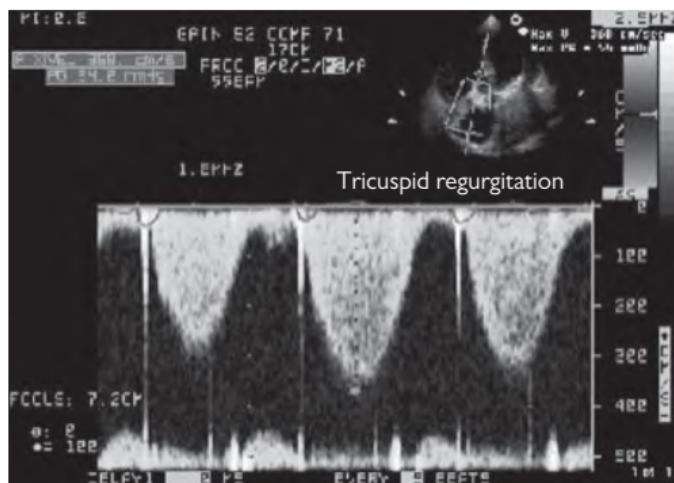


Fig. 6.15 Continuous-wave Doppler of tricuspid regurgitation, with PmA systolic pressure calculated as 54mmHg (plus right atrial pressure). (☞ Colour plate 3.)

Doppler

Doppler is the comparison of transmitted US beam frequency with received US frequency reflected from moving structures, e.g. soft tissues (Doppler tissue imaging) or blood cells. The direction of motion and its velocity can be assessed. When blood cells reflect US as they move towards the transducer, they compress the US wavelength, whereas if they are moving away, the US wavelength lengthens. The change in frequency between the transmitted and reflected wavelengths is the Doppler shift frequency.

Continuous-wave Doppler

Continuous-wave (CW) Doppler acquires velocity data along the US beam's entire path. Blood flow of varying strengths and velocities is demonstrated in blood vessels and through the cardiac valves. The signal is represented graphically on a spectral display. Flow away from the transducer is reflected below the zero line; flow towards the transducer is +ve in deflection. Signal density and shape give an indication of the severity of any abnormalities.

CW Doppler velocity data can be used to measure the pressure gradients between the cardiac chambers according to the Bernoulli equation. The data thus obtained can be used to calculate the severity of valvular stenosis, expressed in terms of mean and peak pressure gradients, and effective orifice area. The pressure half-time can be used to estimate the severity of diastolic valvular lesions, i.e. mitral stenosis and aortic regurgitation. It is defined as the time taken for the peak gradient to fall to half of its original value. It is inversely related to the effective orifice area.

Pulsed-wave Doppler

The velocity of blood is sampled within a small area. Since this involves only a small sample volume, it localizes blood flow. It is particularly useful for evaluating low velocity flow such as cardiac inflow and outflow tract velocities. At higher velocities, it is limited by the problem of aliasing.

Colour Doppler

Colour Doppler colour-codes the direction and velocity of blood flow through cardiac structures. Blood flow in the direction away from the US probe is depicted as blue, whereas blood flowing towards the probe is red. ↑ blood flow velocity is reflected by colour mixing or turbulence. This is a useful screening tool for abnormal jets of blood and can be used to estimate the severity of some abnormalities.

Contrast echocardiography

Microbubbles, consisting of a gas core encapsulated by a protein shell, can be used as contrast agents to improve image quality. Typically, they are used in conjunction with harmonic imaging to allow opacification of the left ventricular volume and thereby assess wall motion. Research is continuing into their use to assess myocardial perfusion.

Transthoracic and transoesophageal echocardiography

TTE, where the US beam is directed to the heart from outside the chest wall, is the most commonly performed examination. In certain clinical situations, more information is obtained by directing the US beam towards the heart from the oesophagus, and this is termed TOE. This allows image acquisition without interference of the chest wall, i.e. ribs, soft tissues, and lungs. US beam attenuation is small and a high-frequency transducer can be used, giving rise to higher spatial resolution than with TTE. This facilitates improved definition of the posterior cardiac structures, i.e. valves, atria, and aorta. Relatively small structures, such as cardiac vegetations, may be visualized with greater accuracy.

Indications

Indications for TTE and TOE are shown in Table 6.2.

Contraindications

TTE is a very safe imaging technique with no known side effects. Contraindications to TOE include:

- Cervical spine instability, e.g. RhA, ankylosing spondylitis.
- Oesophageal disease, e.g. stricture, carcinoma, oesophageal varices.
- Haemodynamically unstable patients, including significant hypoxia.

Patient preparation

For both procedures, the patient is made comfortable on an imaging couch in the left lateral position with the head end raised to at least 60°. Cardiac electrodes should be applied to obtain an ECG trace. Aqueous gel is used on the probe to aid US beam conduction. For TOE, the patient should be nil by mouth for at least 6h prior to the procedure and IV access sited. Any loose teeth or dentures should be removed. The throat should be sprayed with an LAN, e.g. lidocaine, and a bite guard inserted prior to intubation. The patient may be sedated according to the clinical situation, patient preference, or operator recommendation. Antibiotic prophylaxis is not required.

Table 6.2 Indications for TTE and TOE

Condition	TTE	TOE
Congenital heart disease	++	+++
Suitability for percutaneous closure of atrial septal defect	-	++++
Anatomy, size, and function of the cardiac chambers (left atrium, LV, right atrium, RV)	+++	++++
Global and regional left ventricular function	+++	+++
Valvular heart disease (aortic, mitral, tricuspid, and pulmonary)	+++	++++
Assessment for mitral valvuloplasty	+	++++
Prosthetic heart valves	++	++++
Infective endocarditis (and its complications)	++	++++
Guide to safe cardioversion (AF)	-	+++
Intra-operative assessment of valve disease/repair/replacement	-	+++
Pulmonary hypertension	++	+++
Unexplained pulmonary hypertension/right-sided dilatation	+	++++
Cardiac source of embolus in TIA/CVA/peripheral embolism	+	+++
Cardiac masses	++	+++
Pericardial disease	+++	++
Aortic disease	+	+++
Screening (cardiomyopathy)	+++	++

Procedure

For TTE, the probe is applied to the chest in standard imaging positions (parasternal, apical, subcostal, and suprasternal), and the following imaging planes (see Fig. 6.16) are acquired with use of all available modalities:

- Parasternal (long axis and short axis).
- Apical (2-, 3-, 4-, 5-chamber).
- Subcostal and suprasternal.

For TOE, the probe is passed gently over the tongue towards the cricopharyngeal muscles. Gentle continuous pressure is applied, and the patient is encouraged to swallow until the probe lies within the oesophagus. Views of the cardiac structures are acquired from varying levels within the oesophagus, gastro-oesophageal junction, and stomach.

Risks

TTE is extremely safe. TOE is a semi-invasive procedure, and so informed written consent should be obtained. Intubation of the oesophagus carries a risk of ~1 per 2000 of oesophageal trauma. There is a small risk of laryngospasm and cardiac arrhythmia (usually supraventricular). This usually resolves spontaneously on probe withdrawal. Neither technique should be performed on a haemodynamically compromised patient where an interventional procedure is delayed by inappropriate image acquisition, e.g. aortic dissection, cardiac tamponade.

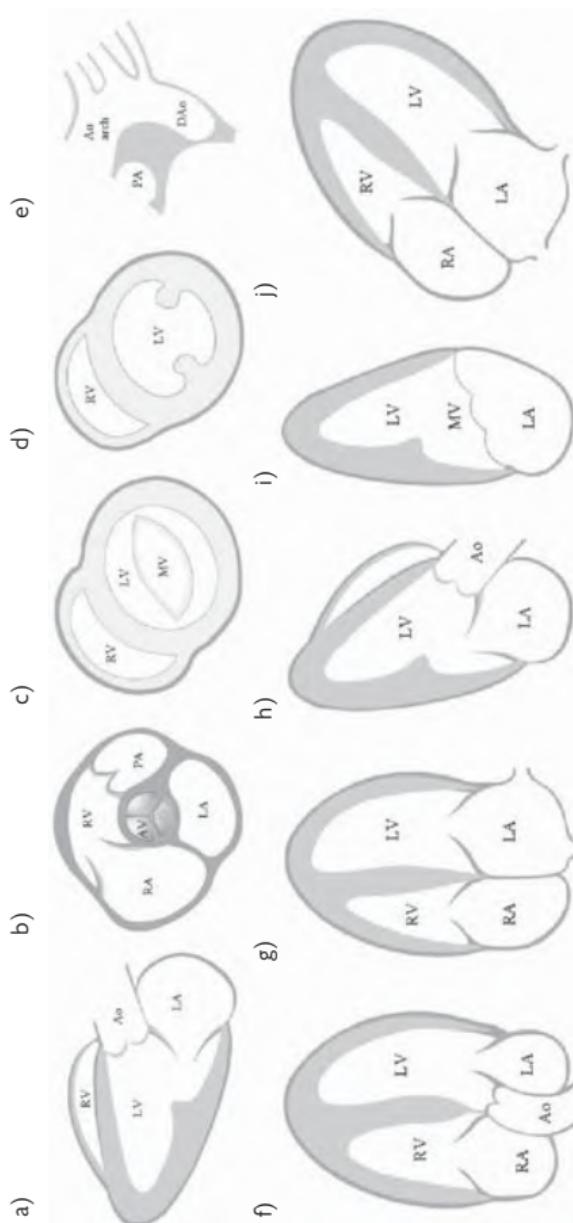


Fig. 6.16 Transthoracic imaging planes: (a) parasternal long axis, (b) parasternal short axis at level of aortic valve, (c) mitral valve, (d) papillary muscles, (e) suprasternal notch, (f-i) apical 5-, 4-, 3-, and 2-chamber views, (j) subcostal plane. LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle; Ao, aorta; PA, pulmonary artery; AV, aortic valve; MV, mitral valve; DAO, descending aorta.

Advantages over other tests

TTE is cheap and non-invasive and requires no ionizing radiation, ensuring that serial examinations are without risk. It is portable and can easily be used at the bedside. The technique provides a comprehensive assessment of cardiac anatomy, function, and blood flow. TOE has similar advantages to TTE, with the difference that it is a semi-invasive technique but offers superior spatial resolution, particularly of structures in close proximity to the probe (e.g. left atrium, mitral valve). It is a powerful tool for intra-operative use and in patients with limited transthoracic windows.

Pitfalls

Transthoracic image quality may be limited by inadequate acoustic windows in patients with obesity, lung disease, and chest wall deformities, and those undergoing artificial ventilation. Structures at the posterior aspect of the heart are not well visualized. Optimal transoesophageal image quality is obtained at the posterior aspect of the heart, and so the apex of the heart is less well seen. Image quality may be compromised in patients with hiatus hernia. Not all patients tolerate the procedure, and so images relating to the suspected pathology should be acquired first in case the procedure has to be abandoned prematurely.

Possible results

Anatomy and size of cardiac chambers

Normal anatomy is demonstrated to exclude congenital heart disease. Each cardiac chamber and its connections are systematically identified and the presence of any shunts excluded. The size of the cardiac chambers and walls are assessed. Normal values are shown in Table 6.3.

Causes of chamber abnormalities include

- *LAt dilatation:* mitral valve disease, systemic hypertension, coronary artery disease, dilated cardiomyopathy, restrictive cardiomyopathy, hypertrophic cardiomyopathy, AF.
- *Left ventricular dilatation:* mitral regurgitation, aortic regurgitation, severe aortic stenosis, systemic hypertension, IHD, dilated cardiomyopathy.
- *Left ventricular hypertrophy:* systemic hypertension, left ventricular outflow obstruction (aortic stenosis, supra-aortic membrane, subaortic membrane), hypertrophic cardiomyopathy (asymmetric), aortic coarctation, infiltrative cardiomyopathy (amyloidosis).

Table 6.3 Normal values for cardiac linear dimensions

Parameter	Normal range
LAt diameter	♂ 3.0–4.0cm ♀ 2.7–3.8cm
Left ventricular diastolic diameter	♂ 4.2–5.9cm ♀ 3.9–5.3cm
Interventricular septum diastolic diameter	0.6–1.2cm
Left ventricular posterior wall diastolic diameter	0.6–1.2cm

- **Aortic root dilatation:** systemic hypertension, collagen disorders, e.g. Marfan's syndrome, syphilitic aortitis, aortic coarctation, aortic valve disease, aortic aneurysm, aortic dissection.
- **Right atrial (RAt) dilatation:** tricuspid valve disease, pulmonary valve disease, pulmonary hypertension, dilated cardiomyopathy, restrictive cardiomyopathy, constrictive pericarditis.
- **Right ventricular dilatation:** 1° pulmonary hypertension, 2° pulmonary hypertension (e.g. mitral stenosis, PEs, lung disease, left-to-right shunts), tricuspid regurgitation, pulmonary regurgitation, right ventricular cardiomyopathy (including arrhythmogenic), right ventricular infarction.
- **Right ventricular hypertrophy:** 1° pulmonary hypertension, 2° pulmonary hypertension (e.g. mitral stenosis, PEs, lung disease, left-to-right shunts), pulmonary stenosis, right ventricular outflow tract obstruction.
- **Pulmonary trunk dilatation:** pulmonary hypertension, collagen disorders, e.g. Marfan's syndrome, pulmonary atresia, pulmonary valve disease, idiopathic PmA dilatation.

Global and regional left ventricular systolic function

The size, shape, and function of the LV are evaluated with 2D/3D imaging ± contrast opacification. Commonly measured echocardiographic parameters of left ventricular function include EF, fractional shortening, stroke volume, and cardiac output. It should be remembered that, when calculated from the M-mode image, only the function of the base of the heart is assessed. This should not be extrapolated to global left ventricular function, unless the entire ventricle is normal. EF can also be calculated from apical diastolic and systolic views (using the modified Simpson's rule). This is more reflective of global left ventricular function but is still limited since it is a 2D measurement and requires geometric assumptions. A more subjective assessment can be made by visually estimating left ventricular function as normal or as having mild, moderate, or severe impairment. Causes of impaired left ventricular systolic function are:

- Ischaemic cardiomyopathy.
- Hypertensive cardiomyopathy.
- Dilated cardiomyopathy.
- Valvular heart disease.

Regional wall motion abnormalities are confined to specific walls or segments of the LV. Systolic wall thickening is defined in Table 6.4.

Table 6.4 Categorization of regional wall motion

Severity	Description
Normal	>50% ↑ in systolic wall thickness, compared with diastole
Hypokinetic	<50% ↑ in systolic wall thickness, compared with diastole
Akinetic	Absent systolic wall thickening
Dyskinetic	Outward wall motion during systole

Causes of regional wall motion abnormalities are almost exclusively related to coronary artery disease

- MI.
- Left ventricular aneurysm.
- Myocardial hibernation.
- Myocardial ischaemia.
- Post-cardiac surgery.
- Cardiac tumour.

Stress echocardiography

Dobutamine stress may be used in conjunction with left ventricular global and regional wall functional assessment. As well as allowing distinction between normal, ischaemic, and infarcted myocardium, it also permits the assessment of myocardial viability (regional dysfunction that will improve with revascularization) vs scar tissue (no effect on function from revascularization). It should be noted that CMR is now proven to be a superior technique for identification of myocardial scar/viability. Resulting interpretation with respect to regional wall motion responses to low-dose and peak-dose dobutamine stress is shown in Table 6.5.

Left ventricular diastolic function

Left ventricular filling during diastole is an important component of left ventricular functional assessment. Normal LAt filling is passive throughout systole (S wave) and diastole (D wave) and is assessed from pulsed-wave (PW) Doppler sampling of pulmonary venous flow. Left ventricular filling is assessed from diastolic mitral valve flow. It is predominantly passive and early in diastole (E wave), with a small later contribution from atrial systole (A wave). Diastolic dysfunction results in elevated left ventricular end-diastolic pressure (LVEDP), and ultimately left atrial pressure (LAP), and so alters measured flow characteristics. There are three types of diastolic dysfunction (see Table 6.6), depending on the degree of raised LAP that occurs in order to drive flow across the mitral valve.

Right heart function

Right heart failure is depicted by a dilated, poorly functioning RV. Raised right-sided pressures are indicated by a dilated right atrium and IVC (seen on subcostal view).

Table 6.5 Interpretation of contractile responses to dobutamine stress

Interpretation	Rest	Low dose	Peak dose
Normal	Normal	↑	Hyperdynamic
Inducible ischaemia	Normal or ↓	No change or ↓ from baseline	↓ from baseline
Scar tissue	Absent	Absent	Absent
Viability	Absent	Improved	Improved or ↓ compared with low dose (biphasic response)

Table 6.6 Echo assessment of left ventricular diastolic dysfunction

Characteristics	Abnormal relaxation	Pseudonormalization	Restrictive
Haemodynamics	<p>↑LVEDP Normal LAP Loss of passive gradient LA:LV Left ventricular filling from atrial systole</p> <p>Mitral valve (E:A reversal E↑ A↑)</p> <p>Pulmonary venous flow</p>	<p>↑LVEDP ↑LAP LA:LV passive filling gradient restored Pulmonary vein: LA gradient lost</p> <p>Normal</p> <p>Diminished S wave, deeper broader A wave</p> <p>Doppler pattern (mitral valve above pulmonary vein below)</p>	<p>↑LVEDP ↑↑↑LAP LA:LV passive filling gradient ↑↑ Pulmonary vein: LA gradient lost</p> <p>(E↑↑, sharp descent, A↑)</p> <p>Diminished S wave, deeper broader A wave</p> 
Left ventricular impairment	Mild	Slow early left ventricular relaxation	Moderate
Significance		Slow early relaxation, reduced compliance	Severe
Symptoms	Well at rest, but shortness of breath if heart rate ↑	Shortness of breath on exercise and limited exercise tolerance	Shortness of breath on minimal exertion

Pulmonary hypertension

Systolic PmA pressure, assuming there is no pulmonary valve stenosis, can be estimated from the pressure gradient between the right atrium and ventricle. This is measured from any tricuspid regurgitant jet (Bernoulli equation) summated with the estimated RAt pressure. Diastolic PmA pressure is measured by substituting the end-diastolic velocity of pulmonary regurgitation into the Bernoulli equation, again summated with the estimated RAt pressure. If the systolic PmA pressure is raised but the diastolic pressure is normal, this represents ↑ flow volume, rather than pulmonary hypertension. RAt pressure is assessed by IVC diameter evaluation and its calibre reduction with inspiration (see Table 6.7).

Valvular heart disease

Echocardiography can identify and quantify valve abnormalities to decide whether long-term follow-up or referral for valve surgery is necessary.

Valves may be

- Stenosed (narrowed).
- Regurgitant (leaky).
- Infected (endocarditis).
- Affected by other cardiac pathological processes, e.g. cardiomyopathy, carcinoid.

Aortic stenosis

Aortic stenosis leads to left ventricular hypertrophy, ↑ left ventricular pressures, and, if untreated, LVF and risk of sudden death (see Table 6.8).

Table 6.7 Estimation of right atrial pressure

IVC size (cm)	IVC change with inspiration	Estimated RAt pressure (mmHg)
<1.5	Collapse	0–5
1.5–2.5	↓ >50%	5–10
1.5–2.5	↓ <50%	10–15
>2.5	↓ <50%	15–20
2.5	No change	>20

Table 6.8 Parameters of aortic stenosis severity

	Mild	Moderate	Severe
Mean gradient (mmHg)	<25	25–40	>40
Peak gradient (mmHg)	<36	36–64	>64
Effective orifice area (cm ²)	1.5–2.0	1.0–1.4	<1.0

- **Aetiology:** congenital abnormality (bicuspid, unicuspido, or quadricuspid valve), calcific degenerative disease, rheumatic heart disease.
- **Differential diagnosis:** hypertrophic obstructive cardiomyopathy, subaortic membrane, supra-aortic membrane.
- **2D/3D findings:** thickened, calcified, and/or fused aortic cusps with reduced excursion, left ventricular outflow tract dimension (to calculate the aortic valve area), effects on the LV (hypertrophy/impaired systolic/diastolic function), post-stenotic dilatation of the ascending aorta/aortic coarctation.
- **Colour Doppler findings:** turbulent bright colour is seen through the valve.
- **CW/PW Doppler findings:** peak and mean valvular gradients; aortic valve area; the dimensionless severity index (DSI) is a useful parameter, particularly where left ventricular function is impaired.

Aortic regurgitation

Aortic regurgitation leads to mild left ventricular hypertrophy, left ventricular dilatation, and LVF.

- **Aetiology:** congenital abnormality (bicuspid, unicuspido, or quadricuspid valve), rheumatic heart disease, aortic leaflet prolapse, calcific or idiopathic degeneration, subaortic VSD, infective endocarditis (presence of vegetations), aortic dissection (aortic root dissection flap), ascending aortic dilatation (hypertension, aortic stenosis, age), aortitis (syphilis, ankylosing spondylitis, GCA, RhA, Reiter's syndrome), degenerative disease, including collagen disease, e.g. Marfan's syndrome.
- **2D/3D findings:** aortic valve anatomy (including calcification, prolapse, vegetations, presence of subaortic VSD), size of aortic root/aortic dissection flap/aortic coarctation, effects on the LV (hypertrophy/impaired systolic/diastolic function).
- **Colour Doppler findings:** a diastolic regurgitant jet is seen. The width of the jet (colour M-mode) comparative with left ventricular outflow tract diameter and its extent into the left ventricular cavity is measured.
- **CW/PW Doppler findings:** the density of the diastolic signal is assessed, together with the pressure half-time. Diastolic flow reversal in the aortic arch or descending aorta is indicative of significant aortic regurgitation, unless the left ventricular diastolic pressure is high from a separate aetiology, e.g. MI. It can be difficult to differentiate between mild and moderate aortic regurgitation, especially with transthoracic imaging (see Table 6.9).

Table 6.9 Parameters of aortic regurgitation severity

	Mild	Moderate	Severe
Jet width (as % of left ventricular outflow tract)	<25		65
Pressure half-time (ms)	>500		<250
Vena contracta width (cm)	<0.3		>0.6
Diastolic flow reversal	None	Aortic arch	Descending aorta

Mitral stenosis

Mitral stenosis causes LAt dilatation, ↑ pulmonary venous pressures, pulmonary oedema, pulmonary hypertension, right heart failure, and functional tricuspid regurgitation. TOE should be used to assess the suitability of the patient for percutaneous mitral valvuloplasty (see Table 6.10).

- **Aetiology:** rheumatic heart disease/calcification (valve leaflets, chordae, or papillary apparatus), congenital abnormality (very rare), SLE (rare).
- **Differential diagnosis:** LAt myxoma, obstruction of the valve by thrombus or vegetations.
- **2D/3D findings:** anatomy and degree of any calcification/fusion of the mitral valve leaflets and apparatus for suitability for valvuloplasty, effective orifice area (evaluated with planimetry), LAt size (dilated), presence of LAt thrombus, right heart size (hypertrophy) and function.
- **Colour Doppler findings:** turbulent flow is seen through the valve.
- **CW/PW Doppler findings:** peak and mean valvular gradients are calculated, the pressure half-time is measured, and the effective orifice area calculated. Pulmonary hypertension is estimated.

Mitral regurgitation

Mitral regurgitation causes LAt dilatation, ↑ pulmonary venous pressures, pulmonary oedema, LVF, pulmonary hypertension, right heart failure, and functional tricuspid regurgitation. TOE gives an optimal assessment of severity (see Table 6.11).

Table 6.10 Parameters of mitral stenosis severity

	Mild	Moderate	Severe
Mean gradient (mmHg)	<5	5–10	>10
Pressure half-time (ms)	71–139	140–219	>219
Mitral orifice area (cm ²)	1.6–2.0	1.0–1.5	<1.0

Table 6.11 Parameters of mitral regurgitation severity

	Mild	Moderate	Severe
Jet area (cm ²)	<4		>10
Signal density on CW Doppler	+	++	+++
Vena contracta width (cm)	<0.3		0.7
Pulmonary venous flow	Normal	Absent systolic component	Reversed systolic component

- **Aetiology:** rheumatic heart disease, mitral valve prolapse/redundant tissue, IHD (papillary muscle rupture/infarction/restriction of posterior leaflet), dilated/ischaemic cardiomyopathy (annular dilatation), hypertrophic obstructive cardiomyopathy (systolic anterior motion), infective endocarditis (presence of vegetations), congenital abnormality (very rare; note the association of cleft mitral valve and primum atrial septal defect), SLE (rare).
- **2D/3D findings:** mitral valve anatomy (calcification, prolapse, redundant tissue, leaflet excursion, vegetations, annular size, apparatus integrity), left ventricular size and function (hypertrophy, dilatation), LAt size (dilatation), right heart function (hypertrophy, dilatation), and PmA pressures.
- **Colour Doppler findings:** an abnormal systolic jet is seen through the mitral valve into the left atrium. The size of this is assessed. This can be deceptive with very eccentric jets associated with mitral valve prolapse.
- **CW/PW Doppler findings:** signal density and shape are characterized. Analysis of pulmonary venous flow with PW Doppler can be helpful.

Tricuspid stenosis

Tricuspid stenosis causes RAt dilatation and ↑ systemic venous pressures.

- **Aetiology:** rheumatic heart disease (occurs in 10% of patients with mitral stenosis), carcinoid disease, endomyocardial fibrosis, SLE.
- **2D/3D findings:** tricuspid valve anatomy and degree of any doming, calcification, fusion of tricuspid valve leaflets and apparatus, size of the right atrium, and IVC (dilated).
- **Colour Doppler findings:** turbulent flow is seen through the valve.
- **CW/PW Doppler findings:** peak and mean valvular gradients are calculated, the pressure half-time is estimated, and the effective orifice area calculated. Pulmonary hypertension is calculated from the velocity of any tricuspid regurgitant jet. Peak E wave velocity is elevated (normal peak is <0.7ms⁻¹) and there is a slow deceleration time. A mean gradient of 2–3mmHg may be clinically significant.

Tricuspid regurgitation

Tricuspid regurgitation leads to RAt dilatation, right ventricular hypertrophy, and failure (see Table 6.12).

Table 6.12 Parameters of tricuspid regurgitation severity

	Mild	Moderate	Severe
Jet area (cm ²)	<5	5–10	>10
Vena contracta width (cm)		<0.7	>0.7
Signal density on CW Doppler	+	++	+++
Shape of CW Doppler signal	Pansystolic	Pansystolic	Triangular
Hepatic venous flow reversal	None	Absent systolic component	Pansystolic flow reversal

- **Aetiology:** rheumatic heart disease, infective endocarditis (IV drug abuse), functional (2° to right ventricular dilatation, e.g. cardiac left-to-right shunts, right ventricular cardiomyopathy, pulmonary hypertension, permanent pacing system), carcinoid disease, Ebstein's anomaly, SLE, myxomatous degeneration, cardiac amyloidosis.
- **2D/3D findings:** tricuspid valve anatomy (site, calcification, prolapse, redundant tissue, leaflet excursion, vegetations, annular size, apparatus integrity), right ventricular size (dilated) and function, size of the right atrium, IVC (dilated), presence of pacing wires.
- **Colour Doppler findings:** abnormal systolic jet is seen through the tricuspid valve into the right atrium. The size of this is assessed, compared with the right atrium.
- **CW/PW Doppler findings:** the density of the signal and shape is assessed. Analysis of hepatic flow with PW Doppler can be helpful. Pulmonary arterial pressure can be measured but may be underestimated if there is severe tricuspid regurgitation.

Pulmonary stenosis

Pulmonary stenosis causes right ventricular hypertrophy and, if left untreated, right heart failure (see Table 6.13).

- **Aetiology:** congenital, rheumatic heart disease, carcinoid.
- **Differential diagnosis:** right ventricular outflow obstruction (infundibular stenosis).
- **2D/3D findings:** pulmonary valve anatomy (calcification, leaflet thickening, doming), size of the pulmonary trunk (post-stenotic dilatation), effects on the RV (hypertrophy/impaired function).
- **Colour Doppler findings:** turbulent systolic flow through the pulmonary valve.
- **CW/PW Doppler findings:** The maximum gradient and pulmonary valve area are calculated.

Pulmonary regurgitation

- **Aetiology:** congenital, infective endocarditis (IV drug abuse), pulmonary hypertension, PmA dilatation, carcinoid disease, post-pulmonary valvotomy.
- **2D/3D findings:** pulmonary valve anatomy (calcification, prolapse, vegetations), size of the pulmonary trunk, effects on the RV (hypertrophy/impaired function).
- **Colour Doppler findings:** a diastolic regurgitant jet is seen. The width of the jet and its extent into the right ventricular outflow tract and cavity are measured.

Table 6.13 Parameters of pulmonary stenosis severity

	Mild	Moderate	Severe
Peak gradient (mmHg)	<40	40–75	>75

- CW/PW Doppler findings: the density of the diastolic signal and its duration are estimated. Pulmonary regurgitation is haemodynamically significant if the jet is broad relative to the width of the PmA, extends >2cm into the right ventricular outflow tract, and persists throughout diastole.

Prosthetic heart valves

Echocardiography is used for follow-up of prosthetic heart valves and potential dysfunction. It is important to consult tables of normal flow pattern values for each valve type according to its make and size. TOE is superior to TTE. The following parameters are assessed:

- Direct 2D/3D imaging of the valve ring and leaflets.
- Presence of any rocking motion suggestive of valvular dehiscence.
- Forward blood flow through the valve.
- Valvular regurgitation (typical regurgitation on valve closure is normal).
- Infective endocarditis.
- Thrombus/pannus.

Infective endocarditis

The following features are characteristic of infective endocarditis:

- Predisposing abnormal structure (valve lesion/congenital abnormality).
- Regurgitant lesion.
- Mobile echogenic masses (vegetations, usually in the path of regurgitant jets).
- Spread of infection to other valves (usually along the path of a regurgitant jet).
- Abscess (particularly around the aortic root, prosthetic valves).
- Embolic potential (large, mobile vegetations, especially the aortic valve).
- Valve destruction (degree of regurgitation and effect on cardiac chamber size and function).
- Chamber perforation and shunting.
- Prosthetic valve dehiscence.

Pericardial disease

The normal pericardium is poorly visualized with echocardiography, since it is a very thin structure. Pericardial abnormalities that may be identified are:

- Pericardial effusion (fluid between the pericardial layers).
- Pericardial thickening or calcification (constrictive pericarditis).
- Pericardial masses, e.g. cysts or tumour.

Pericardial effusion

A pericardial effusion gives rise to echo-free space around the heart. The anatomical relationship of pericardial fluid with the descending aorta distinguishes pericardial (anterior) and pleural (posterior) effusions. If the effusion is organizing or contains a thrombus or tumour, then echodense structures may be identified within it. The width of this space is measured to give a rough guide as to the size of the effusion:

- Small: <1cm
- Moderate: 1–2cm.
- Large: >2cm.

The most important assessment is whether fluid is causing any haemodynamic compromise, i.e. cardiac tamponade. This may occur regardless of the size of the effusion, particularly if it has accumulated rapidly. Features suggestive of a tamponade include:

- Dilatation of the IVC ($>2.5\text{cm}$), poor collapse with inspiration ($<50\%$).
- Exaggerated reduction in transmural velocities on inspiration ($>40\%$).
- Right ventricular diastolic collapse.
- Low-volume, poorly filled LV.

Constrictive pericarditis

This is typically caused by TB and constrains left and right ventricular filling. On 2D imaging, the pericardium is thickened and appears bright when calcification is present. The period of left ventricular diastolic function is shortened. Systolic function is normal. Doppler shows:

- Exaggerated reduction of transmural E wave velocity with inspiration.
- Shortened transmural E wave deceleration time.
- Exaggerated flow reversal in the superior vena cava (SVC) on expiration.

Cardiac masses

The commonest intra-cardiac masses are thrombi and vegetations (infective endocarditis). Thrombus formation is ↑ in conditions causing slow blood flow such as mitral or tricuspid valve stenosis or cardiomyopathy. The commonest cardiac tumours are atrial myxomas and metastatic deposits. An atrial myxoma is typically a pedunculated, frond-like mass that arises from the interatrial septum. Metastatic deposits can be found anywhere within the heart, including the pericardium. Extrinsic masses may cause compression of cardiac structures. Atrial pectinate muscles, Eustachian valve, Chiari network, papillary muscles, fibrin strands, sutures on prosthetic valves, large vascular structures, such as the aorta, coronary sinus, or left ventricular aneurysms, may be incorrectly interpreted as cardiac masses.

- *Atrial masses*: thrombus, myxoma, lipomatous hypertrophy of the interatrial septum, ruptured mitral valve apparatus, 1° benign tumour, 1° malignant tumour, 2° tumour.
- *Ventricular masses*: thrombus, ruptured mitral valve apparatus, 1° benign tumour (fibroma, lipoma, rhabdomyoma, haemangioma), 1° malignant tumour (rhabdomyosarcoma, fibrosarcoma, angiosarcoma), 2° tumour.
- *Valvular masses*: vegetations, thrombus/pannus, fibroelastoma.

Aortic disease

The aorta is a predominantly posterior structure, and so the arch and descending aorta are best visualized by TOE.

- *2D findings*: integrity of the walls is assessed for atheroma (irregular wall thickening), intramural haematoma, or dissection flap. The latter may be indirectly suspected from the presence of pericardial effusion or aortic regurgitation. Its anatomy and extent are noted to allow classification. Presence of regional left ventricular wall motion abnormalities may indicate coronary artery involvement. The size of the aorta is measured at several sites to assess dilatation or coarctation (see Table 6.14). The normal aortic wall thickness is 4mm.

Table 6.14 Normal aortic dimensions

Site	Normal range (mm)
Annulus	20–31
Sinus of Valsalva	29–45
Sinotubular junction	22–36
Tubular ascending aorta	22–36
Descending	20–30

- Doppler findings: colour, CW, and PW Doppler can all be used to distinguish between true (normal systolic velocity flow) and false (low or absent systolic flow) lumens in aortic dissection, to identify and determine the severity of aortic regurgitation and aortic coarctation.

Congenital heart disease

It is important to recognize situs and connections of cardiac chambers and great vessels.

- The RV is recognized by the following features:
 - It is associated with the tricuspid valve.
 - The tricuspid valve is sited more towards the apex than the mitral valve.
 - The tricuspid and pulmonary valves are not continuous (compare the aortic and mitral valves).
 - It is trabeculated and contains a moderator band.
- The ventricles should be connected to the correct outflow tract:
 - The aorta is a single vessel.
 - The PmA bifurcates soon after its origin, unless hypoplasia/atresia is present.
- The atria should be connected to the correct inflow:
 - The pulmonary veins normally drain into the left atrium. Drainage into alternative structures, e.g. SVC or IVC, hepatic veins, is referred to as anomalous pulmonary venous drainage and is associated with a left-to-right shunt.

There should be no shunts between systemic and pulmonary systems; such shunts include atrial septal defect (abnormal connection between the left and right atria; see Table 6.15), VSD (abnormal connection between the LV and RV; see Table 6.16), or patent ductus arteriosus (abnormal connection between the aorta and PmA).

If shunts are identified, they are quantified. Pulmonary artery pressure is assessed, together with evidence of shunt reversal (right to left), indicating Eisenmenger's syndrome. Fallot's tetralogy is a relatively common congenital condition, composed of a subaortic VSD, an overriding aorta, right ventricular outflow tract or pulmonary valve stenosis, and right ventricular hypertrophy.

Table 6.15 Types of atrial septal defect

Atrial septal defects	Site	Associations
Ostium secundum	Fossa ovalis	Mitral valve prolapse
Ostium primum	Low septum	AV valve abnormalities
Sinus venosus	Upper septum	Anomalous pulmonary venous drainage

Table 6.16 Types of ventricular septal defect

VSD	Site	Associations
Membranous	Infundibular septum	
Subaortic	Below aortic valve	Aortic regurgitation Fallot's tetralogy
Muscular	Muscular septum	
AV	Posterior septum near AV valves	AV valve abnormalities

Valves and outflow tracts should be morphologically normal

- **Aortic valve:** may be unicuspid, bicuspid, quadricuspid, or associated with a subaortic or supra-aortic membrane.
- **Pulmonary valve/right ventricular outflow tract:** may be obstructed.
- **Mitral valve:** cleft leaflet associated with primum atrial septal defect, mitral stenosis, parachute valve.
- **Tricuspid valve:** ventricularization of site = Ebstein's anomaly.
- **Aortic and pulmonary arteries:** should be normal situs, size, and anatomy, and unobstructed:
 - Left-sided aortic arch, aortic coarctation.
 - Pulmonary hypoplasia, atresia, affecting either of the PmAs after trunk bifurcation.

Further reading

British Society of Echocardiography. <http://www.bsecho.org>.

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Leeson P, Augustine D, Mitchell ARJ, Becher H. *Echocardiography*, 2nd edn. Oxford: Oxford University Press, 2012.

Otto CM. *Textbook of Clinical Echocardiography*, 5th edn. Philadelphia: Elsevier Saunders, 2013.

Electrocardiogram

Principle

The ECG records the heart's electrical activity. It can provide valuable information about not just arrhythmias, but also a host of other disorders that affect the electrical activity of the myocardium such as ischaemia, cardiomyopathy, and electrolyte disturbances.

Indications

- Investigation of suspected arrhythmias, both to 'capture' the cardiac rhythm, whilst the patient is experiencing symptoms, and also to look for predisposing abnormalities, e.g. short PR interval, ventricular pre-excitation (delta waves), conduction abnormalities, long QT interval.
- Investigation of chest pain, e.g. myocardial ischaemia or infarction, pericarditis, PE.
- Assessment of suspected cardiomyopathy and/or LVF (a normal ECG is unusual in the presence of left ventricular systolic dysfunction).
- Assessment of electrolyte disturbances, particularly where these might have pro-arrhythmic potential, e.g. hyperkalaemia.
- Assessment of drug effects on the heart, e.g. digoxin, tricyclic antidepressants.

Contraindications

None. However, always check if the patient has a known allergy to the self-adhesive pads used to attach the electrodes to the skin.

Patient preparation

- Explain what the procedure involves.
- Ask the patient to lie supine on a bed or examination couch.
- Prepare the skin by shaving, where necessary, and cleaning with alcohol wipes.
- Ask the patient to relax and lie still, whilst the recording is in progress.

Procedure

- Having prepared the patient for the test, attach the chest and limb electrodes in the appropriate positions.
- Before recording the ECG, check that the calibration settings of the ECG machine are appropriate. Standard settings are an amplitude of 10mm/1mV and a paper speed of 25mm/s. Ensure that these settings are noted on the ECG.
- After recording the ECG, ensure that the patient's identification details and the time and date of the recording are noted on it.
- It is good practice to make a record on the ECG of any symptoms (e.g. chest pain, palpitations) that the patient was experiencing at the time of the recording or to write 'asymptomatic' where appropriate.
- Ensure that the ECG is seen and reported by an appropriate staff member as soon as practicable.

Reporting the findings

Always use a systematic approach to ECG reporting to ensure nothing is overlooked.

Report the ECG in the following order

- **Heart rate:** bradycardia vs tachycardia.
- **Rhythm:** regular vs irregular, supraventricular vs ventricular, broad complex vs narrow complex.
- **QRS axis:** left or right axis deviation.
- **P wave:** presence or absence, inverted, tall (peaked), or wide (bifid).
- **P–R interval:** long, short, or variable.
- **Q waves:** are pathological Q waves present?
- **QRS complex:** large, small, broad, or abnormally shaped.
- **ST-segment:** elevated or depressed.
- **T wave:** tall, small, or inverted.
- **QT interval:** short or long.
- **U wave:** are prominent U waves present?
- **Additional waves:** are delta waves or J waves present?

Possible results

Heart rate

Heart rate can be calculated in one of two ways

- By counting the number of large squares between two successive QRS complexes and dividing this number into 300, e.g. four large squares = $300/4 = 75\text{bpm}$. This method is preferred when the heart rhythm is regular.
- By counting the number of QRS complexes along a 25cm rhythm strip (50 large squares) and multiplying this number by 6, e.g. 14 complexes in 25cm = $14 \times 6 = 84\text{bpm}$. This method is preferred when the heart rhythm is irregular.

Bradycardia is arbitrarily defined as a heart rate <60bpm, and *tachycardia* as a heart rate >100bpm.

If the patient is bradycardic, consider

- Sinus bradycardia.
- Sick sinus syndrome.
- Second- and third-degree AV block.
- Escape rhythms, e.g. AV junctional escape rhythms, ventricular escape rhythms, asystole, and drug-induced conditions.

If the patient is tachycardic, consider

- **Narrow complex tachycardia:** e.g. sinus tachycardia, atrial tachycardia, atrial flutter, AF, AV re-entry tachycardias.
- **Broad complex tachycardia:** e.g. narrow complex tachycardia with aberrant conduction, ventricular tachycardia, accelerated idioventricular rhythm, torsades de pointes.

Cardiac rhythm

To identify the cardiac rhythm, ask the following questions

- How is the patient?
- Is ventricular activity (QRS complexes) present?
- What is the ventricular rate?
- Is the ventricular rhythm regular or irregular?
- Are the QRS complexes narrow or broad?
- Are there P waves (atrial activity)?
- What is the correlation between P waves and QRS complexes?

Being able to describe the cardiac rhythm in these terms will narrow down the range of possible diagnoses in most cases and you will, at least, be able to describe the key features of the rhythm clearly over the telephone to an expert.

Rhythms to consider include

- *Sinoatrial nodal rhythms:*
 - Sinus rhythm.
 - Sinus bradycardia.
 - Sinus tachycardia.
 - Sinus arrhythmia.
 - Sick sinus syndrome.
- *Atrial rhythms:*
 - Atrial tachycardia.
 - Atrial flutter (see Fig. 6.17).
 - AF (see Fig. 6.18).
 - AV junctional rhythms.
 - AV re-entry tachycardias (see Fig. 6.19).
- *Ventricular rhythms:*
 - Accelerated idioventricular rhythm.
 - Ventricular tachycardia (see Fig. 6.20).
 - Polymorphic ventricular tachycardia (torsades de pointes; see Fig. 6.21).
 - Ventricular fibrillation (see Fig. 6.22).



Fig. 6.17 Atrial flutter with 4:1 AV block.

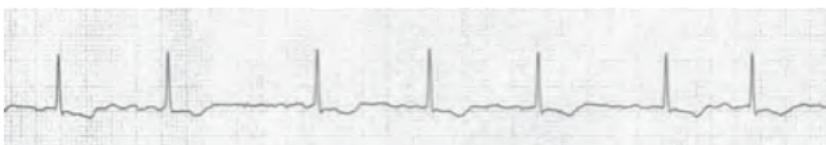


Fig. 6.18 Atrial fibrillation.



Fig. 6.19 AV re-entry tachycardia.

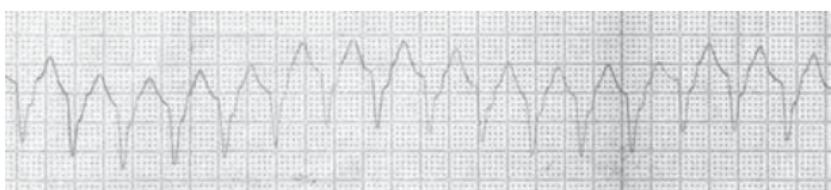


Fig. 6.20 Ventricular tachycardia.

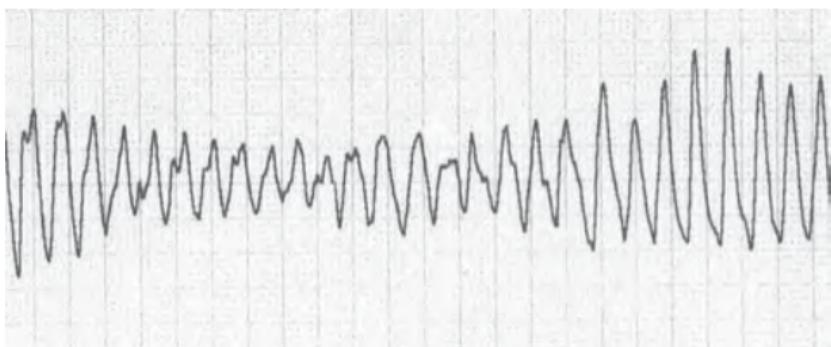


Fig. 6.21 Polymorphic ventricular tachycardia.

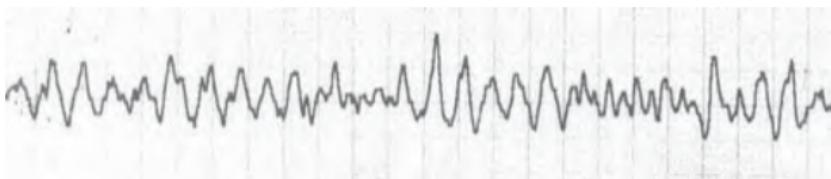


Fig. 6.22 Ventricular fibrillation.

- Conduction disturbances.
- Escape rhythms.
- Ectopic beats.

QRS axis

- *Left axis deviation*: left anterior hemiblock, Wolff–Parkinson–White syndrome, inferior MI, ventricular tachycardia (with left ventricular apical focus).
- *Right axis deviation*: left posterior hemiblock, right ventricular hypertrophy, Wolff–Parkinson–White syndrome, anterolateral MI, dextrocardia.

P wave

- *P waves absent*: AF, sinus arrest or sinoatrial block (persistent or intermittent), hyperkalaemia.
- *P waves inverted*: dextrocardia, retrograde atrial depolarization, electrode misplacement.
- *Tall or peaked P waves*: RAt enlargement.
- *Wide, often bifid P waves*: LAt enlargement.

PR interval

- *Short PR interval (<0.12s)*: AV junctional rhythm, Wolff–Parkinson–White syndrome (see Fig. 6.23).
- *Long PR interval (>0.2s)*: first-degree AV block (see Fig. 6.24), e.g. IHD, hypokalaemia, acute rheumatic myocarditis, Lyme disease, digoxin, β -blockers, certain calcium channel blockers.
- *Variable PR interval*: second-degree AV block (Mobitz type I (see Fig. 6.25), Mobitz type II, 2:1 AV block), third-degree AV block (see Fig. 6.26).

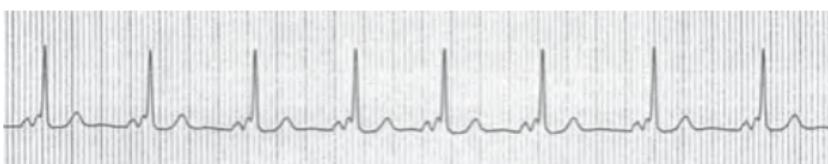


Fig. 6.23 Wolff–Parkinson–White syndrome.



Fig. 6.24 First-degree AV block.



Fig. 6.25 Second-degree AV block (Mobitz type I).



Fig. 6.26 Third-degree AV block.

Q waves

- *Pathological Q waves*: MI, left ventricular hypertrophy, bundle branch block.

QRS complex

- *Large R or S waves*: incorrect ECG calibration, left ventricular hypertrophy, right ventricular hypertrophy, posterior MI, Wolff–Parkinson–White syndrome (left-sided accessory pathway), dextrocardia, bundle branch block.
- *Small QRS complexes*: incorrect ECG calibration, obesity, emphysema, pericardial effusion.
- *Broad QRS complexes (>0.12s)*: bundle branch block, ventricular rhythms, hyperkalaemia.
- *Abnormally shaped QRS complexes*: incomplete bundle branch block, fascicular block, Wolff–Parkinson–White syndrome.

ST-segment

- *Elevated ST-segments*: acute STEMI (see Fig. 6.27), left ventricular aneurysm, Prinzmetal's (vasospastic) angina, pericarditis ('saddle-shaped' appearance), high take-off.
- *Depressed ST-segments*: myocardial ischaemia, acute posterior MI, drugs, e.g. digoxin (reverse tick), ventricular hypertrophy with 'strain'.

T wave

- *Tall T waves*: hyperkalaemia, acute MI.
- *Small T waves*: hypokalaemia, pericardial effusion, hypothyroidism.
- *Inverted T waves*: normal (aVR, V1, sometimes V2–V3 and III), myocardial ischaemia, MI, ventricular hypertrophy with 'strain', digoxin toxicity.

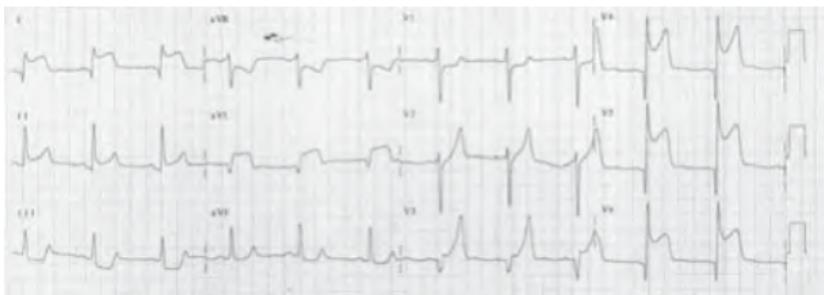


Fig. 6.27 Anterolateral STEMl.

QT interval

- *Short QT interval:* hypercalcaemia, digoxin effect, hyperthermia.
- *Long QT interval:* hypocalcaemia, drug effects, acute myocarditis, hereditary syndromes (Jervell and Lange–Nielsen syndrome, Romano–Ward syndrome).

U wave

- *Prominent U waves:* hypokalaemia, hypercalcaemia, hyperthyroidism.

J wave

- *If present:* hypothermia.

Pitfalls

- A common error is to interpret an ECG in isolation. To avoid this error, always consider the clinical context in which it was recorded. Begin your assessment of the ECG by asking, ‘How is the patient?’ before rushing to conclusions about the clinical relevance of any abnormalities that may be present.
- A normal ECG does not necessarily exclude a significant cardiac problem, particularly when recorded whilst the patient is asymptomatic. This is particularly the case when investigating palpitations and chest pain.
- Technical artefacts are often mistaken for significant abnormalities. Ensure adequate patient preparation and correct electrode placement to minimize the risk of artefact.

Further reading

Houghton AR, Gray D. *Making Sense of the ECG*, 4th edn. Boca Raton: CRC Press, 2014.

Electrocardiographic monitoring

Principle

ECG monitoring allows continuous observation of a patient's ECG in an ambulatory setting over an extended period of time. This is typically from 24h all the way up to a year or more, depending upon the technique used. There are three types of device available for ambulatory ECG monitoring:

- 24h ambulatory ECG (Holter) monitor.
- External loop recorder.
- Insertable cardiac monitor (e.g. Medtronic Reveal LINQ™).

The choice of device is largely determined by how frequently the patient experiences symptoms, as the key to successful ECG monitoring is to maximize the chances of capturing a typical symptomatic event during the monitoring period. A Holter monitor is typically worn for 24–48h but becomes somewhat impractical over longer periods. It is therefore ideally suited to patients with frequent (daily) symptoms. An external loop recorder (often called an event monitor) is carried for around 7 days and is therefore used for patients with less frequent symptoms. Instead of recording a continuous ECG, they capture brief periods of the ECG, usually when activated by the patient. Transtelephonic monitors allow captured loops to be relayed to a cardiac centre by telephone, allowing an immediate analysis of the recorded ECG.

An insertable cardiac monitor (ICM) is used to detect infrequent events. It is used to monitor a patient's cardiac rhythm over an extended period (up to its battery life, usually around 3 years). An ICM is implanted SC and contains a battery, a digital memory, and diagnostic software to analyse the ECG recording. It records the ECG on a digital 'loop', continuously overwriting older ECG data with the most current data. Should a symptomatic event occur, the patient can 'freeze' the loop using an external hand-held device that is held over the ICM ('patient-activated' recordings). Additionally, the device can be programmed to detect and store abnormal cardiac rhythms automatically ('auto-activated' recordings). The device will usually store the ECG leading up to an event and also a short segment of ECG following the event. Stored ECG loops can subsequently be downloaded for further analysis via a telemetry device at the hospital clinic or via a wireless device in the patient's own home.

Indications

Where patients have symptoms suggestive of a paroxysmal arrhythmia (palpitation and/or dizziness/syncope), ECG monitoring can provide a diagnosis by capturing the cardiac rhythm during a typical event. This allows the underlying arrhythmia to be identified or, where the rhythm proves to be normal, an arrhythmic aetiology to be ruled out. The choice of method depends upon the frequency of the symptoms.

Contraindications

None. However, always check if the patient has a known allergy to the self-adhesive pads used to attach the electrodes to the skin.

Patient preparation

For external monitoring, no specific preparation is required, apart from ensuring that the electrodes make good contact with the patient's skin, so that an ECG of diagnostic quality can be recorded. For the implantation of an ICM:

- Explain what the procedure involves.
- Obtain written informed consent.
- Check the FBC (and clotting profile if bleeding risk).
- LAn.
- Sedation if the patient is anxious.

The best site for the device is established by optimal ECG signal measurement prior to insertion.

Procedure

External monitors are attached to the patient's skin via electrodes, ensuring good contact is maintained. The recorder itself is carried on a belt or in a pouch. The patient should be given a diary with clear instructions on how to operate the monitor and how to note the timing and nature of any symptoms that occur. Some event monitors are carried by the patient and only applied to the skin during symptomatic episodes. The patient should be given clear instructions on how to operate such a monitor and a 'test run' should be conducted.

An ICM is implanted SC under aseptic technique and using LAn. The implantation takes around 15min and can be done as a day case procedure. The device is self-contained since, unlike a pacemaker, there are no associated leads. The ICM is commonly implanted near the left deltopectoral groove or below the left breast. Once implanted, the device is interrogated using an external programmer to ensure that a high-quality ECG is being recorded. The incision is closed using surgical glue or butterfly closures.

Risks

External monitoring carries no significant risks. Implantation of an ICM carries a risk of:

- Infection.
- Erosion through the skin (if the patient is thin).

Possible results

Depending upon the underlying cause of the patient's symptoms, almost any cardiac rhythm disturbance (or indeed no rhythm disturbance whatsoever) may be revealed by ECG monitoring. The most important aspect of interpreting the results is to correlate recordings with symptoms. Patient-activated recordings are, as one might expect, usually made in relation to a symptomatic episode. In this case, it is essential to find out precisely what symptoms were experienced at the time (including a witness account where appropriate). Auto-activated recordings may be asymptomatic and made by the device without the patient being aware of a problem. The most useful outcome is to assess the ECG recorded during a typical symptomatic event. It is then usually straightforward to make a diagnosis and plan further treatment as appropriate.

Advantages over other tests

Ambulatory ECG monitoring allows the chance of capturing paroxysmal arrhythmias, an opportunity that is unlikely to arise with a 12-lead ECG recording unless the patient happens to be symptomatic at the time. Each of the methods of ECG monitoring has advantages over the others. External monitoring is non-invasive but can only be performed for relatively short periods. An ICM allows continuous ECG monitoring over a much longer period, making it extremely useful for investigating patients with infrequent, but nonetheless troublesome, symptoms. It does, however, involve an invasive procedure (with the attendant risks) and is more expensive than other forms of ambulatory monitoring. It can, however, prove very cost-effective if it avoids the need for multiple short-term ambulatory recordings.

Pitfalls

As with any other form of ambulatory monitoring, failure to correlate symptoms with recorded events can lead to inappropriate diagnoses.

Further reading

- Crawford MH, Bernstein SJ, Deedwania PC, et al. ACC/AHA guidelines for ambulatory electrocardiography: executive summary and recommendations. *Circulation* 1999; 100: 886–93.
National Institute for Health and Care Excellence (2010). *Transient loss of consciousness ('blackouts') in over 16s*. Clinical guideline CG109. <http://www.nice.org.uk/guidance/cg109>.
Task Force for the Diagnosis and Management of Syncpe; European Society of Cardiology (ESC); European Heart Rhythm Association (EHRA); Heart Failure Association (HFA); Heart Rhythm Society (HRS), Moya A, Sutton R, Ammirati F, et al. Guidelines for the diagnosis and management of syncope (version 2009). *Eur Heart J* 2009; 30: 2631–71.

Exercise testing

Principle

Exercise testing permits the dynamic assessment of cardiac function. There are many different indications for exercise testing, but the commonest is the investigation of suspected or known CHD.

Indications

- Assessment of likelihood of CHD in patients with chest pain.
- Risk stratification of patients with known CHD and hypertrophic cardiomyopathy.
- Evaluation of response to treatment or revascularization in CHD.
- Assessment of exercise-induced arrhythmias.
- Assessment of symptoms in valvular heart disease.
- Objective assessment of exercise capacity.

Contraindications

The absolute and relative contraindications to exercise testing are listed in Box 6.1.

Box 6.1 Absolute and relative contraindications to exercise testing

Absolute contraindications

- Recent MI (within 2 days)
- Unstable angina (rest pain within previous 48h)
- Uncontrolled cardiac arrhythmias (causing symptoms or haemodynamic compromise)
- Symptomatic severe aortic stenosis
- Uncontrolled symptomatic heart failure
- Acute PE or pulmonary infarction
- Acute myocarditis or pericarditis
- Acute aortic dissection

Relative contraindications

- Left main stem coronary stenosis
- Moderate valvular stenosis
- Electrolyte abnormalities
- Uncontrolled hypertension (systolic >200mmHg, diastolic >110mmHg)
- Tachyarrhythmias or bradyarrhythmias
- Outflow obstruction, e.g. hypertrophic cardiomyopathy
- Inability to exercise adequately
- High-degree AV block

Patient preparation

Unless the exercise test is being performed to assess response to treatment, patients should be advised to discontinue anti-anginal drugs, e.g. β -blockers, calcium channel blockers, long-acting nitrates, nicorandil, and also digoxin 48h prior to the test. Patients should attend for the test wearing suitable clothing and footwear.

Procedure

The test should be carefully explained to the patient, so that they are familiar with the exercise protocol being used. A resting ECG is recorded, and the patient's BP measured. An appropriately trained team comprising at least two personnel, trained in advanced life support, should supervise the test. Full resuscitation and defibrillation facilities must be readily available. Exercise can be performed using either an exercise treadmill or an exercise bicycle. A variety of protocols are available, of which the commonest are the Bruce protocol and the modified Bruce protocol (see Table 6.17). The workload during exercise normally \uparrow at 3min intervals, with the BP and ECG being recorded at each stage. The patient should be asked to report any symptoms during the test.

Table 6.17 Modified Bruce and Bruce protocols

Protocol	Modified Bruce			Standard Bruce				
	01	02	03	1	2	3	4	5
Stage	01	02	03	1	2	3	4	5
Speed (kph)	2.7	2.7	2.7	2.7	4.0	5.5	6.8	8.0
Slope (°)	0	1.3	2.6	4.3	5.4	6.3	7.2	8.1

Exercise should be stopped if there is

- A fall of $>10\text{mmHg}$ in systolic BP from baseline associated with ischaemia.
- Moderate to severe angina.
- \uparrow ataxia, dizziness, or near syncope.
- Evidence of poor perfusion.
- Difficulty in monitoring the ECG or BP.
- Onset of arrhythmias (ventricular tachycardia, supraventricular tachycardia, AF, worsening ventricular ectopics).
- 1.0mm or more ST elevation (in leads without Q waves, other than V1 or aVR).
- A request from the patient to stop the test.

One should also consider stopping the exercise if there is

- A fall of $>10\text{mmHg}$ in systolic BP from baseline, even in the absence of ischaemia.
- $>3\text{mm}$ of ST-segment depression or marked axis shift.
- Development of bundle branch block that cannot be distinguished from ventricular tachycardia.

- ↑ chest pain.
- Rise in BP above 250/115.
- Fatigue, breathlessness or wheezing, leg cramps, claudication.

At the end of the exercise, the patient may be permitted to sit. Monitoring of the ECG and BP must continue until the heart rate and BP have returned to baseline and any ECG changes have resolved.

Risks

Exercise testing is generally well tolerated, with a morbidity of 2.4 in 10,000 and a mortality of 1 in 10,000 (within 1 week of testing). Risks include arrhythmias and cardiac arrest, MI, and cardiac rupture, and are more likely in those with a recent history of ACS. Facilities for resuscitation and defibrillation must be immediately available.

Possible results

Myocardial ischaemia is indicated by 1mm horizontal or downsloping ST-segment depression 80ms after the J point. Some cardiologists use 2mm of ST-segment depression as the diagnostic criterion—this ↑ the specificity of the test but reduces the sensitivity. Upsloping ST-segment depression and T wave changes are not reliable indicators of ischaemia. A fall in BP (or a failure of BP to rise) during exercise can also indicate ischaemia, particularly if accompanied by ST-segment depression and chest pain.

Generally speaking, the earlier and the more marked the ST-segment changes, the more severe the underlying coronary artery disease. The prognostic value of exercise testing is well established. Patients can be risk-stratified using the Duke treadmill score, calculated as follows:

$$\text{Duke treadmill score} = \text{Exercise time (Bruce protocol)} - [5 \times \text{ST depression (mm)}] - (4 \times \text{exercise angina index})$$

where: 0 = no exercise angina; 1 = exercise angina; 2 = exercise angina that led to termination of the test.

The Duke treadmill score defines a high-risk group with a score of ≥ -11 , with an annual cardiovascular mortality of 5%. Low-risk patients have a score of $\geq +5$, with an annual cardiovascular mortality of 0.5%.

Almost any arrhythmia or conduction disturbance can occur during exercise testing. If the exercise test is being performed to investigate arrhythmias, this can indicate a diagnostic result.

Advantages over other tests

Exercise testing is a relatively simple and inexpensive investigation, with a strong evidence base that it is useful in a large number of clinical situations. Alternative tests for myocardial ischaemia include stress echocardiography, CMR, and myocardial perfusion imaging.

Pitfalls

Exercise test results are commonly reported as 'positive' or 'negative', giving the erroneous impression that the results are 'black or white'. The sensitivity and specificity of exercise testing vary widely between different patient populations, and false -ve and false +ve results are not uncommon. If the pretest probability of CHD is low, e.g. an asymptomatic young woman, exercise testing is of little value as even a 'positive' result is unlikely to be true. Similarly, if the pretest probability of CHD is high, e.g. a ♂ in his 60s with typical anginal symptoms, a 'negative' result is also unlikely to be true. Guidelines from NICE state that exercise testing should not be used for the *de novo* diagnosis of IHD, but only for the assessment of patients with known IHD.

Further reading

National Institute for Health and Care Excellence (2016). *Chest pain of recent onset: assessment and diagnosis*. Clinical guideline CG95.  <http://www.nice.org.uk/guidance/cg95>.

Myocardial perfusion imaging

Principle

Since myocardial perfusion abnormalities occur early following the onset of ischaemia, evaluation of regional myocardial perfusion heterogeneity is a sensitive marker for the presence of coronary artery disease. Myocardial perfusion imaging is most commonly performed with radionuclide imaging. Alternative modalities include contrast echocardiography and contrast CMR imaging.

The radioisotopes thallium-201 or technetium-99m are taken up by the myocardium in proportion to blood flow. Images are then acquired by a gamma camera. The images are processed to provide colour mapping of myocardial perfusion. Information is obtained regarding the presence of reversible or fixed myocardial ischaemia. Late repetition of image acquisition allows redistribution of the isotope in areas of slow blood flow for assessment of myocardial viability.

As with all investigational methods for evaluation of ischaemia, perfusion imaging is enhanced by the addition of cardiac stress. This may be in the form of physical exercise, e.g. treadmill or bicycle, or with use of pharmacological stressors. The latter are particularly useful if the patient is physically unable to exercise sufficiently or has ECG abnormalities that prohibit accurate interpretation, e.g. left bundle branch block or ventricular pacing. The most commonly used pharmacological stressor is the vasodilator adenosine, which has a very short half-life. Dobutamine can also be used in patients with contraindications to adenosine, but it is a less effective vasodilator. Adenosine gives rise to a 4- or 5-fold hyperaemia, whereas dobutamine only has a 2-fold vasodilatory effect. During adenosine stress, there is a 4- to 5-fold ↑ in blood flow to normal myocardial territories, compared with the basal state. In the presence of coronary artery stenosis, there is impaired vasodilatation and a reduction in the stress:rest ratio, precipitating a myocardial perfusion mismatch.

Indications

- To assess the presence and degree of coronary artery stenoses in patients with suspected coronary artery disease.
- To assist in the management of patients with known coronary artery disease:
 - To determine the likely prognosis and probability of future cardiac events, e.g. following MI or during proposed non-cardiac surgery.
 - To guide proposed revascularization procedures by determining the physiological significance of known coronary artery lesions, including the effects of anomalous coronary arteries, muscle bridging, and coronary artery ectasia in Kawasaki's disease.
 - To assess the success of performed revascularization strategies.
- To differentiate between areas of myocardial scar tissue and viable myocardium prior to proposed revascularization.

Contraindications and risk

Pregnancy is a contraindication to nuclear imaging. Contraindications to physical exercise testing are listed in Box 6.1.

Contraindications to adenosine are

- Known hypersensitivity to adenosine.
- Untreated second- or third-degree heart block, sick sinus syndrome, long QT syndrome.
- Asthma, chronic obstructive airways disease with known bronchospasm.
- Hypotension (systolic BP <90mmHg).
- ACS not successfully stabilized with medical therapy.
- Decompensated heart failure.
- Concomitant use of dipyridamole (within last 24h) or xanthines (within last 12h).

Contraindications to dobutamine include those for physical exercise testing and

- Known hypersensitivity to dobutamine.
- Glaucoma.
- Hypokalaemia.
- Concomitant use of β -blockade.

Patient preparation

β -blockers and rate-limiting calcium antagonists should be withdrawn for 48h prior to the test if physical exercise or dobutamine stress is planned. Xanthines and dipyridamole should be withdrawn for 24h prior to adenosine stress, and any foods or drugs containing caffeine should be avoided. Peripheral IV access should be sited.

Procedure

The stress study is generally performed first, since if this is normal, there may be no need to acquire resting images. The radioisotope is injected at peak stress, so that myocardial uptake of the tracer reflects maximal blood flow and optimizes visualization of any perfusion deficit. The protocols for the varying forms of stress are given in Table 6.18.

Table 6.18 Radionuclear exercise and imaging protocols

Stress	Protocol	Injection time of radioisotope
Physical exercise	As directed by physician, e.g. Bruce, Sheffield, to 85% max predicted heart rate (MPHR)	1–2min prior to cessation of peak exercise
Adenosine	140 μ g/kg/min for 6min	3–4min after start of infusion
Dipyridamole*	140 μ g/kg/min for 4min	4min after infusion completion
Dobutamine	In 3min stages: 5–10, 20, 30, 40 μ g/kg/min	When 85% MPHR and/or maximal dose dobutamine

* See Pellika PA, Nagueh SF, Elhendy AA, et al. American Society of Echocardiography recommendations for performance, interpretation, and application of stress echocardiography. J Am Soc Echocardiogr 2007; 20: 1021–41.

Heart rate and BP should be measured throughout physical or pharmacological stress. A 12-lead ECG should be observed continuously for evidence of ST-segment or T wave changes suggestive of ischaemia and arrhythmias.

Redistribution imaging for assessment of myocardial viability can be performed 3–4h after stress imaging. To enhance redistribution imaging, particularly if any perfusion deficits seen with stress are severe, sublingual nitrate can be given, followed by a further resting injection of the radioisotope and image acquisition an hour later. This is known as a stress–redistribution–reinjection protocol.

A single- or dual-head gamma camera is used for image acquisition. This rotates 180° round the patient from 45° in the right anterior oblique position to 45° in the left posterior oblique position. The tomographic data are reconstructed into double oblique imaging planes. Stress and rest images are aligned carefully with accurate image registration for comparison. Image quality is assessed, and then the long and short axis images are evaluated for myocardial perfusion deficits.

Risks

It should be remembered that the patient is exposed to ionizing radiation, especially if sequential studies are planned. Physical or pharmacological stress may induce severe myocardial ischaemia, infarction, and potentially life-threatening arrhythmias (0.01–0.05%).

The test should be stopped if the patient is physically unable to complete the test or if s/he develops

- Severe angina.
- ST-segment elevation of >0.1mV in leads without Q waves.
- A fall in systolic BP >10mmHg below baseline.
- A severe hypertensive response (BP >250/115).
- Clinical loss of peripheral perfusion, i.e. pallor or cyanosis.
- Dizziness or near syncope.

Possible results

Perfusion deficits are identified as areas of reduced tracer uptake. These may be assessed qualitatively or semi-quantitatively. Semi-quantitative classification expresses regional myocardial uptake as a percentage of the maximal uptake seen, according to the following scale:

- Absent: 10–9%.
- Severely reduced: 10–29%.
- Moderately reduced: 30–49%.
- Mildly reduced: 50–69%.
- Normal: 70–100%.

Perfusion deficits may be categorized as either reversible (present on stress imaging alone) or fixed (present on stress and rest imaging). When the redistribution protocol is followed, areas of reduced perfusion can be examined for the presence of viability (revascularization will improve regional function) or scar tissue (revascularization is futile). The size of the LV and RV can also be determined (see Table 6.19).

Table 6.19 Possible results for myocardial perfusion imaging

	Stress	Rest	Clinical conclusion
Myocardial perfusion	Normal/↑	Normal	Normal
	Reduced	Normal	Myocardial ischaemia (reversible defect)
	Reduced/absent	Reduced/absent	MI (fixed defect)
Late redistribution	Reduced	↑ from baseline	Viable myocardium
	Reduced	Reduced	MI (scar)

Advantages over other tests

Radionuclide imaging is readily available and non-invasive. It is inexpensive, compared with coronary angiography. In contrast to MRI, where the number of imaging planes that can be acquired may be limited, radionuclide imaging provides full myocardial coverage. There are many studies supporting the ability of the technique to give accurate diagnostic information and prognostic data.

Pitfalls

Qualitative or semi-quantitative analytical techniques, whereby signal intensity is compared with the area of maximal myocardial uptake, may limit accuracy in the presence of triple-vessel disease where there is globally reduced myocardial perfusion. Additionally, the study may be suboptimal if peak stress is not achieved. Radionuclide imaging has poor spatial resolution in comparison with other techniques. Perfusion defects limited to the subendocardium may not be visualized. Image quality can be degraded by patient movement and artefacts. Such artefacts include attenuation from breast tissue in the anterior wall and inferior signal loss.

Further reading

- Anagnostopoulos C, Harbinson M, Kelion A, et al. (2004) Procedure guidelines for radionuclide myocardial perfusion imaging. *Heart* 2004; **90**(Suppl 1): i1–10.
- Bourque JM, Beller GA. Stress myocardial perfusion imaging for assessing prognosis: an update. *JACC Cardiovasc Imaging* 2011; **4**: 1305–19.
- Hendel RC, Berman DS, Di Carli MF, et al. ACCF/ASNC/ACR/AHA/ASE/SCCT/SCMR/SNM 2009 appropriate use criteria for cardiac radionuclide imaging. *Circulation* 2009; **119**: e561–87.

Radionuclide ventriculography

Principle

Radionuclide ventriculography (RVN) is a technique to provide accurate assessment of cardiac chamber size, morphology, and function. The patient's RBCs are radiolabelled with $^{99m}\text{technetium}$ pertechnetate *in vitro* or *in vivo*. The labelled blood pool within the cardiac chambers is then imaged with a gamma camera, gated to the ECG. Multiple image acquisitions are acquired throughout the cardiac cycle, typically over at least 16 systolic and 32 diastolic frames. These images can be assessed either based on either the radioactive count or by geometric analysis.

Indications

- Prognostic estimation in patients with heart failure or coronary artery disease.
- Estimation of operative coronary risk for non-cardiac surgery.
- Diagnosis of coronary artery disease where conventional exercise testing is inadequately performed or result equivocal.
- Evaluation of the efficacy of revascularization or medical management strategies in patients with coronary artery disease.
- Monitoring of cardiac function in patients undergoing chemotherapy.

Contraindications

The technique is contraindicated in pregnant or lactating women.

Patient preparation

No special preparation is required for a resting study. If an exercise study is to be performed, the patient should fast for 3–4h prior to the procedure. If pharmacological stress agents are used, the same preparation as for myocardial perfusion imaging should be followed. A resting ECG is helpful to exclude arrhythmias.

Procedure

For a resting study, the patient lies supine whilst anterior and left anterior oblique images are acquired. Stress studies may be performed with either physical exercise, e.g. bicycle ergometry, or pharmacological stressors, e.g. dobutamine. Images are acquired at intervals once the heart rate has stabilized at each new level of exercise or stress. The patient should have haemodynamic and ECG monitoring throughout. Cardiopulmonary resuscitation facilities should be available. Images are then analysed to obtain the required morphological and functional parameters. High activity areas, such as the spleen or aorta, may be filtered out for optimal assessment of cardiac parameters.

Risks

Technetium has a 6h half-life. Although the heart receives the largest dose, 5% of the total radiation dose is sequestered by the BM, the most radiosensitive body tissue. Radiation dose is up to 1100MBq, and so a typical examination carries a fatal cancer risk of 1 in 3300. Serial studies should be avoided where alternative forms of imaging suffice.

Possible results

- Dilatation or hypertrophy of the cardiac chambers and great vessels may be identified.
- Left and right ventricular EFs can be measured. Normal left ventricular EF is 60–80% at rest and slightly more during exercise. Right ventricular EF is 46–70%. Both values decline with age. The extent of any global left ventricular dysfunction can therefore be identified.
- Regional wall dysfunction at rest, at low- and peak-dose stress, and during recovery may be described in a manner analogous to stress echocardiography, in order to identify areas of reversible myocardial ischaemia, myocardial hibernation, or scar.

Advantages over other tests

RNV is non-invasive and repeatable and provides serial measurements, especially in patients who are difficult to scan echocardiographically or who cannot tolerate MRI. It can be used in critically ill patients soon after an acute MI.

Pitfalls

Patients receive a significant radiation dose. Echocardiography is safer, and CMR is likely to replace this technique as the gold standard. Red cell labelling may be inefficient in chronic renal failure. Technical factors are important; in particular, radioactivity in the left atrium must be separated from that in the LV to obtain an accurate EF. A poor ECG signal and inappropriate gating may render data uninterpretable; heart rate variability may compromise diastolic filling indices, and inadequate frame counts ↓ statistical reliability.

Further reading

Hendel RC, Berman DS, Di Carli MF, et al. ACCF/ASNC/ACR/AHA/ASE/SCCT/SCMR/SNM 2009 appropriate use criteria for cardiac radionuclide imaging. *Circulation* 2009; 119: e561–87.

Pulmonary artery catheterization

Principle

A PmA (Swan–Ganz) catheter is a multi-lumen catheter that is passed percutaneously from a central vein, e.g. femoral, subclavian, or jugular, to the right heart structures. It can be used to measure venous, RAt, right ventricular, PmA, and LAt (indirect) pressures to obtain blood samples for O₂ saturation estimation, to measure cardiac output and systemic vascular resistance, and additionally to act as a central venous infusion port.

Indications

- Aid in the diagnosis of cardiovascular shock and pulmonary oedema.
- Management of complicated acute MI, especially right ventricular infarction, cardiogenic shock.
- Management of patients with cardiac failure.
- Fluid therapy/inotropic delivery in severely ill patients, e.g. sepsis, burns, multi-organ failure, cardiac surgery, trauma.
- Diagnostic right heart catheterization, including congenital heart disease, pulmonary hypertension, intra-cardiac shunts.

Contraindications

- Right-sided endocarditis.
- Prosthetic tricuspid or pulmonary valve.
- Right heart tumour or thrombus.
- Unstable ventricular arrhythmia.

Patient preparation

LAn is injected into the skin at the site of venous access. The patient is positioned flat on a couch, generally with a head-down orientation if cephalad access is to be used. Pressure transducers are made ready and zeroed for accurate measurements. Fluoroscopic screening should be available, if required.

Procedure

An access sheath is placed in the vein using a Seldinger technique. The PmA triple-lumen catheter is flushed with saline, and the integrity of the flotation balloon assessed by inflation with air. Under fluoroscopic guidance or by observation of intra-cardiac pressure traces, the catheter is passed through the venous system towards the right heart and into a branch of the PmA. At each stage, pressure and O₂ samples can be measured. The balloon can be inflated to assist passage through the right heart. The balloon is wedged briefly into a PmA branch to obtain an assessment of indirect pressure (PmA wedge pressure). Cardiac output can be calculated using a thermodilution method. Iced saline at a known temperature is injected through the proximal lumen and a thermistor at the catheter tip measures the temperature rise in the blood-warmed saline as it passes through the tricuspid valve, RV, and pulmonary valve. Systemic peripheral resistance can also be estimated.

Risks

- Arterial puncture, pneumothorax, haemothorax.
- Sepsis.
- PE or infarction (if the right heart contains masses or if the balloon remains inflated in wedge pressure position).
- PmA rupture (balloon over-inflation).
- Arrhythmia.

Possible results

PmA catheterization can be used to assess pulmonary and systemic venous filling pressures and fluid status, right and left cardiac function, and also, where indicated, to provide information on valve dysfunction, intra-cardiac shunts, tamponade, and pulmonary hypertension.

Advantages over other tests

This technique has traditionally been a useful adjunct to patient monitoring in the intensive care setting, in particular for accurate pressure evaluation of the right heart and left atrium and for continuous cardiac output assessment. However, recently several less invasive devices have been designed for cardiac output monitoring, e.g. oesophageal Doppler.

Pitfalls

The procedure is generally well tolerated, but it is an invasive procedure not without risk. It is essential that the PmA catheter is inserted only by suitably trained individuals to assist diagnosis and monitor treatment in carefully selected patients. If a non-invasive alternative is available, then this should be preferentially employed. Care must be taken in data interpretation, as misleading results may be obtained if the system is not systematically and accurately zeroed for serial measurements. Indirect LAt pressure measurements may be inaccurate in patients with pulmonary disease.

Further reading

Kelly CR, Rabbani LE. Pulmonary-artery catheterization. *N Engl J Med* 2013; **369**: e35.

Tilt table testing

Principle

On standing, gravity redistributes up to 800mL of blood to the legs. The normal compensatory response is ↑ sympathetic and ↓ parasympathetic stimulation, which maintains BP with a small ↑ in heart rate. Head-up tilt table testing uses gravity-induced venous pooling to assess autonomic control and to attempt to reproduce symptoms of autonomic dysfunction of dizziness or collapse, i.e. neuro-cardiogenic (vasovagal) syncope.

Indications

Testing is appropriate in the investigation of sudden, unpredictable loss of consciousness thought to be neurally mediated (vasovagal syncope, carotid sinus syncope, or situational syncope) in the absence of structural heart disease.

Contraindications

- Severe mitral stenosis.
- Severe left ventricular outflow tract obstruction.
- Severe proximal cerebral or coronary artery disease.
- Testing is not appropriate for frail patients who cannot weight-bear for up to an hour.

Patient preparation

Fasting is not required. Patients should continue all suspected culprit medications.

Procedure

The test should take place in a quiet room at a constant temperature. The patient is lightly strapped to a table with a weight-bearing footboard. Pulse and beat-to-beat BP are recorded throughout the test. The patient is laid supine for 10min (20min, if cannulated), and then the table is mechanically tilted to 70° for 20min of passive tilt. If positivity or discontinuation criteria have not been reached by this point, 400µg of sublingual glyceryl trinitrate (GTN) are administered whilst upright, and the tilt is continued for a further 15min. If the patient has a history of adverse reaction to nitrates, or if a diagnosis of psychogenic or hyperventilation syncope is suspected, then a 40min tilt protocol should be used instead with no GTN provocation.

Risks

Syncopal symptoms (or, in extreme cases, loss of consciousness), hypotension, and bradycardia may be induced, albeit transiently, so full cardiopulmonary resuscitation facilities and an appropriately trained supervising team should be available.

Possible results

A normal response is a <20% ↓ in BP associated with a modest rise in pulse rate. The test is -ve in the absence of a fall in BP, fall in heart rate, and lack of syncopal symptoms, and +ve if syncopal symptoms are induced by hypotension and/or bradycardia. A cardio-inhibitory response is characterized

by a fall in heart rate (asystole in extreme cases), a vasodepressor response by a fall in BP with no pulse change, and a mixed response by a fall in both pulse and BP. A diagnosis of postural orthostatic tachycardia syndrome (POTS) is indicated by a rise in heart rate of ≥ 30 bpm and/or to ≥ 120 bpm, within 10min of upright tilt.

Advantages over other tests

Monitoring of ECG and BP during a 24h ambulatory period or during a Valsalva manoeuvre—measurements of plasma catecholamines, mineralocorticoids, and glucose have a role in the investigation of autonomic dysfunction and syncope, but only tilt table testing provides a dynamic objective, witnessed assessment.

Pitfalls

The test is time-consuming and requires technical and medical personnel trained in the conduct and interpretation of the procedure and in resuscitation.

Further reading

- National Institute for Health and Care Excellence (2010). *Transient loss of consciousness ('blackouts') in over 16s*. Clinical guideline CG109. www.nice.org.uk/guidance/cg109.
- Parry SW, Reeve P, Lawson J, et al. The Newcastle protocols 2008: an update on headup tilt table testing and the management of vasovagal syncope and related disorders. *Heart* 2009; **95**: 416–20.
- Task Force for the Diagnosis and Management of Syncope; European Society of Cardiology (ESC); European Heart Rhythm Association (EHRA); Heart Failure Association (HFA); Heart Rhythm Society (HRS), Moya A, Sutton R, Ammirati F, et al. Guidelines for the diagnosis and management of syncope (version 2009). *Eur Heart J* 2009; **30**: 2631–71.

Gastroenterology

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Endoscopy

This allows direct visualization of the GIT mucosa and offers further diagnostic investigations to obtain tissue for histology, cytology, or microbiology, as well as therapeutic possibilities.

Consent (general)

Consent is a vital component of the endoscopy process. Patients should receive written information before attending for the procedure. This should describe pretest preparation, the procedure itself, risks and possible complications, after-care advice (particularly for those patients requiring sedation), and contact details in the event of problems and include the consent form that the patient will be asked to sign. In the UK, the *Montgomery v Lanarkshire* case of 2015 has established that the patient should be told whatever they want to know rather than what the clinician thinks they should be told, so covering issues of importance to the patient is paramount.

Sedation

Whilst the majority of OGD procedures can be performed using a local anaesthetic spray to the oropharynx, either sedation or patient-controlled Entonox® (an inhaled mix of medical nitrous oxide and O₂) will be required for more complex or prolonged procedures. An IV combination of a sedative with amnesic effects, such as midazolam, is usually combined with an analgesic such as fentanyl. Addition of hyoscine butylbromide may act to reduce intestinal motility, which is useful for colonoscopy, ERCP, or enteroscopy. An alternative to hyoscine is glucagon, which may be used for patients with glaucoma or IHD where hyoscine is contraindicated. Rarely, for patients who are relatively intolerant of procedures and require prolonged interventions at ERCP or enteroscopy, general anaesthetic (GA) is an option, depending on patient fitness and the availability of an anaesthetist and anaesthetic support.

Antibiotic prophylaxis pre-procedure

Pre-procedure prophylaxis is only indicated for patients undergoing:

- Percutaneous endoscopic gastrostomy or jejunostomy placement.
- ERCP where biliary drainage is unlikely to be achieved at the first procedure.
- Severe neutropenia ($<0.5 \times 10^9/L$) and/or severe immunocompromise undergoing procedures with a high risk of bacteraemia such as oesophageal dilatation or variceal sclerotherapy.

Anticoagulation guidelines pre-procedure

With the advent of novel oral anticoagulants and greater use of PY212 receptor antagonists (including clopidogrel and ticagrelor), this has become a more complex area. Please see British Society of Gastroenterology (BSG) guidelines for further details.

In summary, procedures are divided into:

- Low risk: diagnostic procedures (OGD, colonoscopy, flexible sigmoidoscopy, enteroscopy), with simple mucosal biopsies possible if needed.
- High risk: polypectomy, ERCP with sphincterotomy, dilatation of strictures, variceal therapy, percutaneous endoscopic gastrostomy (PEG) placement, endoscopic ultrasound (EUS) with FNA, stenting of the GIT.

PY212 receptor antagonists

Can be continued (\pm aspirin) for low-risk procedures but should be discontinued 5 days before high-risk cases. If there is a high thrombotic risk, continue aspirin and liaise with a cardiologist about risk/benefit of discontinuing.

Warfarin

For low-risk procedures, continue the usual dose of warfarin and go ahead if the INR is within the normal range. For high-risk procedures, stop warfarin 5 days beforehand. If there is a low risk of thrombosis, then check INR pre-procedure and go ahead if INR <1.5 . If there is a high thrombosis risk, then cover with low-molecular-weight heparin from 2 days after stopping warfarin, with the last dose $\geq 24\text{h}$ pre-procedure.

Direct-acting oral anticoagulants (DOACs)

For low-risk procedures, omit on the morning of the procedure. For high-risk procedures, the last dose should be taken $\geq 48\text{h}$ pre-procedure. For patients on dabigatran with an estimated glomerular filtration rate (eGFR) of 30–50mL/min, the last dose should be taken 72h pre-procedure. Specialist haematology advice should be sought if renal function is worse than this.

Risks and points for consent

Table 7.1 shows the common complications of endoscopic procedures.

Table 7.1 Risks and complications of the most commonly performed endoscopic procedures

Procedure	Risks	Morbidity for each complication	Overall mortality	Other issues
OGD	Perforation Haemorrhage	0.03% 0.002%	0.0001%	Greatest risk for therapeutic procedures Cardiorespiratory sedation-related complications— 0.005%
Flexible sigmoidoscopy/ colonoscopy	Perforation Haemorrhage	0.005% 0.001%	0.001%	Cardiorespiratory complications related to sedation—0.01%
Colonic polypectomy	Perforation Haemorrhage	0.06% 0.26%	0.007%	As above
PEG placement	Perforation Haemorrhage Infection	Overall risk 5–10%	1–2%	30-day mortality of ~10% (often resulting from underlying condition)
ERCP	Perforation Haemorrhage Cholangitis Pancreatitis	1.1%, 0.9% 5% 3.8% Cardio- respiratory complications related to sedation—2.3%	1.0%	Greatest risks overall with dilated bile duct, placement of stent, and high-dose hyoscine butylbromide Risk of pancreatitis greatest with age <40 years, placement of stent, and dilated bile duct

Safety considerations before prescribing oral bowel cleansing pre-colonoscopy/CT colonography/barium enema

In view of the risk of electrolyte disturbance and renal failure using oral bowel-cleansing agents, the National Patient Safety Agency (NPSA) advice from 2009 suggests the following:

- Check U&E, creatinine, and eGFR before oral bowel cleansing. If evidence of renal impairment (eGFR <30mL/min), then assess risks and benefits of procedure. Polyethylene glycol-based preparations (Klean-Prep® or Moviprep®) are safer if eGFR 30–50mL/min.
- If safe to do so, omit ACE inhibitors, angiotensin-2 inhibitors, diuretics, and/or NSAIDs on the day of starting oral bowel preparation for 3 days. If unable to stop (e.g. heart failure), then consider the risks and benefits of the procedure and seek advice from a cardiologist.

Further reading

- Allison M, Sandoe ATM, Tighe R, et al. Antibiotic prophylaxis in gastrointestinal endoscopy. *Gut* 2009; **58**: 869–80.
- Connor A, Tolan D, Hughes S, Carr N, Tomson C (2009). *Consensus guidelines for the prescription and administration of oral bowel cleansing agents*. http://www.bsg.org.uk/attachments/960_obca_draft_10.pdf.
- Veitch AM, Vanbiervliet Gj, Gershlick AH, et al. Endoscopy in patients on antiplatelet or anticoagulant therapy, including direct oral anticoagulants: British Society of Gastroenterology (BSG) and European Society of Gastrointestinal Endoscopy (ESGE) guidelines. *Gut* 2016; **65**: 374–89.

Oesophagogastroduodenoscopy

- Endoluminal visualization: oropharynx to second part of duodenum.
- Allows direct testing for *Helicobacter pylori*.
- In preparation for OGD, patients should not eat for 4–6h beforehand, with clear fluids allowed up to 2h before the procedure.
- They should remain nil by mouth for at least 30min afterwards to allow the LAn spray or sedation to wear off.
- Stop proton pump inhibitors 2 weeks before an elective diagnostic OGD to prevent masking of appearances (risk of partial healing and thus misdiagnosing malignant oesophageal or gastric ulcers).

Alternative investigations

As an alternative procedure to OGD or enteroscopy, barium swallow, meal, or follow-through may be performed, depending upon symptoms (for oesophageal, gastric, or duodenal pathology, respectively). Investigations are limited by their lower sensitivity for mucosal pathology and inability to obtain tissue for histology or undertake therapeutic procedures.

Indications

Symptoms/signs

- Dysphagia.
- Haematemesis and melaena.
- Dyspepsia despite appropriate therapy.
- Iron deficiency anaemia (see Fig. 7.1a).
- Weight loss.
- Vomiting or nausea.
- Investigation of suspected giardia or bacterial overgrowth to obtain duodenal aspirates.
- Obtaining duodenal biopsies in suspected coeliac disease.
- Investigation to obtain histology and cultures of *H. pylori*.
- Abnormal barium swallow, meal, or early follow-through.

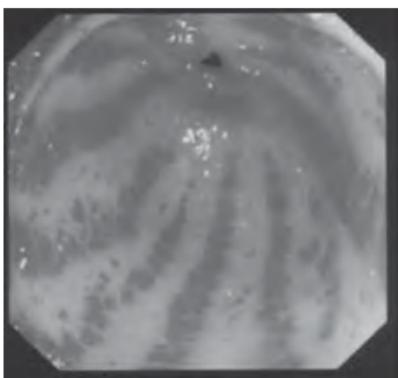
Surveillance/screening

- Ensures healing of oesophageal and gastric ulceration.
- Barrett's oesophagus.
- Establishes response to a gluten-free diet in coeliac disease.
- Diagnosis of polyps in familial polyposis syndromes.

Therapeutic

- Treatment of bleeding lesions (peptic ulceration, angiodysplasia, varices, vascular malformations).
- Palliation of oesophageal cancers using stent placement, argon plasma coagulation, or Nd:Yag laser therapy.
- Placement of PEG or jejunostomy tubes (see Fig. 7.1b).
- Direct placement of nasogastric or nasojejunal feeding tubes.
- Dilatation of strictures of the oesophagus or pylorus.
- Polypectomy.

(a)



(b)

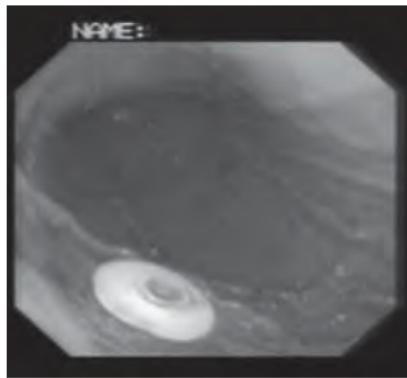


Fig. 7.1 (a) Endoscopic view of gastric antral vascular ectasia, one cause for blood transfusion-dependent iron deficiency anaemia, which may be treated using argon plasma coagulation. (Colour plate 4.) (b) Endoscopic view of internal bumper of percutaneous endoscopic gastrostomy (PEG) holding this *in situ* in the stomach to allow feeding. PEG placement is a therapeutic possibility by using OGD to allow direct visualization.

Enteroscopy

Conventional enteroscopy

- Similar to OGD, but allows views of the distal duodenum and jejunum using a 2.4m instrument.
- An overtube allows less looping but ↑ complications.
- Preparation as for OGD; usually requires sedation, analgesia, and hyoscine butylbromide.

Single- or double-balloon enteroscopy

- Allows views of the entire small bowel from an oral or rectal approach.
- Preparation is as for OGD or colonoscopy and requires deep sedation or GA, with the addition of hyoscine butylbromide.
- Consists of one or two balloons, respectively. For single- and double-balloon procedures, one is attached to a transparent overtube sliding over the endoscope, allowing movement forward by telescoping the small bowel by gripping and pleating it over the endoscope. For the double balloon, the second balloon is attached to the distal endoscope to act as an anchor.

Alternative investigations to enteroscopy

Barium investigations (follow-through or small bowel enema) are limited by a lower sensitivity for mucosal pathology.

MRI small bowel studies are good for young patients, as these present no radiation risk, with contrast allowing clear serosal and mucosal images and views of the whole length of the small bowel.

Wireless capsule endoscopy (WCE) is a more effective (but less frequently available) alternative. However, each of these is limited by an inability to obtain tissue or provide therapeutic intervention.

Which type of enteroscopy to choose?

Single- or double-balloon enteroscopy allows visualization and treatment of lesions within the full length of the small bowel and may replace conventional enteroscopy, although procedure length and intensity will require deep sedation or GA. Conventional enteroscopy will diagnose and treat lesions within the upper small bowel (to the jejunum) but is limited by patient tolerance as conscious sedation is used.

Indications for enteroscopy

- Investigation and/or treatment of obscure GI bleeding or severe anaemia (following non-diagnostic OGD and colonoscopy).
- Investigation and/or removal of lesions found on CT scan abdomen/ MRI small bowel/capsule endoscopy/barium investigation (e.g. polypectomy).
- Treatment of bleeding lesions found at enteroscopy or by WCE.

Flexible sigmoidoscopy and colonoscopy

- Allows examination from anus to splenic flexure (flexible sigmoidoscopy) or from anus to caecum/terminal ileum (colonoscopy).
- Preparation for flexible sigmoidoscopy: phosphate enema 30–60min prior to the procedure.
- Preparation for colonoscopy: oral preparation using an oral bowel-cleansing agent. Need to bear in mind both the patient's fitness and willingness to undergo the purgative preparation, and also the risk of renal failure and electrolyte disturbance associated with administration of these agents (for advice,  Endoscopy, pp. 498–501). Ideally, a low-residue diet should be followed for the 3 days pre-procedure to obtain the best views.
- Whilst colonoscopy usually requires sedation/analgesia, and ideally a smooth muscle relaxant (hyoscine butylbromide), both colonoscopy and flexible sigmoidoscopy are possible using Entonox® with or without hyoscine butylbromide. For patients who are intolerant of such procedures, GAn may rarely be available with appropriate anaesthetic specialist support.

Alternative investigations

Alternative procedures are radiological—mainly CT colonography, which has largely superseded barium enema. Neither allows tissue or polypectomy to be taken for histology nor other therapeutic procedures to be performed. For these reasons, colonoscopy is considered the 'gold standard' for investigating likely colon cancer, diarrhoea, anaemia, and rectal bleeding. Sensitivity is lower for detecting adenomas of $\leq 10\text{mm}$ using CT colonography (90%) than colonoscopy (99%). These alternative procedures all require the use of oral bowel-cleansing agents too.

For patients not fit to undergo oral bowel cleansing, then an unprepared CT abdomen and pelvis is a possibility. However, it is significantly less sensitive for lesions of $<10\text{mm}$; it may have a role for elderly or medically unfit patients where prognosis is important.

Indications

Symptoms and signs

- Rectal bleeding (bright red = flexible sigmoidoscopy, and dark red = colonoscopy), but beware as some right-sided colonic lesions do present with bright red bleeding.
- +ve faecal occult bloods (colonoscopy).
- Abnormal barium enema (depends upon site of pathology found).
- Iron deficiency anaemia (colonoscopy).
- Diarrhoea (colonoscopy with biopsies from right and left colon).

Surveillance/screening for specific disease states

- Extensive ulcerative colitis or colonic Crohn's disease for >10 years, then ongoing follow-up determined by disease extent and severity.
- High risk of adenomatous colonic polyps or carcinoma, or previous history of adenomatous polyps or carcinoma.
- Familial polyposis syndrome (FAP), hereditary non-polyposis colorectal carcinoma (HNPCC), or other family cancer syndromes.
- For further information, please see BSG guidelines.¹

Therapeutic

- Treatment of bleeding lesions (angiodynplasia, vascular abnormalities, haemorrhoids).
- Dilatation of benign strictures.
- Palliation of malignant strictures (placement of stents, argon plasma coagulation, or Nd:Yag laser therapy).
- Decompression of sigmoid volvulus and non-malignant toxic megacolon.
- Endoscopic mucosal resection of tumours.

National screening programmes**National Bowel Cancer Screening Programme**

Offers 2-yearly screening using FOB testing (due to change to faecal immunochemical testing shortly) with kits sent to everyone from 60–74 years of age. Face-to-face review for all patients with a +ve result allows either colonoscopy or CT colonography to be offered, depending on patient fitness and wishes. Aim: to allow diagnosis and removal of polyps (adenomas) before development of carcinoma.

Bowel Scope Screening Programme

Currently in roll-out phase across the UK to offer one-off flexible sigmoidoscopy for all 55-year-olds. Aim: to diagnose and remove polyps (adenomas) and reduce future bowel cancer risk. Full colonoscopy is offered to those with >3 adenomas, those with a villous component to one or more polyps, and anyone with an adenoma of >1cm.

¹ Cairns SR, Scholefield JH, Steele R, et al. Guidelines for colorectal cancer screening and surveillance in moderate and high risk groups (update from 2002). Gut 2010; **59**: 666–90.

Endoscopic retrograde cholangiopancreatography

- Side-viewing endoscope used to find the ampulla of Vater and guide cannulation of the biliary and pancreatic ducts by injecting radio-opaque contrast medium using fluoroscopy.
- MRCP has replaced ERCP in 1° diagnosis. ERCP is used for interventional procedures and to obtain biopsy and cytology specimens.
- ERCP requires sedation, analgesia, and hyoscine butylbromide or GA (which requires anaesthetic support).

Alternative investigations

MRCP allows imaging of the biliary and pancreatic systems and is the best (and safest) option for diagnosis, although no therapeutic procedures are possible. A percutaneous transhepatic cholangiogram (PTC) allows imaging, stent placement, and drainage of a dilated biliary tree using a transabdominal approach. PTC is indicated if therapeutic ERCP fails, albeit with greater risk of complications.

Indications

Diagnostic

- Endoscopic diagnosis of periampullary polyps and tumours.
- Obtain bile/brushings for cytology in suspected cholangiocarcinoma.
- Investigation of dilated biliary ducts found on USS with contraindications to MRCP.
- Assessment of the sphincter of Oddi pressures in suspected sphincter of Oddi dysfunction (SOD) syndromes—at specialist centres.

Therapeutic

- Biliary stenting:
 - Palliation of pancreatic, ampullary, and cholangiocarcinomas.
 - Treatment of a biliary leak following surgery.
 - Benign biliary stricture.
- Biliary sphincterotomy:
 - Gaining access to perform diagnostic or therapeutic procedures.
 - Choledocholithiasis.
 - Ampullary carcinoma.
 - Treatment of a biliary leak following surgery.
 - Treatment of SOD (types I and possibly II only)
 - Treatment of acute severe pancreatitis 2° to gallstones.
- Pancreatic stenting:
 - Drainage of pseudocysts (via the stomach).
 - Following sphincterotomy for SOD.
- Pancreatic sphincterotomy:
 - Pancreatic stone disease.
 - Gaining access prior to stent placement.
 - Minor duct papillotomy in pancreatic divisum.

Endoscopic ultrasound

- Combines endoscopy with US imaging to allow visualization of organs, such as the pancreas, and accurate assessment of the degree of invasion of luminal tumours in the oesophagus, stomach, and duodenum.
- Higher-frequency US probe allowed by proximity of the probe to the organ results in ↑ spatial resolution, compared with transabdominal US, CT, or MR scanning.
- Two different modes of imaging are possible: radial (which allows a 360° view around the shaft of the instrument) or linear (which is in line with the endoscope and allows 90° and up to 270° views).
- Pre-procedure preparation is as for OGD.
- Consent procedures differ, depending upon indications and potential pathology.

Alternative investigations

Transabdominal US, CT, and MRI scanning allow views of the pancreas and liver but do not allow such fine detail for diagnosis or therapy.

Indications

- Staging of oesophageal, gastric, pancreatic, and distal biliary tumours.
- Diagnosis and staging for GI stromal tumours.
- FNA of 'Trucut' biopsy of mediastinal or coeliac axis lymph nodes, pancreatic lesions, or submucosal lesions.
- Defining mucosal abnormalities such as Barrett's oesophagus.
- Coeliac axis nerve block to treat pancreatic pain (chronic pancreatitis or pancreatic carcinoma).
- Evaluation and treatment of pancreatic pseudocysts.
- Detection of CBD stones.

Further reading

Allum WH, Blazeby JM, Griffin SM, et al. Guidelines for the management of oesophageal and gastric cancer. Gut 2011; 60: 1449–72.

Wireless capsule endoscopy

- Allows imaging of the entire small bowel by using an 11 × 27mm capsule (e.g. PillCam® SB) with up to 7.5h of battery life.
- When swallowed, the capsule transmits images to aerials attached by adhesive pads to the abdominal wall and stored on a recorder attached round the waist.
- Propelled by the patient's own peristalsis, so symptom-free.
- Specialist procedure, but with a higher diagnostic yield for mucosa pathology than other small bowel investigations (see Table 7.2).
- If there is suspicion of stricturing where WCE could precipitate obstruction, then a dissolvable patency pill will verify adequate patency, allowing subsequent WCE. Prior barium radiology would also rule out stricturing.
- New capsules for investigation of the colon and oesophagus (PillCam® COLON and PillCam® ESO) are not in widespread use.

Indications

- WCE has a role in visual diagnosis but is unable to obtain samples for subsequent analysis or allow therapeutic management.
- Occult GI bleeding undiagnosed at endoscopy.
- Possible small bowel polyposis (e.g. Peutz–Jegher syndrome).
- Unexplained diarrhoea or malabsorption undiagnosed at endoscopy.

Points for consent

- A 0.5% risk of obstruction reported, especially if stricturing (e.g. small bowel Crohn's disease) or previous small bowel surgery. However, prior use of the patency pill will rule out stricturing significant enough to cause capsule retention.

Table 7.2 Diagnostic yield of small bowel investigations for occult bleeding lesions

	Sensitivity (%)	Specificity (%)	Diagnostic yield (%)
Enteroscopy	37	97	30–32
WCE	64	92	55–68

Further reading

- Mylonaki M, Fritscher-Raven A, Swain CP. Wireless capsule endoscopy: a comparison with push enteroscopy in patients with gastroscopy and colonoscopy negative gastrointestinal bleeding. *Gut* 2003; 52: 1122–6.
- Swain P. Wireless capsule endoscopy. *Gut* 2003; 52: 48–50.

Tests for *Helicobacter pylori*

Indications for testing

- 'Test and treat': patients <55 years of age with a low probability of significant pathology and dyspeptic symptoms do not require OGD. Non-invasive testing for *H. pylori* allows treatment of those found to be +ve, with acid suppression using a proton pump inhibitor to assess response for all patients.
- Ensure effective eradication in patients with confirmed peptic ulceration or mucosa-associated lymphoid tumour (MALT) lymphoma.

Non-invasive tests

Faecal antigen test

- *H. pylori* antigens in faeces measured using an immunochromatographic test.
- Sensitivity exceeds 91%, and specificity exceeds 92%.
- Commercially available kits make an initial diagnosis and confirm eradication.

Urea breath test

- Expired air is collected after ingestion of ¹³C-labelled urea.
- If *H. pylori* present, bacterial urease breaks down urea → ammonium and bicarbonate, then → CO₂ and ammonia.
- Expired ¹³CO₂ is collected into a tube 30min after ingestion, measured by a mass spectrophotometer, and compared with one collected prior to ingestion.
- Test almost 100% sensitive and specific, and remains the gold standard to confirm eradication.

Serology

- Serum IgG antibodies to *H. pylori* may be detected by ELISA with sensitivity of 90% and specificity of 70–90%.
- IgG levels persist up to 1 year, so fail to confirm eradication.
- Other organisms may cause cross-reactivity.
- False –ves occur in elderly or immunocompromised patients.

Invasive tests (performed at OGD)

- Investigations are based on biopsy samples.
- *H. pylori* density is usually the greatest in the antrum, but during acid suppression, the greatest concentration is found in the corpus.
- Colonization is patchy so may yield sampling errors.
- So for patients taking proton pump inhibitors, one biopsy should be taken from each area (two in total) for the greatest yield for each of the tests below.

Histology

- *H. pylori* can be detected on routine histology using the modified Giemsa stain.
- Sensitivity is 85%, with a specificity of almost 100%.

Culture

- This is useful in those few patients who have failed eradication to establish antibiotic sensitivities.
- Sensitivity is over 95%, with specificity of almost 100%.

Rapid urease test

- Biopsy is placed into a urea solution containing phenol red.
- *H. pylori* contains urease, releasing ammonia from urea, thus changing the pH and detected as a colour change with phenol red dye turning from straw to pink/purple.
- Commercial kits, such as the *Campylobacter*-like organism (CLO) test, are available.
- Specificity is 97%, and sensitivity between 70 and 95%.

Further reading

National Institute of Health and Care Excellence (2014). *Gastro-oesophageal reflux disease and dyspepsia in adults: investigation and management*. Clinical guideline CG184.  <https://www.nice.org.uk/guidance/cg184>.

Faecal occult blood testing

Faecal occult blood

Indications

- Used in the current National Health Service (NHS) England National Bowel Cancer Screening Programme as population screening for 60- to 74-year-olds every 2 years.
- Early diagnosis of cancer pathway for primary care (NICE guidelines, 2015). General practitioners advised to offer testing for adults without rectal bleeding if:
 - 50 years or over with unexplained abdominal pain or weight loss.
 - Under 60 years with a change in bowel habit or iron deficiency anaemia.
 - Aged 60 or over with anaemia in the absence of iron deficiency.

Investigation

- Simple and inexpensive, performed by the patient in their own home.
- Samples taken onto a test card from three consecutive bowel motions and card sent to a screening centre.
- Test card uses the 'guaiac' reaction with pseudoperoxidase in Hb, causing a colour change in an indicator dye.
- Dietary changes advised to avoid red meat, horseradish, broccoli, and turnips, as their high peroxidase activity may cause false +ve results, and avoid vitamin C tablets (high ascorbic acid activity).

Results

- Sensitivity of the non-hydrated test is 70%; this ↑ to 90% with rehydration, but at the loss of specificity. Sensitivity improves with the number of samples taken.
- Individuals who are +ve require full colonoscopy.
- Multicentre UK trial invited 486,355 for screening using FOB (take up 56%), finding 2% FOB +ve, and of these, 10.9% have carcinoma, 35% adenoma, and 54.1% normal.

Limitations

Polyps and carcinomas may bleed intermittently, but FOB are more likely to be +ve with early-stage cancers than polyps.

False positives

Non-colorectal blood source such as the upper GIT or nosebleed; diet containing red meat, broccoli, or turnips (peroxidase activity).

False negatives

'Old' sample with bacterial degradation of Hb, the presence of ascorbic acid, and reduced or absent bleeding at time of testing.

Faecal immunochemical test (FIT)

- This will replace FOB testing used by the NHS England National Bowel Cancer Screening Programme and has already happened in Scotland.
- A single sample is collected via a brush into a pot; this appears to encourage more people to take part in screening (currently just 55% of those invited to participate).
- Tests for human Hb protein from the colon in stool, with less reactivity for blood from the upper GIT or nose.
- +ve result means that colonoscopy or CT colonography is required.
- Higher sensitivity to lower concentrations of blood than FOB.

Further reading

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Faecal and serological testing in inflammatory bowel disease

Faecal calprotectin

- Neutrophil granulocyte cytosol protein, which acts as a marker of intestinal inflammation.
- +ve correlation with inflammation in Crohn's disease and ulcerative colitis.
- Non-specifically elevated in neoplasia and radiation proctitis too.
- Sensitivity for diagnosing pathology is 93%, with specificity of 100%, with a result of $\leq 50\mu\text{g/g}$ classed as -ve, effectively excluding IBD.
- A normal result of $\leq 50\mu\text{g/g}$ reduces the need for invasive investigations, such as colonoscopy, for patients of 16–40 years of age with no alarm symptoms of pathology (weight loss, bleeding, anaemia, nocturnal diarrhea, or a significant family history of bowel cancer).

Faecal lactoferrin

- Neutrophil-derived protein; marker of intestinal inflammation.
- May be used for non-invasive testing to differentiate those who require colonoscopy from those more likely to have functional disease such as IBS.
- Not as effective as calprotectin, as sensitivity for diagnosing pathology is 82% and specificity is 84%.

Perinuclear antineutrophil cytoplasmic antibodies

- Occur in the serum of 50–80% of patients with histologically confirmed ulcerative colitis, but only 10% of those with Crohn's disease. This is likely to be genetically determined.
- Antibodies are particularly associated with 1° sclerosing cholangitis in association with ulcerative colitis.
- Overall sensitivity is 55.3%, with a specificity of 88.5%.
- In a paediatric cohort with -ve antibodies to *Saccharomyces cerevisiae* (ASCA), sensitivity ↑ to 70.3%, with 93.4% sensitivity.

Antibodies to *Saccharomyces cerevisiae*

- Found in 60% of patients with Crohn's disease, but only 5% of those with ulcerative colitis.
- In Crohn's disease, the presence of high titres of ASCA is associated with early age of onset, fibrostenosing and fistulating disease types.
- Sensitivity is 54.6%, with specificity of 92.8% if pANCA is -ve.

Further reading

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Reese GE, Constantinides VA, Simillis C, et al. Diagnostic precision of anti-*Saccharomyces cerevisiae* antibodies and perinuclear antineutrophil cytoplasmic antibodies in inflammatory bowel disease. *Am J Gastroenterol* 2006; 101: 2410–22.

Tumour markers

- As a result of generally low sensitivities and specificities, these should not be first-line investigations.
- They are best used for tracking patients following a diagnosis of carcinoma through subsequent surgery, and chemo-radiotherapy when levels should fall to normal unless disease recurs.

Carcinoembryonic antigen

- ↑ in 60% of those with localized colorectal carcinoma (CRC) and 80–100% of those with metastatic disease.
- Non-specific and non-diagnostic—also ↑ in bronchial carcinoma, heavy smokers, and IBD.
- Levels do not relate to tumour load, but rising levels, which were previously low/normal, may imply recurrence of CRC.

Alpha-fetoprotein

- Normally produced by fetal liver.
- High levels in non-pregnant adults imply hepatocellular carcinoma (raised in over 90% cases).
- ↑ serological concentrations in pregnancy suggest neural tube defect.
- Blood levels may be ↑ in hepatocyte regeneration, acute viral hepatitis, cirrhosis, choriocarcinoma, and teratoma.
- May be used 6-monthly (with liver USS) to screen cirrhotic patients for development of hepatocellular carcinoma.

Carbohydrate antigen 19-9 (CA 19-9)

- ↑ in pancreaticobiliary obstruction, with the highest levels in pancreatic carcinoma.
- Levels >40IU/L have 75–90% sensitivity and 80–95% specificity for ductal pancreatic carcinoma.
- Serum levels may be elevated in jaundice, cholangitis, choledocholithiasis, and chronic pancreatitis.

Cancer antigen 125 (Ca-125)

- Glycoprotein antigen to an epidermal growth factor receptor (p110 sEGFR).
- Blood levels ↑ with menstruation, endometriosis, pelvic inflammatory disease, pregnancy, and ascites of all types.
- Highest levels with ovarian malignancy, but also ↑ with ovarian, pancreas, breast, lung, and colon cancers.
- 20% of ovarian cancers have little/no expression of Ca-125.
- Useful in monitoring response of ovarian carcinoma to treatment (if initially +ve), as rising level of Ca-125 precedes clinical recurrence by up to 3 months (>90% of cases).
- As isolated values lack sensitivity and specificity, serial Ca-125 readings may be used to achieve specificity of 99.6%, but with a sensitivity of only 80%.

Investigations for small bowel pathology

General investigations

- Presenting features of small bowel pathology include diarrhoea, steatorrhoea, abdominal pain, weight loss, and nutritional deficiencies.
- Investigation of diarrhoea is shown in Fig. 7.4.
- Occasionally, occult GI bleeding may originate in the small bowel, and an algorithm for investigation of anaemia is shown in Fig. 7.5.

Serology

- Anti-tTG antibodies have almost 100% sensitivity for coeliac disease but may be false -ve in presence of low IgA.
- Diagnosis should be confirmed using duodenal or jejunal biopsies taken at OGD, which would reveal partial or total villous atrophy.

Endoscopy

- OGD and enteroscopy allow views of the proximal small intestine to obtain tissue and allow therapeutic possibilities.
- WCE permits diagnosis from the whole small intestine but does not allow therapeutic options.

Radiology

- MRI of the small bowel has mostly replaced barium radiology, particularly in younger patients, with a lack of radiation, greater sensitivity for mucosal detail by using contrast, and abilities to use cine views to determine motility.
- Barium follow-through involves ingestion of dilute barium, with images taken every 10–30min until barium reaches the caecum.
- Small bowel enema (enteroclysis) uses insertion of a nasoduodenal tube to infuse barium slowly, creating a column, which may be followed continuously using fluoroscopy. This allows focusing on areas including the terminal ileum (see Fig. 7.2).

Nuclear medicine

Radiolabelled white cell scintigraphy for inflammation/infection

- Uses ^{99m}Tc -HMPAO to show intensity and extent of inflammation or infection 1 and 3h after injection of autologous radiolabelled leucocytes.
- Delineates disease extent in Crohn's disease and ulcerative colitis (see Fig. 7.3).
- False -ve results may occur in small bowel Crohn's disease.
- Sensitivity of 96% and specificity of 97% for IBD, and sensitivity of 85–100% and specificity of 100% for detecting abscesses.

Radionuclide studies to detect Meckel's diverticulum

- IV ^{99m}Tc pertechnate accumulates in gastric mucosa with a time course of 5–60min.
- Uptake in ectopic mucosa (e.g. Meckel's diverticulum) occurs simultaneously.
- Imaging at 5–10min intervals up to 2h after injection allows localization of the site of ectopic mucosa.
- False +ves are caused by early gastric emptying.
- Sensitivities vary from 90% in children to 60% in adults.



Fig. 7.2 Barium follow-through image showing a terminal ileal stricture.

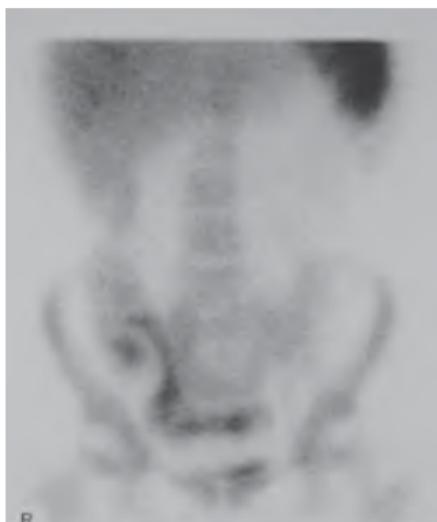


Fig. 7.3 Radiolabelled white cell scintigraphy using ^{99m}Tc -HMPAO to show inflammation of the terminal ileum consistent with Crohn's disease (taken at 3h).

SeHCAT scan for bile salt malabsorption

- The labelled bile acid $^{75}\text{selenium homotaurocholate}$ (SeHCAT) is administered orally. Retention measured at 7 days by whole body counting.
- Retention of >15% SeHCAT is normal (less indicates malabsorption).
- Commonest reason for abnormal result is post-cholecystectomy, presence of ileal disease, or post-ileal resection.
- Useful second-line investigation in patients with diarrhoea of unknown aetiology.

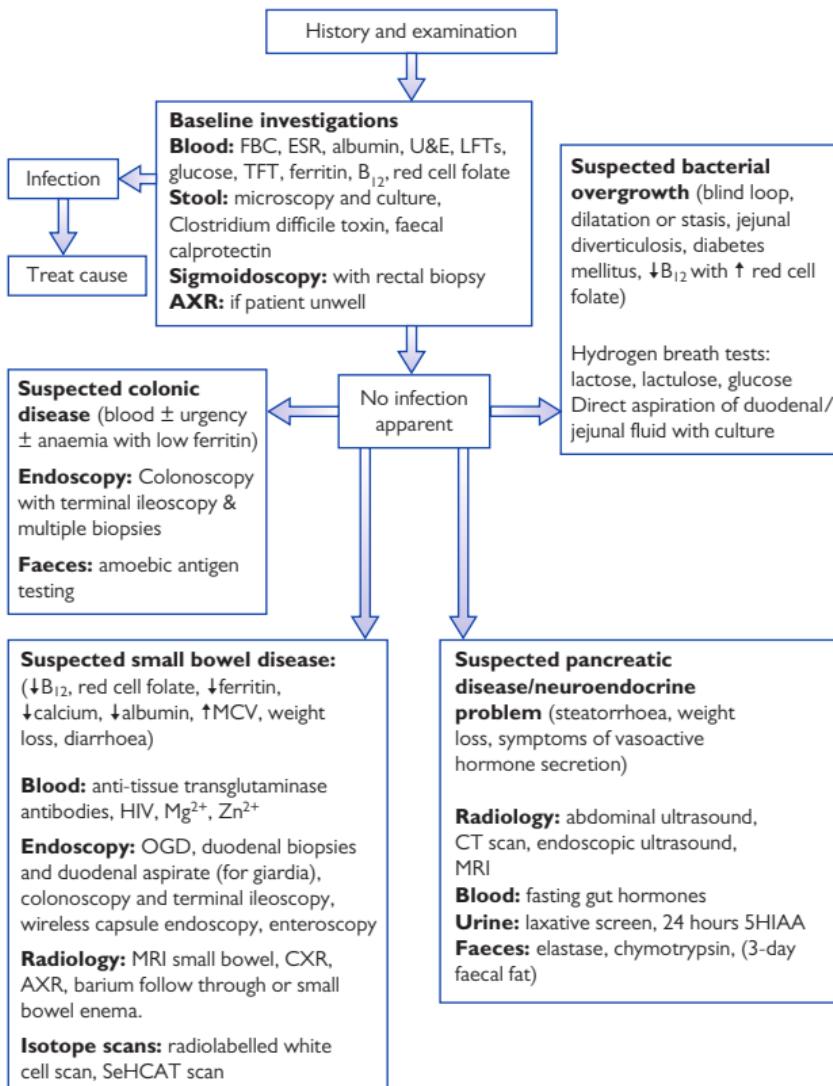


Fig. 7.4 Investigation of diarrhoea with specific tests for likely sites of pathology.

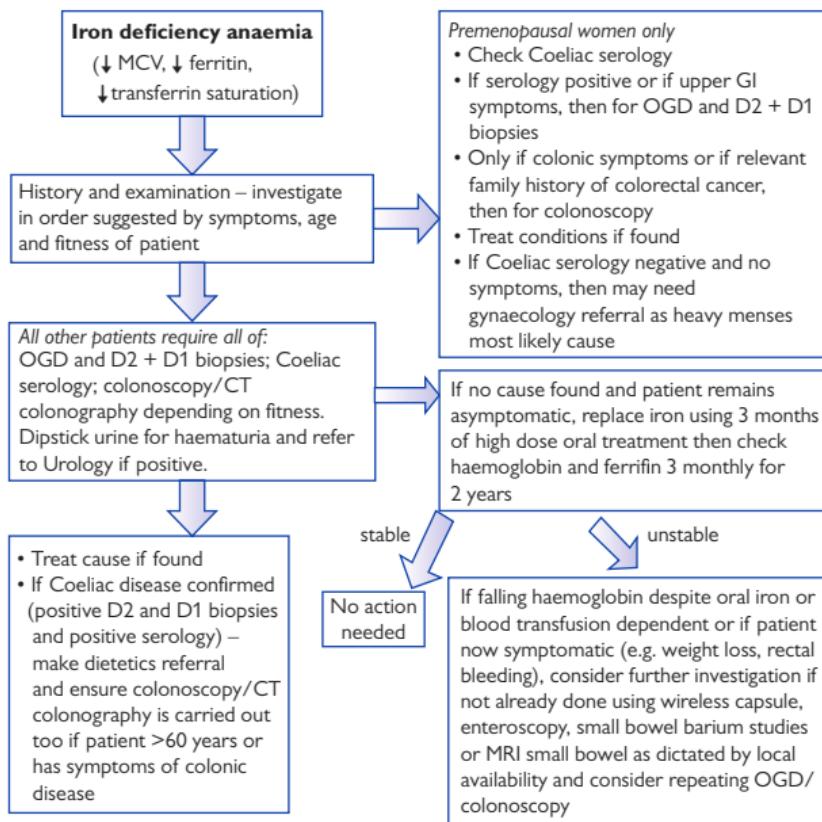


Fig. 7.5 An algorithm for investigation of iron deficiency anaemia.

Tests of small bowel absorption

Tests of carbohydrate malabsorption

D-xylose tolerance test

Xylose is absorbed from the proximal small bowel, and urinary excretion and blood levels reflect absorption. It is used mainly in paediatric practice and has a low sensitivity.

Lactose tolerance test

Oral administration of 50g of lactose is followed by blood sampling every 30min for 2h. A rise in blood glucose of $<1.1\text{mmol/L}$ suggests deficiency of disaccharidases, especially with colicky abdominal pain and diarrhoea.

Lactose hydrogen test

This is similar to the hydrogen breath test for bacterial overgrowth. Lactase-deficient individuals fail to metabolize lactose, which then undergoes luminal metabolism by lactase-producing colonic bacteria to yield hydrogen. Glucose and fructose may be used as alternative short-chain carbohydrates to determine the presence of malabsorption.

Tests of fat malabsorption (may occur in pancreatic malabsorption too)

Patients with fat malabsorption 2° to small bowel diseases, such as coeliac disease or tropical sprue, may malabsorb between 10 and 20g/day of fat, whilst patients with pancreatic insufficiency may malabsorb 30–50g/24h. A normal faecal fat excretion is $<6\text{g/day}$ (17mmol/day).

3-day faecal fat

- Unpleasant test, fallen into disuse, but remains gold standard.
- Fat content measured in a 3-day faecal collection.
- Patient takes a standard diet of 100g of fat per day.
- Does not differentiate small bowel and pancreatic malabsorption.

Tests for bacterial overgrowth

Deep duodenal/jejunal aspiration

- Samples may be collected through the biopsy channel at OGD.
- Scanty bacteria are present in normal upper small bowel.
- Numbers in excess of 10^6 /mL of aspirated fluid are pathological.
- This technique may culture *Giardia* and *Strongyloides* species.

Hydrogen breath tests

Rationale

In mammals, the only source of breath hydrogen is bacterial fermentation of carbohydrates. Hydrogen is absorbed from the intestinal lumen and expired during breathing. In bacterial overgrowth, hydrogen production can occur in the small intestine, as well as in the colon.

Method

- A mouthwash is given beforehand to reduce contamination by oral bacteria.
- Test dose of glucose/lactose (50g of either) or lactulose (10–15mL) and breath hydrogen measured.
- An early peak (e.g. 40min) suggests bacterial overgrowth.
- This test can be used for other short-chain carbohydrates, such as fructose or lactose, to determine if a patient is malabsorbing one or more.

Limitations

- Sensitivity 60–90%, with specificity of 80%.
- False –ves result from variations in microflora present in the small intestine or antibiotic administration within 3 weeks.
- False +ves occur in patients with IGT, those with rapid transit to the colon, and smokers.
- Patients should avoid eating pulses for 48h prior to the test (normal digestion of pulses liberates excess hydrogen).

Tests of pancreatic exocrine function

- Symptoms of pancreatic exocrine dysfunction include diarrhoea or steatorrhoea and weight loss.
- Investigations aim to determine the degree of pancreatic insufficiency, although biochemical and radiological investigations are more reliable with greater sensitivity and specificity in advanced disease.

Faecal testing

Elastase

- Proteinase produced by pancreatic acinar cells and remains un-degraded during gut transit.
- Measured using immunoassay of non-liquid faeces sample.
- Levels of $>200\mu\text{g/g}$ of faeces being normal, 100–200 representing mild insufficiency, and <100 being diagnostic of severe disease.
- Specificity is 93%, whilst sensitivity is 63% and 100% for mild and severe disease, respectively.
- False +ves may occur with high-volume watery stools.
- The result is not affected by pancreatic enzyme supplements.

Chymotrypsin

- Less sensitive (64%) and specific (89%) than elastase.
- Used mainly in children to screen for cystic fibrosis.
- Result is affected by pancreatic enzyme supplements.

Direct investigations

Secretin test

- Direct intubation of the duodenum allows collection of pancreatic juice, following IV injection of either secretin (allowing measurement of volume and bicarbonate content) or cholecystokinin (allowing measurement of amylase, trypsin, and lipase).
- Whilst highly sensitive and specific, even with mild pancreatic disease, this is only available in specialist centres.

Indirect investigations

PABA (*N*-benzoyl-*L*-tyrosol *p*-aminobenzoic acid) test

- Synthetic peptide, which is given orally.
- In normal patients, pancreatic chymotrypsin hydrolyses this peptide, yielding free PABA, which is absorbed, metabolized, and excreted in urine.
- In pancreatic insufficiency, free PABA levels are reduced, resulting in reduced absorption and excretion with lower urinary and serum PABA concentrations.

Pancreolauryl test

- Fluorescein dilaurate (an ester) is taken orally with a set diet.
- In the presence of normal pancreatic function, aryl esterases release fluorescein, which is absorbed, partially conjugated in liver, and excreted in urine.
- A 24h urine collection for excreted levels shows close correlation with pancreatic exocrine function.

Testing for neuroendocrine tumours

- Specialist area where clinical suspicion results from baseline investigations, and radiology should allow targeting of more specific biochemical investigations, depending on which syndrome is suspected.
- Histology is required to confirm the diagnosis.

Baseline biochemical tests

Urinary 5-hydroxyindole acetic acid

- 24h collection of urine for 5-hydroxyindole acetic acid (5HIAA).
- High levels imply carcinoid syndrome.
- High specificity, but false +ves result from serotonin-rich bananas, tomatoes, or drugs such as phenothiazines.

Chromogranin A

- Marker of neuroendocrine tumours (NETs) found in high concentrations, regardless of presence or absence of hormone-related clinical features.
- May be falsely elevated in proton pump inhibitor use and atrophic gastritis.

Baseline radiological/endoscopic investigations

- CT or MRI of the chest, abdomen, and pelvis. The 1° tumour is identified in only 50–70%.
- Radiolabelled somatostatin receptor scintigraphy (OctreoScan).
- Positron emission tomography (PET) using gallium-68 with concurrent CT (PET/CT) if unknown 1° or to look for 2°.
- Endoscopy or EUS may allow tissue diagnosis.

Vasoactive peptides and amines

- Measured using fasting serum gut hormone assays: insulin (paired with a serum glucose sample), glucagon, chromogranin A and B, VIP, pancreatic polypeptide (PP), gastrin, and somatostatin.
- Single tumours often secrete >1 type of hormone.
- Up to 50% of slow-growing tumours are thought to be non-functional.
- Several types of tumour occur as part of MEN-1.
- Concurrent therapy with a proton pump inhibitor will falsely raise serum gastrin and chromogranin A levels, so these should be stopped for at least 1 week prior to measuring serum levels.

Further reading

Ramage JK, Ahmed A, Ardill J, et al. Guidelines for the management of gastroenteropancreatic neuroendocrine (including carcinoid) tumours (NETs). Gut 2012; 61: 6–32.

Gastrointestinal physiology

Sphincter of Oddi (SOD) manometry

- A water-perfused pressure catheter is used during ERCP to measure sphincter of Oddi pressures to formally diagnose SOD.
- SOD is triad of abnormal liver function, biliary-type abdominal pain, and dilatation of the biliary tree in the absence of gallstone disease.
- Diagnosis is confirmed by high resting pressure, retrograde peristalsis, and failure of contrast to drain from the biliary tree within 45min.
- Whilst patients with SOD have an ↑ risk of pancreatitis post-ERCP, biliary sphincterotomy may relieve symptoms of pain in selected cases.

pH monitoring

Indications

- Assessment of gastro-oesophageal reflux disease (GORD).
- Atypical symptoms such as asthma or non-cardiac chest pain.
- Before consideration of anti-reflux surgery.
- Poorly controlled GORD to confirm diagnosis on or off treatment.

Investigation

- Ambulatory 24h test that places a pH electrode through the nose to sit 5cm above the lower oesophageal sphincter.
- Connected to a portable microprocessor that records episodes where the pH dips below 4.
- Patient fills in a simultaneous event diary to correlate symptoms and episodes of low pH.
- For most patients, H₂ receptor antagonists and proton pump inhibitors are stopped 7 days beforehand. A few patients require testing on medication to establish treatment response.

Results

- Measure frequency and duration of episodes of pH <4.
- Duration of longest episode.
- Assess correlation between symptoms and pH.
- Excessive oesophageal acid reflux is defined as a total duration of pH <4 of 4–6% of recording time.

Oesophageal manometry

Indications

- Diagnosis and assessment of motility disorders suggested by symptoms/ OGD/barium swallow findings.
- Defining the precise location of the lower oesophageal sphincter prior to 24h pH monitoring.
- Before consideration of anti-reflux surgery.

Investigation

- Static test using a nasogastrically placed multiple channel, water-perfused catheter, which is gradually withdrawn during a series of wet and dry swallows.
- Pressure is measured through the length of the oesophagus, at the upper and lower oesophageal sphincter, and the duration and frequency of contractions are recorded (see Table 7.3).

Table 7.3 Results from oesophageal manometry studies

Non-specific motility disorder	Abnormal and incomplete peristalsis with normal body contractions
Achalasia	Incomplete relaxation and high pressure of lower oesophageal sphincter with aperistalsis of the oesophageal body
Nutcracker oesophagus	High amplitude and duration of contractions, normal peristalsis
Oesophageal spasm	Disordered peristalsis with simultaneous prolonged contractions throughout

Gastric emptying

- Scintigraphy using the radioactive tracer 99m technetium displays gastric movements with a range of test meals (liquid to solid) to quantify gastric emptying and intestinal filling over time.
- In practice, barium and endoscopic studies often provide enough information when investigating patients with vomiting.

Intestinal transit studies

- Intestinal transit is measured using 50 radio-opaque plastic markers inside a pH-sensitive gel capsule, swallowed by the patient and designed to release its contents in the terminal ileum. An abdominal radiograph at 100h should show <20% of markers present in the colon.
- Measures colonic transit in suspected slow transit constipation.

Anorectal manometry

- Water-perfused catheter measures anorectal pressures to assess voluntary and involuntary sphincter squeeze pressures and reflex responses to balloon distension in the rectum.
- Readings allow assessment of rectal sensation, spinal reflexes, and internal and external sphincter integrity.
- Assesses symptoms of faecal soiling, incontinence, and chronic constipation.

Non-invasive liver investigations

Basic liver function testing

These are basic serological screening tests that establish whether liver inflammation, infection, or obstruction is present.

Alanine transaminase

Alanine transaminase (ALT: cytosol enzyme specific to the liver) and aspartate transaminase (AST: mitochondrial enzyme also present in the heart, muscle, kidney, and brain)—both enzymes are present in hepatocytes and leak into blood with liver cell damage.

Alkaline phosphatase

Alkaline phosphatase (ALP: canalicular and sinusoidal membranes of the liver, but also bone, intestine, placenta)—specific isoenzymes for ALP are produced by different tissues, but simultaneously raised γ -glutamyl transpeptidase (γ GT) and ALP implies a hepatic origin. Extra- and intra-hepatic cholestasis may cause raised ALP and results from benign or malignant disease with or without raised bilirubin. The highest levels result from PBC and hepatic metastases.

Gamma glutamyl transpeptidase

γ GT (microsomal enzyme)—activity can be induced by drugs, such as phenytoin and rifampicin, and alcohol. Mild elevation of γ GT is common with even a small alcohol intake, and isolated elevation does not imply liver disease. It rises in parallel with ALP in cholestasis.

Albumin

A protein that is synthesized in the liver. Plasma concentration partially results from functional capacity within the liver. However, it has a serum half-life of 20 days and may be normal in early phases of acute liver disease. Hypoalbuminaemia may also arise from \uparrow volume of distribution (sepsis, overhydration, pregnancy), \uparrow excretion or degradation (nephrotic syndrome, protein-losing enteropathy), haemorrhage, or catabolic states such as malignancy or burns.

Prothrombin time

Test of plasma clotting activity and reflects the activity of vitamin K-dependent clotting factors synthesized by the liver. PT may be elevated in acute or chronic liver disease. In vitamin K deficiency with normal liver function, PT will return to normal within 18h of administration of parenteral vitamin K.

Bilirubin

In liver disease, a raised bilirubin is usually associated with other liver function abnormalities. There are many causes of raised serum bilirubin. Bilirubin may be conjugated or unconjugated, although in practice, this conjugation state only differentiates congenital hyperbilirubinaemias. In Gilbert's syndrome (the commonest benign cause of an isolated raised serum bilirubin), an elevated unconjugated bilirubin, which rises during fasting and mild illness, diagnoses the condition. Haemolysis is another cause of hyperbilirubinaemia, which is covered in  Chapter 3.

Immunoglobulins

- IgG ↑ in viral hepatitis, chronic AIH, and cirrhosis.
- IgM ↑ in PBC, non-biliary cirrhosis, and acute viral hepatitis.
- IgA is ↑ in alcoholic liver disease, with β-γ fusion seen on electrophoresis.

Specific biochemical tests

These are summarized in Table 7.4, with serological tests, diagnosis, and definitive investigations required to confirm the diagnosis. The best investigations for an individual patient are established from history, examination, and basic biochemical parameters.

Table 7.4 Specific biochemical tests

Test	Condition	Findings	More specific tests
Serum ferritin	Haemo-chromatosis	Serum iron >30 µmol/L	HFE gene testing (83–90% of patients have Cys 282 Tyr mutation; 25% have His 63 Asp; 187G in complete linkage disequilibrium with Cys 282 Tyr). Liver biopsy—with dry weight of iron
Serum iron		Serum ferritin >500 µg/L	
Transferrin saturation		Transferrin saturation >60%	
24h urinary copper excretion	Wilson's disease	Serum copper and caeruloplasmin levels are usually reduced but can be normal	Liver biopsy—with dry weight of copper
Serum copper			
Caeruloplasmin			
α ₁ -antitrypsin	α ₁ -antitrypsin deficiency	Levels of <10% of normal in homozygotes and 60% in heterozygotes	Liver biopsy and lung function testing for emphysema
AMA	PBC	AMA present in titres >1/160 Specific M2 antibody (possible non-specific ↑ ANA/SMA) ↑ serum IgM and ALP	Liver biopsy
ANA/SMA	Type I AIH	↑ ANA ± SMA ↑ total IgG	Liver biopsy
Anti-LKM1 or anti-liver cytosol antibodies	Type II AIH	↑ antibodies titres ↑ total IgG	Liver biopsy
Fasting cholesterol and glucose, glycosylated Hb	Non-alcoholic fatty liver disease/ non-alcoholic Steatohepatitis	IGT	Fibroscan®/fibrosis score/liver biopsy

Specific virological tests

Hepatitis A

- Acute infection: +ve IgM antibodies to HAV.
- Chronic infection: does not occur.
- Markers of clearing virus: +ve IgG antibodies to HAV and anti-HAV IgM.

Hepatitis B

Acute infection with subsequent clearing of virus

- HBsAg appears in blood from 6–12 weeks after infection, then disappears.
- HBeAg appears early, then declines rapidly.
- Anti-HBs appears late and indicates immunity.
- Anti-HBc is the first antibody to appear, and IgM anti-HBc may persist for many months as the only marker of ongoing viral replication when HBsAg has disappeared and anti-HBs is not yet detectable.
- Anti-HBe appears after anti-HBc and indicates ↓ infectivity.

Acute infection leading to chronic hepatitis B

- HBsAg persists and indicates chronic carrier state.
- HBeAg persists, correlating with ↑ severity and infectivity.
- Anti-HBe indicates seroconversion (if this occurs) with disappearance of HBeAg and a rise in ALT.
- HBV DNA suggests continued viral replication.

Hepatitis C

- Acute infection: hepatitis C HCV RNA is +ve 1–2 weeks after infection, with HCV antibodies developing after ~12 weeks.
- Chronic infection: >50% of patients with persistent HCV RNA, which can be measured as viral load.
- Hepatitis C has six genotypes that determine response to treatment.

Hepatitis E

- Similar to hepatitis A with no chronic or carrier state.

Investigation of liver disease

Figure 7.6 shows an algorithm for investigation of liver disease. This differentiates obstructive from parenchymal liver disease to suggest further investigation and treatment.

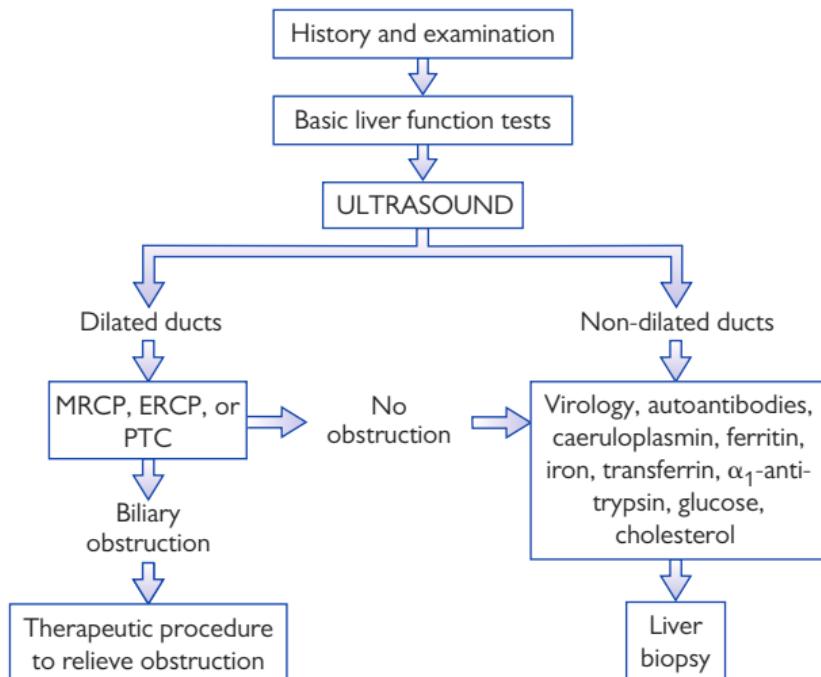


Fig. 7.6 An algorithm for investigation of liver disease.

Non-invasive testing of liver fibrosis

Transient elastography (Fibroscan®)

- Stages liver disease by assessing stiffness using the velocity of a vibration wave.
- Measures liver inflammation and fibrosis, with ↑ stiffness correlating with disease severity.
- Requires a minimum of ten valid readings; results are expressed in kPa.
 - Normal result: <7.0kPa.
 - Fibrosis: >7.0kPa (with an 85% probability).
 - Cirrhosis: >14.0kPa (with a 90% probability).
- Falsely high results: liver inflammation (active hepatitis), cholestasis, mass lesion or tumour, liver congestion in heart failure.
- Unreliable results: presence of ascites.
- Not suitable: pregnancy and patients with a pacemaker.
- Benefit: avoid risks of liver biopsy.

Liver biopsy

- Obtains tissue for diagnosis of diffuse or localized parenchymal disease.
- Severity of histological liver dysfunction cannot be predicted from basic LFTs.
- Consent should be obtained based on risks.
- Transjugular liver biopsy overcomes many contraindications.

Indications

- Unexplained persistently abnormal LFTs.
- Staging of disease in hepatitis B or C infection, and prior to considering antiviral treatment.
- Acute hepatitis of unknown aetiology.
- Cirrhosis of unknown aetiology.
- Alcohol-related liver disease.
- PBC/chronic active hepatitis.
- Targeted liver biopsy of lesions (not if resection/transplant is a possibility).
- PUO.
- Haemochromatosis/Wilson's disease.
- Storage diseases.
- Post-liver transplant to rule out acute or chronic rejection.

Methods for obtaining tissue (risks and benefits)

Percutaneous with or without ultrasound viewing

(See Table 7.5.)

- Standard method for obtaining tissue.
- Ultrasonography of liver and biliary tree pre-procedure to identify anatomical variations ↑ risk and to rule out obstruction.
- Most complications <2h but can occur up to 24h.
- No evidence that direct US-guided biopsy is safer, but US should definitely be used if a targeted biopsy is required.

Contraindications

- An uncooperative or confused patient.
- PT prolonged by >3s, platelets $<80 \times 10^9/L$, or bleeding diathesis.
- Ascites.
- Hydatid cysts (risks of anaphylaxis and abdominal seeding).
- Extrahepatic cholestasis.
- Higher risk of bleeding if amyloidosis present.

Minor complications

Shoulder tip pain, minor intra-abdominal bleeding, or mild abdominal pain (up to 30%)—usually settles with analgesia.

Major complications

- Perforation (0.01–0.001%: pneumothorax, gall bladder puncture, kidney, colon).
- Intra-abdominal haemorrhage; haemobilia (0.05%: a triad of biliary colic, jaundice, and melaena within 3 days of liver biopsy).
- Mortality varies between 0.001 and 0.0001% and results from intraperitoneal haemorrhage or biliary peritonitis.
- Risk of tumour seeding if a malignant lesion is biopsied; if curative resection/transplantation is planned, needle biopsy should be avoided.

Table 7.5 Percutaneous liver biopsy—practical procedure

Procedure	Should be carried out by an experienced doctor using aseptic precautions
Check	Clotting, FBC, and take group and save (G&S) within 24h before procedure (cancel if platelets $<80 \times 10^9/L$ or PT prolonged $>3s$)
Patient	Lies flat on his/her back
Liver margins	Delineated using percussion and/or US
1% lidocaine	5mL injected at the point of maximal dullness down to the liver capsule through intercostal space in expiration
Menghini or Trucut needle	Used to obtain sample with patient's breath held in expiration (up to two passes may be used). Menghini needles use suction, have a lower rate of complications, and allow more rapid biopsy, but they have a lower yield for tissue than Trucut (a cutting needle)
Sample	Placed into 10% formalin (or into a dry pot to estimate dry weight of iron or copper or for culture)
Patient	Nurse in supine position or right lateral for $>6h$, with regular BP and pulse measurements (every 15min for 2h, every 30min for the next 2h, then hourly), with urgent medical review if any sign of deterioration
If stable	At 6h, the patient can be discharged home as long as s/he can return to hospital within 30min and have a responsible adult with him/her overnight

Transjugular

- Specialist technique carried out using fluoroscopic guidance.
- Catheter passes from the right internal jugular vein, through the right atrium and IVC, and into the hepatic veins.
- Patient holds his/her breath, whilst the biopsy needle is advanced through the catheter then rapidly pushed forward by 1–2cm into the liver to obtain a small core of liver parenchyma.
- Safe technique with few complications (1.3–2% morbidity and 0.5% mortality), usually performed in higher-risk patients.
- Complications range from neck haematoma, puncture of intrathoracic arteries, transient Horner's syndrome, cardiac arrhythmias, infection, and perforation of the liver capsule.
- Test of choice in patients excluded from percutaneous biopsy by coagulopathy, bleeding diathesis, ascites, portal hypertension, and amyloidosis.
- Transjugular cannulation allows measurement of portal venous pressures.

Laparoscopic

- Allows sampling of the liver when an operation is planned.
- Allows targeted biopsies, as well as parenchymal biopsies.
- Bleeding is directly controlled and perforation is avoided.
- Risks of surgery and anaesthesia should be considered.

Further reading

Grant A, Neuberger J, Day C, Saxseena S (2004). *Guidelines on the use of liver biopsy in clinical practice*.
 © http://www.bsg.org.uk/images/stories/docs/clinical/guidelines/liver/liver_biopsy.pdf.

Investigation of ascitic fluid

(See Table 7.6.)

Investigation requires diagnostic aspiration

- 10mL is sent for cell count, Gram stain, ZN stain culture (add 10mL to a pair of blood culture bottles to ↑ diagnostic yield).
- 10mL is sent for cytology.
- 10mL for biochemical investigation of protein, glucose, LDH, TGs (if chylous ascites is suspected), and amylase (if pancreatic ascites is suspected).

Results

Cell count

- Polymorphonuclear leucocytes (neutrophils) of $>250 \text{ cells/mm}^3$ suggest underlying spontaneous bacterial peritonitis (SBP) (or 2° infection).
- Total leucocytes of $>500 \text{ cells/mm}^3$ imply bacterial peritonitis (SBP) if a specific neutrophil count is not available.
- Lymphocyte count of $>500 \text{ cells/mm}^3$ implies tuberculous peritonitis (with raised protein, positive ZN stain/TB culture, and low glucose).

Gram stain and culture

- Gram staining for early identification of bacteria and bacterial culture allow targeting of antimicrobial therapy.

Protein

- Using ascitic fluid protein levels to aid diagnosis is best achieved using the serum ascites albumin gradient (SAAG) by subtracting ascites albumin concentration from serum albumin concentration. Levels of $\geq 11 \text{ g/L}$ suggest cardiac failure, cirrhosis, and nephrotic syndrome, whilst levels of $< 11 \text{ g/L}$ suggest malignancy, TB, or pancreatitis as causes.
- Risk of SBP is greatest if ascitic protein $< 10 \text{ g/L}$.

Cytology

May be diagnostic in cases of peritoneal malignancy.

Amylase

↑ ascitic amylase ($>2000 \text{ IU/L}$) is typical of pancreatic ascites.

Triglycerides

↑ in chylous ascites.

Table 7.6 Ascitic fluid aspiration—practical procedure

Patient	Lies on his/her back, tilting towards side planned for aspiration
Percussion	Used to find shifting dullness in left or right lower quadrant
Chlorhexidine	Used to clean the site
Lidocaine	2–3mL of 1% used to infiltrate a site in the left or right lower quadrant where shifting dullness is detected. It should be possible to aspirate ascitic fluid using a standard 19G needle
50mL syringe	Sterile 19G needle placed through the same site to obtain the samples required
Rare complications	Include bowel perforation, 2° bacterial peritonitis, or haemorrhage

Respiratory medicine

- Airway hyperresponsiveness test or histamine/methacholine challenge test 536
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Airway hyperresponsiveness test or histamine/methacholine challenge test

Clinical indications

Suspected asthma in patients with normal spirometry.

Patient preparation

1. Explain the procedure to the patient.
2. Obtain informed consent.
3. Warn that wheezing and shortness of breath (SOB) may occur and that a bronchodilator may be given.
4. Baseline FEV₁ measured.
5. Patient breathes in a nebulized aerosol of histamine (or methacholine) of ↑ concentrations. This stimulates bronchoconstriction in a dose-dependent manner.
6. The FEV₁ is measured after each dose.
7. Patient must remain in the department for 30min following the procedure to observe any delayed reactions.

Possible results

The percentage fall in FEV₁ from baseline is plotted against the dose of inhaled histamine on a logarithmic scale. A dose-response curve is constructed, and the provocation concentration (PC) of inhaled histamine required to reduce the FEV₁ by 20% (PC₂₀) can be derived by linear extrapolation. This figure has been arbitrarily chosen to assess degrees of bronchial reactivity for ease of comparison and safety.

Interpretation

- Asthma suggested by PC₂₀ <8mg/mL (see Fig. 8.1).
- A direct relationship exists between the severity of asthma and requirement for medication, and the PC₂₀ value as an index of bronchial hyperresponsiveness.
- Non-asthmatic subjects almost always have a PC₂₀ >8mg/mL.

Advantages over other tests

- Easy to do.
- Cheap.
- Non-invasive.
- Quick.
- Reproducible.
- Safe—bronchoconstriction may be reversed by an inhaled β-adrenergic agonist. It is important that personnel performing the test are able to recognize severe bronchospasm and that resuscitation equipment is available.

Contraindications

- Documented cholinergic hypersensitivity, e.g. cholinergic urticaria or angio-oedema, or both.
- Allergy to histamine/methacholine.

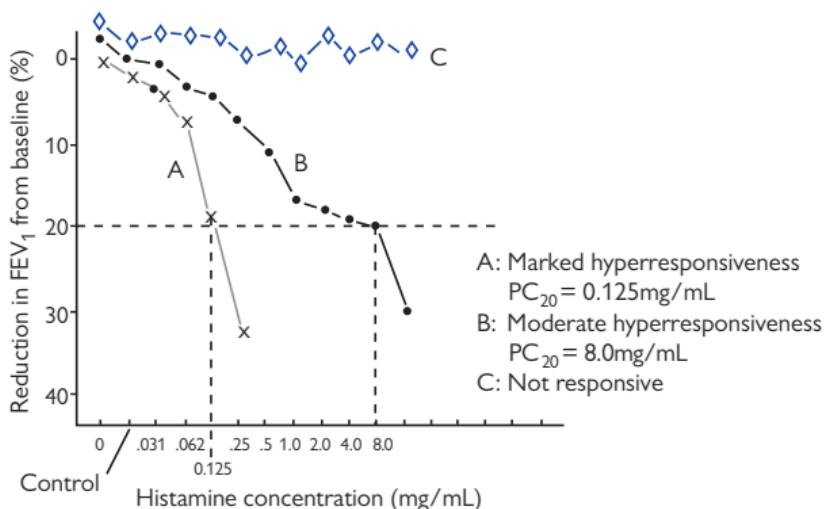


Fig. 8.1 Differing responses to varying concentrations of histamine.

- Inability to perform acceptable-quality spirometry.
- Unstable cardiac status, e.g. recent MI, arrhythmia, or heart failure.
- Uncontrolled hypertension (systolic BP >200 or diastolic BP >100).
- Pregnancy.
- Severe baseline obstruction with $FEV_1 <80\%$ predicted or <1.5L.

Ancillary tests

- PEFR chart: diurnal variation.
- Sputum cytology: eosinophilia.
- Exhaled nitric oxide (NO): elevated levels.

Pitfalls

- Bronchial hyperresponsiveness in asthma is not a static phenomenon and may vary widely from day to day.
- May change quite markedly without any change in symptoms (and vice versa).
- Represents only one component contributing to the symptomatology of asthma. Others include airway oedema and mucus hypersecretion.

Further reading

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 Pratter MR, Irwin S. The clinical value of pharmacologic bronchoprovocation challenge. *Chest* 1984; **85**: 260–5.
 Smith CM, Anderson SD. Inhalation provocation tests using nonisotonic aerosols. *J Allergy Clin Immunol* 1989; **84**: 781–90.

Arterial blood gas sampling

Clinical indications

- Breathlessness (acute or chronic).
- Cardiorespiratory failure.
- Metabolic disturbance.
- Poisoning with drugs.
- Acute asthma with O₂ saturation <92% (on air).

⊕ OHCM 10e, p. 189, p. 665.

Patient preparation

- Informed consent (verbal usually satisfactory).
- Common sites: radial/brachial/femoral arteries.

Contraindications to radial

- Absent ulnar circulation (Allen's test).
- Arteriovenous (AV) fistula for dialysis.
- Fractured wrist.
- Poor peripheral circulation.

Contraindications to brachial

- AV fistula.
- Fractured elbow.
- Poor peripheral circulation.

Contraindications to femoral

- Presence of graft/extensive vascular disease.

Procedure

1. Identify the pulse.
2. Clean the skin with an alcohol swab.
3. Confirm the position of maximum pulsation with the non-dominant hand.
4. LAN reduces pain.
5. Insert a 23G needle attached to a heparinized 2mL syringe.
6. If using low-resistance syringe, this will fill automatically; otherwise aspirate gently.
7. Remove the needle, and apply firm, gentle pressure with a cotton wool ball for 5min.
8. Expel air bubbles from the sample.
9. Label the specimen.

Possible results

- Hypoxaemia with normal CO₂.
- Hypoxaemia with ↑ CO₂.
- Normoxaemia with ↓ CO₂.
- Metabolic acidosis vs compensation.

⊕ OHCM 10e, p. 162, p. 189, p. 771.

Interpretation

Start by looking at the pH. Next check whether CO₂ fits with the pH change; if so, the 1° problem is respiratory. Then check for any metabolic compensation or for a combined respiratory and metabolic process. If CO₂ is not consistent with the pH change, the 1° disturbance is metabolic and you should check whether there is any respiratory compensation. To assess

oxygenation properly, it is essential to record the patient's inspired O_2 concentration (FiO_2) at time of sampling.

Advantages over other tests

- Easy, quick, cheap.
- No real alternative for assessing CO_2 or acid–base balance.
- Greater precision in upper ranges of arterial O_2 saturation (SaO_2) curve (see Fig. 8.2).

Ancillary tests

- **Pulse oximetry:** gives indication of oxygenation status, but not CO_2 levels.
- Arterialized blood sampling.

Complications

- Haematoma.
- Nerve damage.
- Inadvertent venous sampling.

Pitfalls

- If the sample is to be analysed in a laboratory with >5 min transit time, it should be kept on melting ice to slow the metabolic activity of the cells.
- Avoid arterial puncture, if possible, in patients on anticoagulant therapy, those with bleeding disorders, or those who have received thrombolytics in previous 24 h.
- Failure to note the FiO_2 at time of sampling will lead to difficulty in interpretation and potential therapeutic errors.

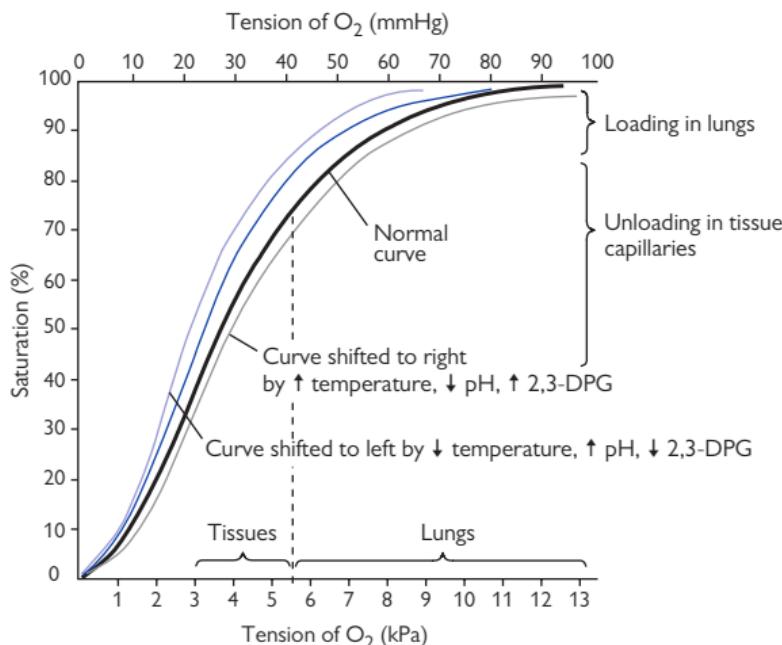


Fig. 8.2 Dissociation curve for oxyhaemoglobin.

Further reading

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- Syabbalo N. Measurement and interpretation of arterial blood gases. *Br J Clin Pract* 1977; 51: 173–6.

Pleural aspiration (diagnostic)

Clinical indications

- Unilateral pleural effusion detected clinically and with imaging, e.g. CXR, USS, CT chest.
- Aspiration should not be performed routinely for bilateral pleural effusions if a transudate is suspected.

Patient preparation

1. Informed written consent.
2. Aseptic technique.
3. Posterior or axillary approach if effusion large (be guided by bedside USS).
4. Clean skin with antiseptic solution.
5. Infiltrate with LAn (1% lidocaine).
6. Insert a fine-bore 21G (green) needle attached to a 50mL syringe under USS guidance. Note: ensure the needle enters immediately above the rib to avoid the neurovascular bundle.
7. Aspirate fluid. If no fluid, then try adjusting the angle of the needle with USS guidance.
8. Remove the needle and apply a plaster.
9. Post-aspiration CXR.

Possible results

- Pleural fluid is normally straw-coloured and odourless.
- Pleural fluid analysed for protein, glucose, LDH, microbiology, cytology, and pH.
- If heavily bloodstained, suspect malignancy, pulmonary infarction, or trauma. A traumatic tap will become progressively less bloodstained.
- If pus present: empyema.
- If creamy, opalescent fluid: chylothorax (lymphoma, trauma to the thoracic duct, yellow nail syndrome, lymphangioleiomyomatosis) or pseudo-chylothorax, e.g. in TB or RhA.

Interpretation

Measure blood LDH and total protein simultaneously for Light's criteria; satisfying any ONE criterion means it is exudative:

- Pleural total protein/serum total protein >0.5 .
- Pleural LDH/serum LDH >0.6 .
- Pleural LDH $>$ two-thirds of upper limit of normal for serum LDH.

(See Table 8.1.)

Advantages over other tests

- Quick and easy.
- Cheap.
- Relatively non-invasive.
- Provides cytological, microbiological, and biochemical data.

Table 8.1 Interpretation

Cytology	+ve in ~60%, especially carcinoma of lung/breast Immunohistochemistry should be used to differentiate between malignant cell types and may guide oncological therapy
Protein	Exudate >30g/L protein Transudate <30g/L protein
pH	<7.0: empyema, oesophageal rupture >7.0 and <7.3: collagen disorders, TB, malignancy, empyema
Glucose	↓ in RhA, TB, malignancy, infection
Microbiology	Organisms, ZN stain
Eosinophilia	>10% in benign asbestos effusions, parasitic hydropneumothorax
Neutrophilia	>1.0 × 10 ⁹ /L in acute inflammation, e.g. pneumonia, infarction
Lymphocytosis	Chronic effusions, e.g. TB, malignancy, lymphoma, or RhA
Amylase	↑↑↑ in pancreatitis ↑ in oesophageal rupture (salivary amylase) ↑ in malignancy
ANA	>1:160 virtually diagnostic of SLE
LDH	↑ in infection
RF	+ve in RhA
Complement	↓ in RhA, SLE, malignancy, infection

Ancillary tests

- Thoracoscopy (medical or surgical).
- Percutaneous pleural biopsy.

Pitfalls

- Traumatic tap.
- May be difficult to locate a loculated effusion, even with USS.

Complications

- Haemorrhage.
- Pneumothorax.
- Visceral injury (e.g. liver).
- Large volumes of pleural fluid (>1000mL) should not be aspirated at one time due to risk of inducing re-expansion pulmonary oedema.

Further reading

- Hooper C, Lee G, Maskell N. Investigation of a unilateral pleural effusion in adults: British Thoracic Society pleural disease guideline 2010. *Thorax* 2010; **65**: ii4–17.
 Light RW. Diagnostic principles in pleural disease. *Eur Respir J* 1997; **10**: 476–81.

Epworth test/Epworth sleepiness scale

Clinical indications

Screening tool for OSA. Measures general level of daytime sleepiness.

Patient preparation

1. Ask patient to fill in questionnaire.
2. Subject rates on a scale of 0–3 the chance that, as part of his usual life in recent times, he would doze in each of eight different situations.

Use the following scale to choose the most appropriate number for each situation:

0 = Would NEVER doze

1 = SLIGHT chance of dozing

2 = MODERATE chance of dozing

3 = HIGH chance of dozing

Situation

- Sitting and reading.
- Watching TV.
- Sitting inactive in a public place (e.g. theatre or a meeting).
- As a passenger in a car for an hour without a break.
- Lying down to rest in the afternoon when circumstances permit.
- Sitting and talking to someone.
- Sitting quietly after lunch without alcohol.
- In a car, whilst stopped for a few minutes in the traffic.

Possible results

Epworth sleepiness scale (ESS) score is the sum of eight item scores and can range from 0 to 24.

Interpretation

Clinically normal score <10. Each ESS item gives an estimate of sleep propensity in one of eight specific situations, whereas the total ESS score gives a measure of more general average sleep propensity. Does not measure 'subjective' sleepiness.

Advantages over other tests

- Cheap.
- Easily administered.

Ancillary tests

- Polysomnography/Visi-Lab studies.
- Stanford sleepiness scale.
- Multiple sleep latency test.
- Maintenance of wakefulness test.

Pitfalls

Limited by patient's ability to read and comprehend the questionnaire and answer questions honestly.

Exercise testing

Clinical indications

- To confirm that reduced exercise tolerance exists.
- To determine the degree of impairment and disability.
- To investigate which system appears responsible for the reduction.
- To evaluate treatment results.
- To plan rehabilitation.

Patient preparation

1. Evaluate the patient's medical history for contraindications to test.
2. Warn the patient of cardiovascular complications (e.g. mortality 1:10,000 tests).
3. Obtain informed written consent.
4. Patient to wear comfortable clothes and shoes.
5. Monitoring: ECG, O_2 saturation, BP.
6. Exercise: treadmill/bike/free run on flat surface.
7. Steady state 5–12min walking test (usually 6min) or stepped stress test.
8. During a maximal exercise test, the patient should be able to achieve 85–90% of predicted maximum heart rate.

Contraindications

- Unstable myocardium (recent MI, unstable angina, arrhythmias, severe valvular heart disease, congestive heart failure).
- Acute asthma.
- Acute febrile illness.
- Uncontrolled diabetes.
- Systemic hypertension (systolic >200mmHg, diastolic >120mmHg).

Possible results

- Cardiac response: ECG, BP, cardiac output, and stroke volume response.
- Ventilatory response: ventilatory limitation (reduced breathing reserve), pattern of response, V_T , minute volume, respiratory rate.
- Gas exchange: ABGs, A-a gradient, P_aCO_2 .
- Ventilatory (anaerobic) threshold: normal or ↑.
- $VO_{2\max}$ (maximum O_2 uptake): normal or ↑.

Interpretation

Useful in making the distinction between exertional dyspnoea 2° to lung disease or fatigue 2° to cardiac dysfunction. In patients known to have asthma, exercise test is +ve in 75% of cases with a single treadmill run and 97% if the test is repeated in –ve responders. A fall of 10% or more from baseline in PEFR or FEV_1 suggests exercise-induced asthma.

Advantages over other tests

Best assessment of exercise capacity. Adds to diagnostic accuracy quantitatively (measurement of work capacity, $VO_{2\max}$, and sustained work capacity) and qualitatively (identification of the cause of exercise limitation).

Ancillary tests

- Static lung function tests.
- For asthma: exhaled NO levels, histamine/methacholine inhalation challenges, and PEFR diary.

Pitfalls

- Dependent on patient effort and compliance.
- Not suitable for patients with severe objective measurement of respiratory impairment.

Complications

- ► *Bronchospasm*: usually easily reversed with an inhaled β -adrenergic agonist.
- ► *Cardiac arrhythmias/arrest*: appropriate equipment and drugs should be available in the exercise testing area. Personnel should be trained in basic and advanced cardiopulmonary resuscitation.

Further reading

- Hughes JMB, Pride NB (eds.). *Lung Function Tests: Physiological Principles and Clinical Applications*. Philadelphia: WB Saunders, 1999.
- König P. Exercise challenge: indications and techniques. *Allergy Proc* 1989; **10**: 345–8.
- Sue DY. Exercise testing in the evaluation of impairment and disability. *Clin Chest Med* 1994; **15**: 369–87.

Exhaled nitric oxide fraction

Clinical indications

Asthma

- Diagnosis.
- Assessment of severity.
- Assessment of treatment response.

Patient preparation

1. Patient breathes directly into the NO analyser.
2. Perform three tests each time, and record the largest value.

Possible results

Exhaled nitric oxide fraction (FeNO) can be detected by chemiluminescence in the range of 5–300ppb.

Interpretation

Elevated FeNO levels are associated with eosinophilic asthma:

- <25ppb: low probability. Asthma unlikely. Consider other possible aetiologies for symptoms.
- 25–50ppb: intermediate probability. Based on clinical judgement, asthma is a possible diagnosis. Consider initiating inhaled corticosteroids and monitoring further FeNO levels.
- >50ppb: high probability. Asthma likely in an appropriate clinical context. Symptomatic patients are likely to benefit from inhaled corticosteroids.

Advantages over other tests

- Simple and quick.
- Non-invasive.
- Objective measure of response to treatment.

Ancillary tests for diagnosis of asthma

- PEFR diary.
- Histamine/methacholine inhalation challenges.
- Sputum cytology: eosinophilia.

Pitfalls

- A –ve FeNO does not exclude asthma; asthma may be caused by neutrophilic airways inflammation.
- FeNO levels are reduced by smoking.

Further reading

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- Saito J¹, Gibeon D, Macedo P, Menzies-Gow A, Bhavsar PK, Chung KF. Domiciliary diurnal variation of exhaled nitric oxide fraction for asthma control. *Eur Respir J* 2014; **43**: 474–84.

Fibreoptic bronchoscopy

Clinical indications

- Any patient with persistent/substantial haemoptysis.
- Suspected lung neoplasm:
 - For histology.
 - To assist with staging.
- Infection:
 - To identify organism.
 - To determine course of recurrence/persistence.
- Interstitial lung disease (ILD):
 - To obtain BAL for cytology; useful in diagnosis of sarcoidosis and hypersensitivity pneumonitis.
 - To obtain endobronchial biopsies (EBBs); useful in diagnosis of sarcoidosis.
 - To obtain transbronchial lung biopsies (TBLBs); useful in diagnosis of sarcoidosis, hypersensitivity pneumonitis, and lymphangitis carcinomatosa.

Pre-assessment

- CXR.
- FBC.
- Spirometry. Patients with obstruction may require nebulized bronchodilators pre-procedure.
- Clotting.
- Pulse oximetry.
- ABGs on air if hypoxaemia suggested by O₂ saturation.

Patient preparation

Endoscopy suite

1. Patient informed and consented. Fasted from solids for 6h and liquids for 3h.
2. Frontal approach with patient lying on couch, trunk at 45°.
3. IV access obtained.
4. Basic monitoring—pulse oximeter and BP.
5. Supplementary O₂ via single nasal cannula.
6. IV sedation: midazolam/alfentanil.
7. Topical lidocaine spray to nose and pharynx.
8. Bronchoscope lubricated with 2% lidocaine gel and passed via nostril or mouth guard.
9. Further boluses of lidocaine (1%) applied to cords and bronchial tree.

Contraindications

- Patients at risk of pulmonary and cardiovascular decompensation, e.g. recent MI, unstable angina, arrhythmias, severe valvular heart disease, uncontrolled congestive heart failure, pulmonary hypertension, severe hypoxaemia.

- Patients at high risk of bleeding, e.g. patients on antiplatelet or anticoagulant therapy or patients with coagulopathies such as thrombocytopenia.
- Bronchoscopists should be cautious when sedating patients with COPD.

Possible results

- Direct inspection of the nares, nasopharynx, and oropharynx.
- Assess movement of vocal cords (ask patient to say 'eee').
- Direct inspection of the bronchial tree down to subsegmental level.
- Able to take endobronchial/transbronchial biopsies and brushings.
BAL: wedge the tip of the bronchoscope into a subsegmental bronchus and instil aliquots of 50mL of sterile saline into the distal airway. Aspirate immediately, aiming to obtain ~50% of instilled volume.
Bronchial washings (BWs) are performed in a similar fashion with 10–20mL of sterile saline instilled into the bronchus.

Interpretation

(See Table 8.2.)

Advantages over other tests

- Well tolerated.
- Quick, cheap.
- Provides histological and immunobiological confirmation (to back up CT/CXR diagnosis).
- Therapeutic—removal of retained secretions, mucus plugs, blood clots.

Table 8.2 Interpretation

Histology	Tumours ILD	EBB TBLB
Cytology	Tumours ILD	Brush/BAL/BW BAL
Microbiology	Gram stain ZN stain Stain for <i>Pneumocystis jiroveci</i> Fungi Virus PCR for <i>Legionella</i> and atypical organisms	BAL/BW

Some appearances diagnostic.

Screening: X-ray-guided biopsy of non-visible lesions.

Ancillary tests

- Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA): allows assessment and biopsy of mediastinal lymph nodes and tumours.
- Rigid bronchoscopy: under GAn:
 - Allows therapeutic interventions, e.g. laser therapy, cryotherapy, stent insertion, debulking of large tumours in the major airways, and better control of haemorrhage.
 - Preferable for removal of foreign body.

Side effects and complications

- Pneumothorax with transbronchial biopsies.
- Haemorrhage post-biopsy.
- Hypoxaemia.
- Post-bronchoscopy fever with BAL (usually resolves in a few hours with paracetamol, but antibiotics may be necessary).
- If performed in a day case unit with sedation, the patient will not be able to drive home and will need a responsible adult in attendance overnight.

Pitfalls

- Only visualizes proximal airways.
- Biopsies may be inadequate or from necrotic areas.
- Not easy to biopsy submucosal tumour.
- Needs good-quality cytology preparation.

Further reading

Du Rand IA, Blaikley J, Booton R, et al. British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults. *Thorax* 2013; **68**: i1–44.

Yasufuku K, Nakajima T, Chiyo M, Sekine Y, Shibuya K, Fujisawa T. Endobronchial ultrasonography: current status and future directions. *J Thorac Oncol* 2007; **2**: 970–9.

'Fitness to fly' assessment

Clinical indications

The following groups of patients should be referred to a chest physician for assessment before flying:

- Severe COPD ($\text{FEV}_1 < 30\%$) or asthma.
- Severe (vital capacity (VC) $< 1\text{L}$) restrictive disease (including chest wall and respiratory muscle disease).
- Cystic fibrosis.
- Recent pneumothorax.
- Bullous lung disease.
- Pre-existing requirement for ventilator support or O_2 therapy.
- Co-morbidity with conditions worsened by hypoxaemia (e.g. coronary artery disease, pulmonary hypertension, cyanotic congenital heart disease, cerebrovascular disease).

Patient preparation

1. Take a history and examine the patient, with particular reference to cardiorespiratory disease and previous symptoms during flights.
2. Perform spirometry.
3. Measure SpO_2 by pulse oximeter. ABGs should be performed if hypercapnia is suspected.
4. A hypoxic challenge test (breathing 15% FiO_2 for 20min via a face mask, followed by ABG sampling) may be necessary, depending on the results of the initial assessment.

Possible results

(See Tables 8.3 and 8.4.)

Table 8.3 Results of initial assessment breathing air

Screening result	Recommendation
Sea-level $\text{SpO}_2 > 95\%$	O_2 not required
Sea-level SpO_2 92–95% and no risk factor*	O_2 not required
Sea-level SpO_2 92–95% and additional risk factor*	Perform hypoxic challenge and ABGs
Sea-level $\text{SpO}_2 < 92\%$	In-flight O_2

* Additional risk factors: hypercapnoea; $\text{FEV}_1 < 50\%$ predicted; lung cancer; lung fibrosis; kyphoscoliosis; respiratory muscle weakness; cerebrovascular or cardiac disease; within 6 weeks of discharge for an exacerbation of chronic lung or cardiac disease; recent pneumothorax; risk of (or previous) VTE disease; recent pneumothorax; and patients with previous significant respiratory symptoms associated with air travel.

Table 8.4 Results of hypoxic challenge test

Hypoxic challenge result	Recommendation
$\text{PaO}_2 \geq 6.6\text{kPa} (>50\text{mmHg})$ or $\text{SpO}_2 \geq 85\%$	O_2 not required
$\text{PaO}_2 < 6.6\text{kPa} (<50\text{mmHg})$ or $\text{SpO}_2 < 85\%$	In-flight O_2 (flow rate 2L/min)

PaO_2 , arterial oxygen tension; SpO_2 , oxygen saturation.

Interpretation

Identifies most patients requiring in-flight O_2 therapy.

Ancillary tests

- Exercise testing.
- In complex cases, patients may require testing in a hypobaric chamber.

Pitfalls

- Infectious patients should not fly.
- Even with in-flight O_2 therapy, travel cannot be guaranteed to be safe.

Further reading

- Ahmedzai S, Balfour-Lynn IM, Bewick T, et al.; British Thoracic Society Standards of Care Committee. Managing passengers with stable respiratory disease planning air travel: British Thoracic Society recommendations *Thorax* 2011; **66**(Suppl 1): i1–30.
 Coker RK, Shiner RJ, Partridge MR. Is air travel safe for those with lung disease? *Eur Respir J* 2007; **30**: 1057–63.

Flow-volume loops/maximum expiratory flow–volume curve

Clinical indications

Patient in whom COPD/small airways disease or upper airway obstruction is suspected.

Patient preparation

- Advised to wear comfortable, loose clothing.
- Technician explains procedure to patient.
- Mouthpiece in position; patient breathes in maximally and then out as hard and fast as possible.
- Three acceptable manoeuvres should be performed. Patients must perform the test with maximal effort each time, and the results should be similar for each of the three attempts.

Interpretation

Particularly useful in recognizing patients with narrowing of the central airway (larynx and trachea). Narrowing at this site has the greatest effect on maximum expiratory flow and also on maximum inspiratory flow, giving rise to a characteristic appearance. Also identifies patients with reduced elastic recoil (bullae, emphysema) or reduced airway lumen (asthma, COPD, bronchiolitis).

Oscillation of flow gives a ‘sawtooth’ pattern. This usually signifies instability of the upper airway and has been observed in OSA, thermal injury to the airway, bulbar muscle weakness, extrapyramidal neuromuscular disorders, upper airway stenosis/tracheomalacia, and snoring.

(See Fig. 8.3.)

Advantages over other tests

- Allows early detection of small airway disease—more sensitive than FEV₁ alone.
- Reproducible.

Ancillary tests

- Spirometry.
- Transfer factor.

Pitfalls

- Dependent on patient understanding and maximal effort.
- Infection control necessary in patients with known or suspected transmissible disease (e.g. active pulmonary TB).

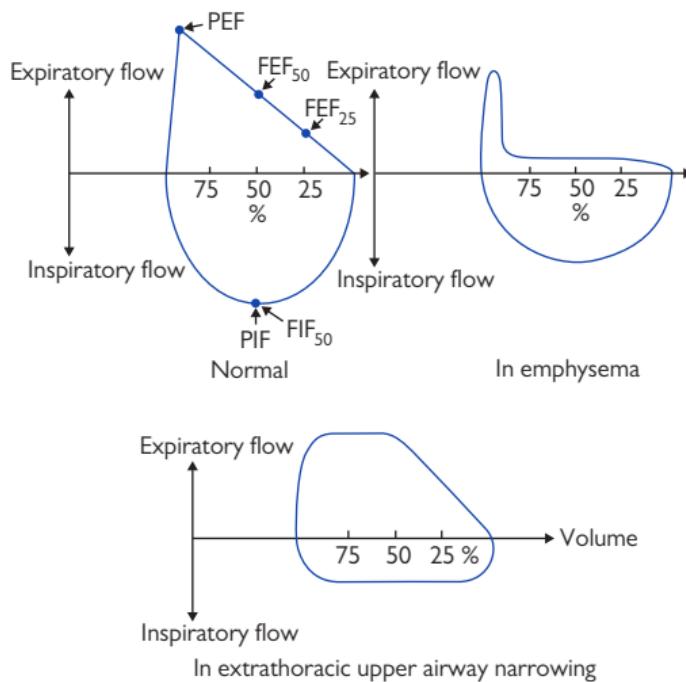


Fig. 8.3 Flow-volume loops.

Nijmegen questionnaire

Clinical indications

Hyperventilation syndrome

- Diagnosis.
- Assessment of severity.
- Assessment of response to therapy.

Patient preparation

1. Ask patient to fill in questionnaire.
2. Subject rates on a scale of 0–4 the frequency of 16 different symptoms.

Use the following scale to choose the most appropriate number for each symptom:

- 0 = Would NEVER experience that symptom
1 = RARELY experience that symptom
2 = SOMETIMES experience that symptom
3 = OFTEN experience that symptom
4 = VERY OFTEN experience that symptom

Symptoms

- Chest pain.
- Feeling tense.
- Blurred vision.
- Dizzy spells.
- Feeling confused.
- Faster or deeper breathing.
- SOB.
- Tight feeling in the chest.
- Bloated feeling in the stomach.
- Tingling fingers.
- Unable to breathe deeply.
- Stiff fingers or arms.
- Tight feelings around the mouth.
- Cold hands or feet.
- Heart racing (palpitations).
- Feelings of anxiety.

Possible results

Nijmegen score is the sum of 16 item scores and can range from 0 to 64.

Interpretation

Clinically normal score <23.

Advantages over other tests

- Cheap.
- Easily administered.

Ancillary tests

- ABGs to identify hypocapnia and respiratory alkalosis.
- Hyperventilation provocation test. (Ask the patient to over-breathe for several minutes to see if it reproduces symptoms.)

Pitfalls

Limited by the patient's ability to read and comprehend the questionnaire and answer questions honestly.

Further reading

Van Dixhoorn J, Duivenvoorden HJ. Efficacy of Nijmegen Questionnaire in recognition of the hyperventilation syndrome. *J Psychosom Res* 1985; **29**: 199–206.

Peak flow charts

Clinical indications

Asthma

- Diagnosis.
- Assessment of severity.
- Assessment of treatment response to β_2 -agonists.

Occupational asthma

- Diagnosis.

Patient preparation

Patients need to be equipped with a peak flow meter and a peak flow and symptom diary, and have a thorough understanding of how to use them.

Guidelines to patients should include

1. Perform the test standing (if possible).
2. Hold the meter lightly and do not interfere with the movement of the marker.
3. Perform three tests each time and record the largest value.

Readings should be taken at various times throughout the day. Limiting the patient to two readings in each day may aid compliance. In occupational asthma, 2-hourly peak flow readings are required during the day and evening.

Possible results

- *Diurnal variability:* as measured by the lowest PEFR value (usually on waking) and the highest PEFR value (usually in the afternoon/evening).
- Patient symptoms and PEFR can be examined together.

Interpretation

Diurnal variation is ↑ in patients with asthma, compared with normals (amplitude >20%), i.e. peak flow falls significantly overnight and in the early morning (see Figs 8.4 and 8.5).

Advantages over other tests

- Cheap.
- Saves time of the respiratory physician and technician.
- Reproducible.
- Objective measure of response to treatment.

Ancillary tests for diagnosis of asthma

- Airway hyperresponsiveness test or histamine/methacholine challenge test.
- Exercise test.
- Sputum cytology: eosinophilia.
- FeNO.

Peak Flow Meter Record

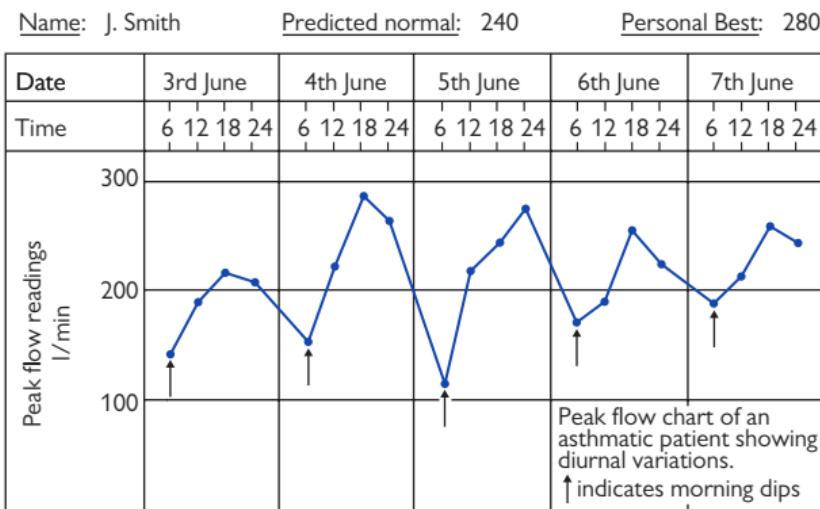


Fig. 8.4 An example of a patient's peak flow record.

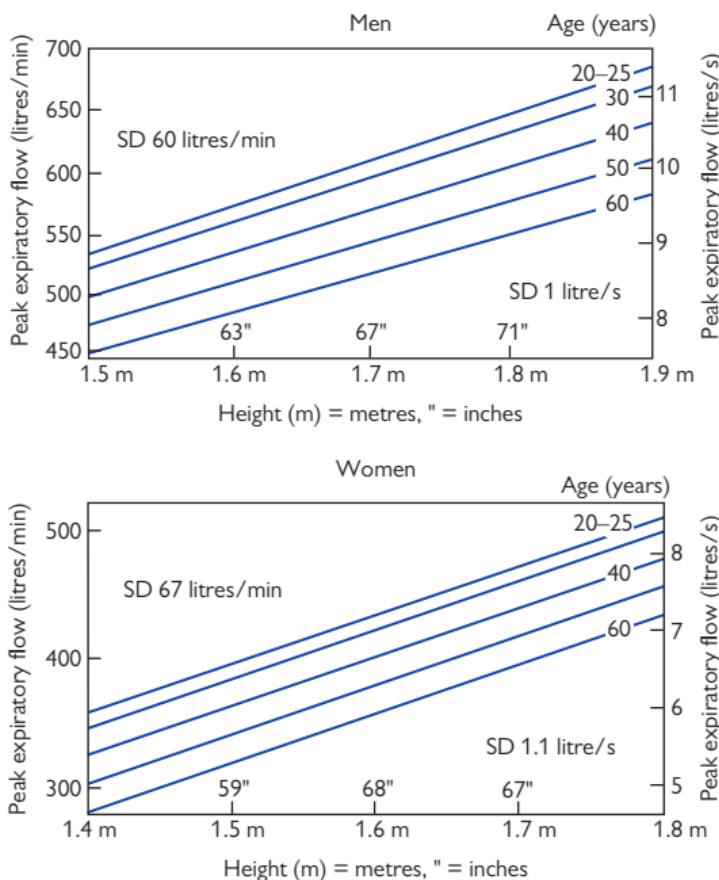


Fig. 8.5 Peak flow readings.

Pitfalls

- Not all asthma exacerbations are associated with ↑ diurnal variability.
- Calculating diurnal variation can be complicated and tedious.
- Time of recording or recent use of β_2 -agonist drugs may result in minor changes in peak flow but can cause large errors in diurnal variability.
- Dependent on patient understanding, cooperation, and accuracy.

Further reading

- Hetzl MR, Clark TJ. Comparison of normal and asthmatic circadian rhythms in peak expiratory flow rate. *Thorax* 1989; 35: 732–8.
- Reddel H, Jenkins C, Woolcock A. Diurnal variability—time to change asthma guidelines? *BMJ* 1999; 319: 45–7.

Percutaneous pleural biopsy

Clinical indications

- Pleural effusion of unknown aetiology, especially if TB or malignancy suspected.

Note: if malignancy is suspected and areas of pleural nodularity are shown on a contrast-enhanced CT, an image-guided cutting needle is the percutaneous pleural biopsy method of choice.

Abrams' needle biopsies are only diagnostically useful in areas with a high incidence of TB.

Patient preparation

1. Informed written consent.
2. Aseptic technique.
3. Posterior or mid-axillary approach using USS guidance.
4. Skin cleaned with antiseptic solution.
5. Lidocaine (1%) infiltrated in rib interspace. Check pleural fluid aspirated.
6. Stab incision with a narrow scalpel.
7. Insert closed Abrams' needle (requires firm pressure to be applied until it penetrates the parietal pleura—*take care not to apply too much force*).
8. Attach a 50mL syringe.
9. Twist open the Abrams' needle.
10. Aspirate fluid to ensure needle in pleural space.
11. Withdraw needle at angle to chest wall until side hole 'snags' parietal pleura.
12. Maintain lateral pressure and rotate to close hole, thereby cutting biopsy. Remove needle and extract biopsy tissue.
13. Repeat with samples taken from 3, 6, and 9 o'clock position (avoid the 12 o'clock position to avoid the neurovascular bundle).
14. May require suture to close.
15. Apply dressing.
16. Obtain CXR post-procedure.
17. Place samples in formalin for histological examination, and saline for microbiological culture.

Possible results

- Slivers of white pleural tissue.
- Examine histology and culture for acid- and alcohol-fast bacilli (AAFB).

Interpretation

- Malignant mesothelioma may be diagnosed on histology, especially with addition of immunohistochemical methods looking at tumour cell markers.
- More sensitive than pleural fluid aspiration in diagnosing TB.
- Carcinoma cells may arise from direct spread from lung 1° or represent 2° carcinoma. In either case, management is palliative.

Advantages over other tests

- Easy, quick, cheap; more reliable than diagnostic pleural aspiration.
- Less invasive than thoracoscopy for diagnosis of TB.

Ancillary tests

- Diagnostic pleural fluid aspiration.
- Thoracoscopy.
- Image-guided cutting needle pleural biopsy is more sensitive than Abrams' pleural biopsy at diagnosing malignancy.

Complications

- Pneumothorax.
- Haemothorax.

Pitfalls

- Skeletal muscle biopsy— inadequate specimen.
- Damage to neurovascular bundle.
- Diagnosis of mesothelioma may remain equivocal.

Further reading

- Benamore RE, Scott K, Richards CJ, Entwistle JJ. Image-guided pleural biopsy: diagnostic yield and complications. *Clin Radiol* 2006; **61**: 700–5.
- Kirsch CM, Kroe DM, Azzi RL, Jensen WA, Kagawa FT, Wehner JH. The optimal number of pleural biopsy specimens for a diagnosis of tuberculous pleurisy. *Chest* 1997; **112**: 702–6.
- Kirsch CM, Kroe DM, Jensen WA, Kagawa FT, Wehner JH, Campagna AC. A modified Abrams needle biopsy technique. *Chest* 1995; **108**: 982–6.
- Maskell NA, Gleeson FV, Davies RJ. Standard pleural biopsy versus CT-guided cutting-needle biopsy for diagnosis of malignant disease in pleural effusions: a randomised controlled trial. *Lancet* 2003; **361**: 1326–30.
- Prakash UB, Reiman HM. Comparison of needle biopsy with cytologic analysis for the evaluation of pleural effusion: analysis of 414 cases. *Mayo Clin Proc* 1985; **60**: 158–64.

Polysomnography

Clinical indications

Note: symptoms alone do not help predict which patient with sleep disturbance has OSA.

- Patients with low-probability sleep disorder, e.g. snores with no other features suggestive of OSA.
- Patients with high-probability sleep disorder, e.g. typical symptoms and physiognomy. Need study for diagnosis and assessment of severity.
- Known OSA—assessing treatment response.
- Assessment of nocturnal hypoventilation syndromes, e.g. scoliosis.
- Patients with unexplained sleep–wake disorders.

Patient preparation

The patient is admitted to the sleep laboratory in the early evening. Monitoring is explained and attached, using some combination shown in Table 8.5.

Table 8.5 Monitoring combinations

Sleep	EEG Electro-oculogram EMG	
Oxygenation	O ₂ saturation probe (ear or finger)	
Breathing pattern	Airflow by: Thoracoabdominal movement by:	Thermocouples Thermistor End-tidal CO ₂ pressure Inductance plethysmography Impedance Strain gauge
Miscellaneous	Snoring Leg movement by:	Microphone EMG Video Movement detector

Possible results

Original diagnosis of OSA based on polysomnography—overnight recording of sleep, breathing patterns, and oxygenation. It is relatively expensive, and most centres use a combination of video to assess the quality of sleep and identify transient arousals and paroxysmal leg movement disorder (PLMD), and oximetry (to detect desaturation), plus some form of measuring the breathing pattern to detect hypopnoea.

Interpretation

OSA diagnosed in the context of multiple (typically >15/h) hypopnoeic/apnoeic events occurring throughout the night and resulting in desaturation.

Advantages over other tests

Demonstrates a number of hypopnoeic (reduction in breathing) or apnoeic (absence of breathing) events occurring per hour. May be used to monitor effectiveness of treatment.

Ancillary tests

- ESS.

Pitfalls

- Expensive.
- Time-consuming.
- Most sleep study systems are poorly validated; therefore, need expert interpretation of results to consider false +ves and -ves.
- Patients need to sleep for >3h/night and have rapid eye movement (REM) sleep.

Further reading

British Thoracic Society (2009). *IMPRESS service specification for investigation and treatment of obstructive sleep apnoea syndrome.*  <https://www.brit-thoracic.org.uk/document-library/clinical-information/sleep-apnoea/impres-service-specification-for-investigation-and-treatment-of-osas/>.

Pulse oximetry

Clinical indications

- Any acutely unwell patient: avoids repeated blood gas measurements, provided that hypercapnia is absent.
- Monitoring of long-term oxygen therapy (LTOT): not suitable for initial assessment.
- Assessment of nocturnal FiO_2 and screening for sleep apnoea syndrome: identification of nocturnal desaturations.
- Exercise walk test.

Patient preparation

1. Clean probe site (ear or finger).
2. Ensure good contact of probe with warm, well-perfused skin.
3. Avoid nail-varnished fingers.

Possible results

- SpO_2 expressed as %.

Interpretation

- Respiratory failure unlikely if $\text{SpO}_2 > 92\%$ on air.
- Provides almost immediate arterial O_2 saturation data.
- Must know the FiO_2 the patient is breathing.

Advantages over other tests

- Non-invasive.
- Easy, cheap.
- Instantaneous.
- Sensitive.
- Portable.

Ancillary tests

- ABG sampling.

Pitfalls

- Does not detect CO_2 levels.
- If COHb or methaemoglobin (MetHb) is present in the blood in elevated levels, the pulse oximeter will give a falsely elevated reading for the arterial O_2 saturation.
- ↑ in jaundice.
- Erroneous information if patient poorly perfused.
- Excessive patient movement can give false readings.

Skin prick tests

Clinical indications

- To evaluate atopy in asthmatic individuals.
- To assess the possible development of ABPA in patients with asthma/other long-standing lung disease.

Patient preparation

1. Explain what the test involves.
2. Use a pen to label the patient's forearm with the antigens to be tested, including +ve and -ve controls (alternatively, numbered adhesive tape may be used).
3. Clean the test area with an alcohol wipe.
4. Place a drop of antigen next to each corresponding label.
5. Use a lancet to puncture the skin. Repeat with other antigens using a new lancet each time.
6. Blot off excess antigen, taking care not to contaminate other test sites.
7. After 10min, measure any resulting wheal reactions using a ruler. Record the mean diameter in mm.

Possible results

A +ve result is indicated by a wheal and flare reaction of 3mm, providing there is no reaction at the -ve control site. -ve results are validated by a wheal reaction at the +ve control site.

Interpretation

A +ve result indicates sensitization to the allergen but does not necessarily mean that this allergen is responsible for the patient's symptoms. All tests should be carefully interpreted in light of the clinical history.

Advantages over other tests

- Cheap, quick.

Ancillary tests

- Specific IgE to allergens (especially where the history is not supported by skin test results).
- Total IgE (affects interpretation of weak +ve specific IgE results, which are less relevant if the total IgE is very high).
- Aspergillus precipitins (IgG).

Pitfalls

- False -ve results if the patient has taken an antihistamine within 5 days of the test.
- False +ve results with dermatographism or inflamed skin.

Spirometry

Clinical indications

- To evaluate symptoms, signs, or abnormal test results.
- Provide objective, quantifiable measures of lung function.
- Evaluate and monitor disease.
- Assess effects of environmental/occupational/drug exposures, both adverse (e.g. amiodarone) and beneficial (e.g. bronchodilators).
- Preoperative assessment.
- Employment/insurance assessment.
- Early detection of bronchiolitis obliterans in lung transplant patients.

Patient preparation

1. Explain what the test involves. Most respiratory technicians demonstrate the technique to ensure maximal effort and co-operation of the patient.
2. Patient must inhale fully before the test.
3. Exhale into the breathing tube. Encourage maximal effort with no breath-holding before the manoeuvre.
4. No cough/glottal closure in the first second.
5. Test should last at least 6s (may need up to 15s with obstruction).
6. No evidence of airflow leak/obstruction of mouthpiece.

Possible results

(See Table 8.6.)

Interpretation

At least three acceptable tracings should be obtained. Examine each tracing to ensure adequate effort is made by the patient, that it is reproducible, and that there are no artefacts (see Table 8.7).

Advantages over other tests

- Cheap, quick.
- Bedside/outpatient test.
- Reproducible.

Ancillary tests

- *Total lung capacity (TLC)*: to confirm interstitial disease with restrictive spirometry.
- *Pre- and post-bronchodilator studies*: an ↑ of 15% in FEV₁ and 20% in FVC suggest reversibility.

Pitfalls

- Need standardization of normal data for height, weight, age, sex, and race.
- Level at which a result may be considered abnormal is contentious, usually accepted to be outside the range of 80–120% of mean predicted.
- FEV₁ may remain relatively normal in early stages of generalized lung disease.

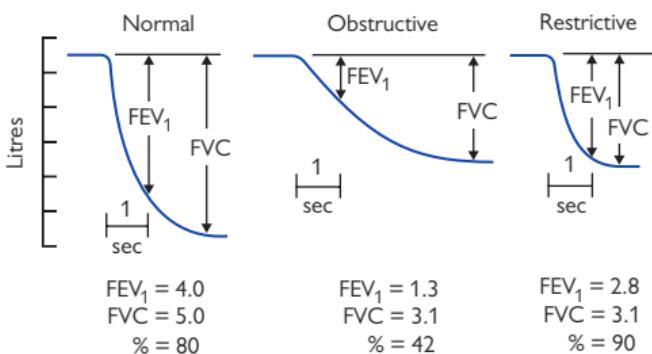
Table 8.6 Possible results

FEV_1	Forced expiratory volume in 1s Test of mechanical function of the lungs Depends on size and elastic properties of the lungs, calibre of the bronchial tree, and collapsibility of airway walls
FVC	Forced vital capacity

Table 8.7 Interpretation

FEV_1/FVC ratio	Index of the presence/absence of airflow limitation Young and middle-aged healthy non-smokers rate 75% Older normal patients ratio 70–75%
$FEV_1/FVC \downarrow$	Obstructive Classify severity using FEV ₁ expressed as % of predicted value, e.g. COPD, asthma
$FEV_1/FVC \leftrightarrow$ or \uparrow	Restrictive, but need reduced thin-layer chromatography (TLC) to confirm, e.g. lung fibrosis, chest wall problems, pulmonary effusion and oedema

If used for monitoring purposes, need an adequate baseline study.

**Fig. 8.6** Examples of spirograms.

- FEV₁/FVC ratio is a good guide to the presence of significant airway narrowing, but as disease progresses, both will fall and correlation with severity of disease is poor.
- Variability (noise) is greater in pulmonary function tests than in most other clinical laboratory tests because of the inconsistency of effort by patients.

For examples of spirograms, see Fig. 8.6 and → OHCM 10e, p. 163.

Further reading

Crapo RO. Pulmonary-function testing. *N Engl J Med* 1994; 331: 25–30.

Sputum microscopy and culture/sputum cytology

Clinical indications

Microbiology

- Productive cough with sputum.
- Infective exacerbations of any chronic lung disease.
- Pneumonia.

Cytology

- Suspected lung cancer: only in elderly/frail patients who are not fit for invasive investigation.
- Sputum eosinophilia in asthma.

Patient preparation

- Explain the need for a sputum sample.
- Provide suitable sputum pots.
- Early morning samples are best.
- Consider induced sputum—use ultrasonically nebulized hypertonic saline to facilitate sputum production, in association with chest physiotherapy.

Possible results

Induced sputum results in successful sputum production in >70% of normal and asthmatic subjects who cannot produce sputum spontaneously (see Table 8.8).

Interpretation

- Commensal organisms common.
- *Streptococcus pneumoniae* and *Haemophilus influenzae* likely pathogens in COPD.
- *S. pneumoniae* commonest organism in community-acquired 1° pneumonia.
- *Staphylococcus aureus* and *Pseudomonas* likely in bronchiectasis.
- Nosocomial infections:
 - *S. aureus*.
 - *Pseudomonas*.
 - *Klebsiella*.
- The sensitivity of sputum cytology varies by location of the lung cancer and is greatest in central endobronchial lesions.

Table 8.8 Possible results

Gram stain	Gram +ve or -ve organisms
ZN stain	AAFB
Microscopy	Differential cell count Eosinophils in asthma
Cytology	Malignant cells Small cell, squamous cell, adenocarcinoma cells

Advantages over other tests

- Cheap, easy.
- Non-invasive.

Ancillary tests

- Bronchoscopy and BAL.
- Serum serology if atypical pneumonia suspected.
- If bronchiectasis suspected, consider high-resolution CT (HRCT) chest ± CT sinuses, and check Igs (IgG, IgA, and IgM). Other investigations depend on the clinical scenario (RF, IgG subclasses, IgE, *Aspergillus* IgE and precipitins, α 1-antitrypsin). Involve the respiratory team early.
- PCR for drug-resistant TB.

Pitfalls

- Sputum may be diluted by saliva.
- Diagnosis of squamous cell carcinoma is not as robust as for small-cell lung cancer or adenocarcinoma. Needs careful cross-referencing to radiology and should be confirmed, if possible, with biopsies.
- -ve results should not preclude further investigations if malignancy suspected.

Further reading

- Pasteur M, Bilton D, Hill A; British Thoracic Society Bronchiectasis non-CF Guideline Group. British Thoracic Society guideline for non-CF bronchiectasis. *Thorax* 2010; **65**(Suppl 1): i1–58.
 Rosia E, Scano G. Association of sputum parameters with clinical and functional measurements in asthma. *Thorax* 2000; **55**: 235–8.

Static lung volumes/whole body plethysmography

Clinical indications

- Differentiate between obstructive and restrictive disease patterns.
- Identify and quantify trapped air (shown by ↑ residual volume (RV)/TLC ratio).
- Assess response to therapeutic interventions (e.g. drugs, radiation, transplantation).
- Identify the presence and amount of unventilated lung.
- Assess chronic lung disease (e.g. sarcoidosis, rheumatoid lung).
- Preoperative assessment.
- Assessment of pulmonary disability.

Patient preparation

1. Ask the patient to wear comfortable clothes.
2. Place the mouthpiece securely in the mouth with the lips tight around it.
3. Breathe in a relaxed manner through the spirometer system (nose clips mandatory).
4. After a total of five tidal breaths with consistent end-expiratory levels, patient asked to maximally inspire to TLC, followed by exhalation with encouragement to force out the last 5–15% of air.
5. A minimum of two attempts should be obtained.
6. More may be needed in the young and elderly to obtain reproducible results.

Most accurate results are obtained with whole body plethysmography.

Possible results

- *Total lung capacity*: volume of air in the lungs at the end of full inspiration.
- *Residual volume*: volume of air remaining in the lungs after maximal expiration.
- *Vital capacity*: the amount of air expired (or inspired) between maximum inspiration and maximum expiration.
- *Functional residual capacity*: the amount of air in the lungs at the end-tidal position.
- *Inspiratory capacity*: the maximum amount of air that can be breathed into the lungs from the end-tidal position.
- *Tidal volume*: the volume of air inspired and expired with each breath.
- *Inspiratory reserve volume*: the volume between the peak inspiratory tidal position and maximum inspiration.

(See Table 8.9 and Fig. 8.7.)

Table 8.9 Causes

Causes of ↑ TLC	Generalized airway obstruction, e.g. COPD Emphysema (including bullae) Bronchiectasis Asthma Other, e.g. acromegaly
Causes of ↓ TLC	<i>Intrapulmonary:</i> <ul style="list-style-type: none">● Pneumonectomy● Collapsed lung● Consolidation● Oedema● Fibrosis <i>Extrapulmonary:</i> <ul style="list-style-type: none">● Pleural disease● Effusion● Thickening● Pneumothorax● Rib cage deformity● Scoliosis● Thoracoplasty● Respiratory muscle weakness
Causes of ↑ RV	Generalized airway obstruction Pulmonary vascular congestion, e.g. mitral stenosis, atrial septal defect Expiratory muscle weakness, e.g. spinal injury, myopathies
Causes of ↑ FRC	Age, lung disease causing air trapping, e.g. asthma, emphysema, COPD
Causes of ↓ FRC	Restrictive lung diseases, e.g. diffuse interstitial pulmonary disease of any aetiology, pneumonectomy

Interpretation

- Only interpret if the test is reproducible, i.e. if the two largest VC values are within 5% or 100mL (whichever is the larger).
- VC may remain within normal range in some pulmonary disease, e.g. emphysema.
- ↓ VC—restrictive pulmonary disease, neuromuscular disease, e.g. amyotrophic lateral sclerosis.
- During the testing process, the patient is enclosed in a chamber equipped to measure either pressure, flow, or volume changes. Because all the gas in the thorax is accounted for, this method is particularly useful in patients with trapped gas, e.g. bullous emphysema.

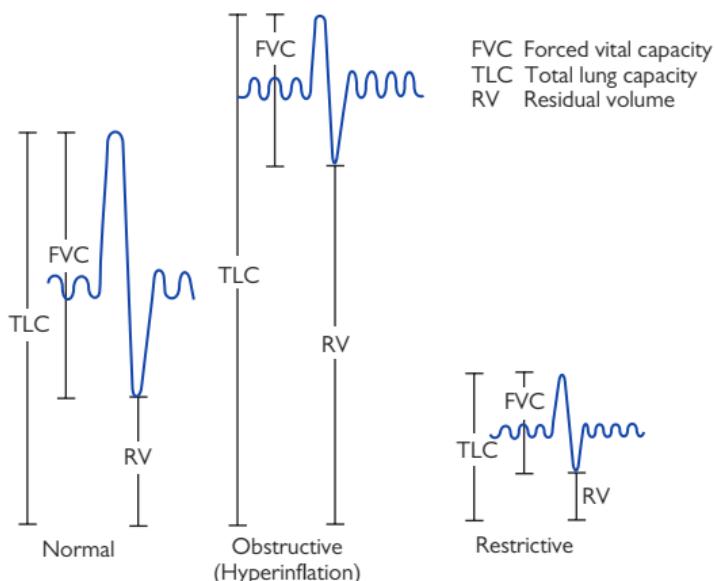


Fig. 8.7 Lung volumes: physiological and pathological.

Advantages over other tests

- Reproducible.

Pitfalls

- Patient co-operation is essential. They must provide maximal effort and be capable of understanding instructions.
- Calibration should take place on a regular basis.
- Risk of disease transmission between patients, and between patient and technician; therefore, avoid if pulmonary TB suspected.

Sweat test

Clinical indications

Suspected cystic fibrosis in the context of

- Bronchiectasis/recurrent chest infections.
- Pancreatic insufficiency/DM.
- Family history.
- Fertility problems.

Patient preparation

1. Obtain informed consent: verbal usually sufficient, but important to discuss the reasons for the test and possible implications. Perform two sweat tests simultaneously on each arm for greater accuracy.
2. Induce sweating by pilocarpine iontophoresis. A weak electrical current aids penetration of pilocarpine into the skin, thus stimulating the sweat glands of the forearm, previously washed and dried, to secrete sweat.
3. Collect sweat on preweighed filter paper ($>100\text{mg}$), then measure eluted Na^+ and chloride (Cl^-).

Possible results

- 98–99% of children homozygous for CyF have sweat Cl^- and Na^+ levels well above 70 and 60 mmol/L, respectively.
- Sweat Na^+ concentrations tend to ↑ with age and show wide variability between individuals.
- Diagnostic accuracy is improved in borderline cases by a suppression test using fludrocortisone.

Interpretation

- A +ve test is virtually diagnostic of CyF. This should lead to counselling and genetic testing.
- Equivocal results are defined as Na^+ or Cl^- concentrations between 50 and 70 mmol/L.
- The diagnosis should never rest on the sweat test alone and should be considered together with the clinical findings and laboratory evidence of pancreatic insufficiency.

Advantages over other tests

- Cheaper than genetic tests.
- Assesses functional deficit, therefore capable of detecting patients who have rare variants of CyF.

Ancillary tests

- Nasal potential difference.
- Pancreatic function tests (3-day faecal collection).
- Genetic studies.

Pitfalls

- A wide discrepancy between the results from each arm suggests a problem with the technique.
- Accurate interpretation of sweat tests requires knowledge of the age-related changes in sweat Na^+ and Cl^- concentrations and should be done in a specialized centre.

False negatives

- Inexperience of operator.
- Low rates of sweating.
- Poor skin preparation.
- Poor iontophoretic contact with the skin.
- Faulty chemical analysis.

False positives

- Evaporation of sweat 2° to inadequate sealing during collection.
- Untreated adrenal insufficiency.
- Nephrogenic DI.
- Hypothyroidism.
- Glycogen storage disease.
- Nephrotic syndrome.
- Severe malnutrition.
- AIDS (some reports of abnormal sweat electrolytes).
- Faulty chemical analysis.

Further reading

- Green A, Dodds P, Pennock C. A study of sweat sodium and chloride: criteria for the diagnosis of cystic fibrosis. *Ann Clin Biochem* 1985; **22**: 171–6.
- Hall SK, Stableforth DE, Green A. Sweat sodium and chloride concentrations—essential criteria for the diagnosis of cystic fibrosis in adults. *Ann Clin Biochem* 1990; **27**: 318–20.
- Heeley AF, Watson D. Cystic fibrosis—its biochemical detection. *Clin Chem* 1983; **29**: 2011–18.

Medical thoracoscopy

Clinical indications

- Pleural effusions when pleural fluid analysis non-diagnostic.
- Pneumothorax.
- Staging of lung cancer.
- Diagnosis of malignant mesothelioma and other pleural abnormalities, e.g. neurinomas, lipomas, plastocytomas.

Pre-assessment

- A recent (<1 month) CT scan of the chest is mandatory. A pre-procedure USS is desirable.
- FBC.
- Clotting.
- Renal function and electrolytes.
- Serum glucose.
- Spirometry.
- Pulse oximetry.
- ABGs on air if hypoxaemia suggested by SpO_2 or if significant hypercapnia is suspected.

Patient preparation

Endoscopy suite

1. Patient informed and consented. Fasted from solids for 6h and liquids for 3h.
2. IV access obtained.
3. Full aseptic technique.
4. Patient positioned in the lateral position with the side to be examined uppermost.
5. Basic monitoring: pulse oximeter, BP, and cardiac monitor.
6. Supplementary O_2 given via face mask or nasal cannulae to maintain saturations >92%.
7. IV sedation—midazolam/alfentanil. LAN: 0.5–1% lidocaine.
8. An absolute prerequisite for thoracoscopy is the presence of an adequate pleural space (i.e. at least 6–10cm diameter).
9. If pleural effusion: drain using a 3-way tap. Replace with equal quantity of atmospheric air.
10. If no effusion: create a pneumothorax. Insert a needle connected to a manometer into the pleural space. Introduce 400–1000mL of air.
11. Skin incision 1cm in fifth intercostal space, mid-axillary line (or guided by USS).
12. Insert 5–10mm pleural trocar and cannula.
13. Introduce the thoracoscope via the trocar into the pleural cavity.
14. After inspection and biopsies, remove the trocar and insert a drain.
15. CXR post-procedure.
16. May need to apply suction to the drain: ↑ in small increments of 5 cmH_2O (0.5kPa), up to a maximum of -20 cmH_2O .

Possible results

- Direct inspection of pleural surfaces.
- Biopsy of parietal pleura—histology/culture, especially AAFBs.
- Pleural fluid → M,C&S → cytology.
- Therapeutic options: pleurodesis, coagulation of blebs, resection of fibrinous loculations in empyemas.
- Drainage of large pleural effusions possible without risk of re-expansion pulmonary oedema due to rapid equalization of pressures by entrance of air into the pleural space.

Interpretation

- Macroscopic appearance of pleura may be diagnostic, e.g. metastatic disease.

Advantages over other tests

- Better than blind pleural biopsy.
- Able to obtain diagnosis in 70–95% of cases.
- Especially good at diagnosing TB.
- Less invasive than thoracotomy.
- Can perform talc poudrage (pleurodesis) at the same time.
- Less expensive than surgical VATS. Does not require a theatre or an anaesthetist.
- Done under sedation, unlike VATS which requires GA and selective one-lung ventilation.

Ancillary tests

- Diagnosis of mesothelioma improved with use of immunohistochemical markers.

Pitfalls

- Biopsies may be inadequate or non-representative.

Contraindications

- Obliterated pleural space.
- Small pneumothorax.
- Patient SOB at rest, unless 2° to pneumothorax or pleural effusion, which can be treated during the procedure.
- Disturbed haemostasis:
 - Platelets $<40 \times 10^9/L$.
 - APTT $>50\%$ of normal.
- Recent MI, arrhythmias, heart failure.

Complications

- Fever 24–36h post-procedure.
- Empyema (<1%).
- Wound infection.
- SC emphysema.
- Air embolism.
- Bronchopleural fistula following lung biopsy.
- Seeding of metastases/mesothelioma along trocar wound.
(Radiotherapy a few weeks post-thoracoscopy should be carried out to prevent this.)
- Haemorrhage.
- Arrhythmias.
- Mortality rate <0.01%.

Further reading

- Blanc FX, Atassi K, Bignon J, Housset B. Diagnostic value of medical thoracoscopy in pleural disease: a 6-year retrospective study. *Chest* 2002; 5: 1677–83.
- Buchanan DR, Neville E. *Thoracoscopy for physicians: a practical guide*. London: Arnold, 2004.
- Colt HG. Thoracoscopy: window to the pleural space. *Chest* 1999; 116: 1409–15.
- Loddenkemper R. Thoracoscopy—state of the art. *Eur Resp J* 1998; 11: 213–21.

Transfer factor

Clinical indications

- Test for abnormalities of pulmonary gas exchange.

Patient preparation

- Avoid smoking 6h prior and strenuous exercise 2h prior.
- Allow 15–30min for test.
- Usually measured by single-breath inhalation technique.
- Patient breathes in air containing a known concentration of carbon monoxide (CO) and holds breath for 10s.

Possible results

- Transfer factor (TLCO).
- Transfer coefficient (KCO).
- May need to correct for anaemia:
 - Result usually standardized to Hb 14.6g/dL.
 - Effect of mild anaemia (Hb >10g/dL) slight but becomes progressively more marked at lower values.

Interpretation

Decrease in TLCO

- Obstructive lung disease, e.g. COPD, emphysema.
- Diffuse ILD, e.g. cryptogenic fibrosing alveolitis (CFA), amiodarone lung.
- Pulmonary involvement in systemic disease, e.g. SLE, RA, Wegener's.
- Cardiovascular disease, e.g. pulmonary oedema, mitral stenosis, PE.
- Others: anaemia, cigarette smoking.

Increase in TLCO

- Diseases associated with polycythaemia.
- Pulmonary haemorrhage.
- Diseases associated with ↑ pulmonary blood such as left-to-right intracardiac shunts.
- Exercise.
- Asthmatics (reasons not clear).

Advantages over other tests

- Quick.
- Relatively easy to perform.
- Reproducible.

Pitfalls

- Breath-holding time may be difficult for some patients to achieve.
- Calculation of TLCO is based on assumption that ventilation and diffusion are homogeneous in the entire lung. With unequal distribution of ventilation and diffusion, the TLCO will be underestimated on the alveolar level.
- With extrapulmonary lung restriction and consequent inability to achieve full inspiration, KCO tends to be higher than normal.

Neurology

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Lumbar puncture

Indications

- Meningitis.
- Encephalitis.
- Polyradiculitis, polyneuritis.
- MS.
- Myelitis.
- Vasculitis.
- Suspected SAH.

Note: in general, a –ve CT does not exclude an SAH.

- Suspected malignancy with meningeal involvement.
- Assessment of CSF pressure:
 - High (e.g. idiopathic or ‘benign’ intracranial hypertension (IIH)).
 - Low (e.g. ‘low pressure’ headache).
- Therapeutic trials, e.g.
 - IIH.
 - Normal pressure hydrocephalus (NPH) (not particularly helpful).
- To seek specific antibodies/markers in CSF, e.g.
 - HIV.
 - Lyme (*Borrelia*).
 - Syphilis.
 - ACE (for neurosarcoid).
 - Tumour markers.
 - Lactate in mitochondrial cytopathies.

Preparation

- Decide exactly what investigations you want. If necessary, alert the appropriate laboratories and organize transport of samples. In particular, samples for xanthochromia and cytology should be rapidly taken to the laboratory to be spun down.
- If the patient is also due to have a neuroradiological investigation with contrast and an LP is not urgent, delay the LP until after the scan, as there may be diffuse meningeal enhancement after the LP.
- If the patient is extremely anxious, he may benefit from 5–10mg of oral diazepam prior to the LP.

Procedure

1. Explain to the patient what you are about to do.
2. Arrange all your equipment on a sterile tray, including assembled CSF manometer.
3. Position the patient on his side, with the back perpendicular to the bed, at the edge of a firm bed. Place the head on one pillow. Draw the knees up, and place one pillow between them.
4. Adjust the height of the bed, so that you are comfortable.
5. Identify the bony landmarks. L3/L4 space is in line with the iliac crests and is most commonly used. L2/L3 to L5/S1 are also used. If you like, mark the target space with the imprint of your thumb nail. Take time over these first four stages.

6. The insertion of the needle should be a sterile procedure. Clean the skin over the lower back. Don sterile gloves and mask.
7. Insert a little (0.25–0.5mL) LA—too much can obscure the bony landmarks.
8. Pass the LP needle horizontally into the space, with the tip angled at about 10–15° (towards the umbilicus), in the midline horizontal plane. At all times, the stylet should be fully inserted and the bevel of the needle facing up.
9. Slight resistance should be felt as the needle passes through the ligamentum flavum and dura, and then a 'give' as it enters the subarachnoid space.
10. Slowly withdraw the stylet. CSF drops should appear.
11. If CSF does not appear, reinser the stylet and slightly rotate the needle—this sometimes frees it of obstructing nerve roots. A gentle cough from the patient can also help.
12. If the needle encounters bone, or the patient complains of pain shooting down the leg, check the position of the needle (is it in the midline? Is it angled correctly?) and then withdraw it entirely.
13. Insert a fresh needle, correcting for any error noted above.
14. If this second pass is unsuccessful, withdraw the needle and inform the patient. If he is happy for you to proceed, then attempt the LP in another space, repeating all steps from 4 down. Use a fresh needle.
15. If you fail again, explain to the patient and seek a more experienced operator to perform the LP. Multiple failed attempts are painful and discouraging (to both you and your patient).
16. If a more experienced operator fails, ask your friendly radiologist to do it under X-ray guidance, but give him the help he requests and precise instructions about the samples required.
17. When CSF collection is complete, gently pull out the needle and place a sterile dressing over the insertion site.
18. Allow the patient to mobilize shortly after the LP.

Measuring the cerebrospinal fluid pressure

As soon as the CSF starts to flow, attach the pre-assembled manometer. Wait until the CSF stops rising. If the patient is very anxious, or uncomfortable, a falsely raised opening pressure may be recorded. Sometimes having the patient slightly relax his legs will help. Using the 3-way tap, let the CSF run into your first prelabelled tube (do not waste the CSF!). Having collected all the CSF you require, if the opening pressure was elevated, note the closing pressure. If the opening pressure is expected to be very raised, e.g. in suspected or confirmed idiopathic (benign) intracranial hypertension, then two or more manometers should be pre-assembled, as the pressure may exceed 40mmCSF.

Collecting samples

- As always, tailor your investigations to the clinical picture. If you are just checking the CSF pressure, then no samples need necessarily be collected. If you suspect an SAH, collect three samples in sequentially labelled bottles and promptly hand-carry to the laboratory for quantitative estimation of xanthochromia and Hb breakdown products.

If you are looking for evidence of malignant cells, then at least one sample should be sent to the laboratory promptly for cytology.

- To avoid contamination, allow the microbiology laboratory to split samples, rather than attempting this yourself.
- Collect at least ten drops in each bottle. The microbiology and cytology laboratories in particular will thank you for greater volumes.
- As soon as the CSF is collected, a blood sample should be obtained (if necessary) for glucose and OCB detection.

Alternative positioning of patient

Sometimes there is a dry tap if the CSF pressure is too low to distend the lumbar cistern. This can sometimes be overcome by performing the LP with the patient sitting on a firm reversed chair, leaning forward to bend over its back. This manoeuvre maximizes the separation of the vertebrae. Again, the needle should be angled slightly (10°) upward relative to the spine at that point. This position does not allow accurate measurement of CSF pressure.

Which needle to use?

A 22G needle usually appropriate. Needles with larger bores tend to cause a greater CSF leak (and thus more headache). Some advocate even finer needles, but these make the collection of CSF take too long. 'Blunt' anaesthetists' needles reduce the risk of post-LP headache.

Clinical record keeping

Record what you did in the notes after the procedure (e.g. if >1 pass was required; which space you used), the opening and closing CSF pressures, and what investigations you have requested. Note the appearance of the CSF (if normal, it will be clear and colourless). If the CSF appears bloody, record this and whether the final bottle collected is clearer than the first.

When NOT to attempt a lumbar puncture

- Risk of herniation:
 - SOLs.
 - Non-communicating hydrocephalus.
 - Cerebral oedema (if in doubt, cranial imaging should be performed first).
- Uncorrected bleeding diathesis/anticoagulant use.
- *Caution:* if previous lumbar spine surgery or known anatomical abnormalities.
- Local skin sepsis.

Note: it is usually safe and *appropriate* to perform an LP in suspected meningitis, *unless* there are specific clinical features to suggest raised ICP, in which case cranial imaging should be performed first.

Complications and what to do about them

Headache

- Usually starts within 24h of LP.
- May last from a few hours to 2 weeks, but typically several days.
- Probably related to persistent CSF leak via the dural tear; therefore, tends to have 'low pressure' characteristics (frontal, worse on sitting up, better on lying down). There may be mild meningism and nausea.

Treatment has traditionally involved bed rest, analgesia, and the encouragement of plenty of fluids.

- If nausea is a major problem, the patient may require IV fluids.
- Rarely, if the headache is severe and persistent, then an anaesthetist may place an autologous blood patch to 'plug' the dural tear. Surgical intervention is very rarely required.

Low backache

A variety of causes of post-LP backache exist; these may usually be treated conservatively.

Infection

Very rare if a sterile technique is used. Occasionally may occur if the needle passes through a region of infection. Meningitis typically develops within 12h. Very rarely, there may be an epidural abscess or vertebral osteomyelitis. Treat with appropriate antibiotics and, if necessary, surgery.

Herniation

- Uncal or cerebellar herniation may occur, particularly in the presence of a posterior fossa mass. ►► An LP should not be performed if there is suspicion of raised ICP without first obtaining cranial CT or MRI.
- Should the CSF pressure be found to be very high (300mmCSF), even after relaxing the patient, and in the absence of idiopathic (benign) intracranial hypertension, manage as follows:
 - Nurse the patient prone with no pillow.
 - Raise the foot of the bed.
 - Start an infusion of 20% mannitol at 1g/kg over 20min.
 - Start a neurological observation chart.
 - Arrange an urgent CT of the brain and notify the neurosurgeons.

►► Do not instil saline into the subarachnoid space.

Haemorrhage

A 'traumatic' tap may cause a little local bleeding that is rarely of clinical significance. Patients with impaired clotting (remember warfarin) or platelet function are at risk of more extensive bleeding, and an LP should not be attempted unless the coagulopathy is corrected. An arachnoiditis, or spinal subdural, or epidural haemorrhage may develop. A spinal SDH is otherwise rare, and an intracranial SDH very rare.

Cerebrospinal fluid constituents: normal values

- White cells: 0–4/mm³.
- RBCs: ideally none!
- Protein: 0.15–0.45g/L.
- Glucose: ~ half to two-thirds of simultaneous blood glucose.
- Opening pressure: 8–20cmCSF.

Note: if there is a traumatic 'bloody' tap, there may be hundreds or thousands of RBCs/mm³. If so, then white cells should be expected in the CSF, but in similar proportions to the peripheral blood.

Rules of thumb

1. Pressure:
 - ↑ by SOLs within the cranial vault such as oedema, masses, chronic inflammation.
 - ↑ by ↑ CVP, e.g. in the anxious patient with tensed abdominal muscles.
 - ↓ if the spinal subarachnoid space is obstructed, thus impeding CSF flow.
2. Cells:
 - *Polymorphs (neutrophils)*: suggest acute bacterial infection.
 - *Lymphocytes and monocytes*: viral and chronic infections or tumours.
 - *Eosinophils*: tumours, parasites, foreign body reactions.
3. Glucose—↓ by non-viral processes causing meningeal inflammation.
4. Total protein—↑ by breakdown of the blood–brain barrier.
5. IgG specific to the CSF, i.e. without matching IgG in a simultaneous blood sample: inflammation within the theca, e.g. MS, infection, tissue damage.

Common patterns

These are shown in Table 9.1.



OHCM 10e, p. 822.

Table 9.1 Common patterns

Condition	Glucose	Protein	Cells	Comments
Acute bacterial meningitis	↓	↑	Often >300/mm ³	Polymorphs; lactate ↑
Acute viral meningitis	N	N or ↑	<300 mononuclear	Culture, antigen detection may be possible
Fungal meningitis	↓	↑	<300 mononuclear	Culture and antigen detection
Tuberculous meningitis	↓	↑	Mixed pleocytosis <300	ZN stain organisms, culture PCR
Herpes simplex encephalitis	N	Mildly ↑	5–500 lymphocytes	PCR
GBS	N	↑	Normal	
SAH [†]	N	May be ↑	Erythrocytes	Look for bilirubin pigments on spectrophotometry; xanthochromia unreliable
Malignant meningitis	↓	↑	Mononuclear	Rapid cytopsin and look for malignant cells
HIV	N	N or ↑	Mononuclear pleocytosis	Culture, antigen detection, antiviral antibodies
Neurosyphilis	N or ↓	↑	<300 lymphocytes	VDRL
Neurosyphilis— early	↑		<i>Treponema pallidum</i>	
Neurosyphilis— late			Immobilization tests	

[†] LP should be done >12h after onset of headache; the CSF should be spun down within 45min; ↓ numbers of RBCs in successive bottles are compatible with SAH.

Reprinted from *Brain Res Bull*, 61: 287–97 Kleine T O et al. 'New and old diagnostic markers of meningitis in cerebrospinal fluid (CSF)' (2003) with permission from Elsevier.

Further reading

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Skull radiograph

Indications

Usually more modern imaging techniques are much more informative, but there are occasions when these may not be speedily available. However, the plain SXR has quite low specificity and sensitivity for detecting many abnormalities of neurological importance.

Used in (suspected) cases of

- Skull fracture.
- Pituitary fossa abnormalities.
- Tumours involving bone.
- Bone changes related to meningioma.

Procedure

- Lateral view in the first instance.

Consider

- Occipitofrontal.
- Towne's (half axial).
- Basal (submentovertical).
- Specific views (e.g. orbits).

What to look for (what you see will depend on the pathology)

See  Chapter 13.

- Shape and symmetry of the vault.
- Pituitary fossa.
- Position of calcified pineal (midline shift?).
- Bone density changes (e.g. tumour, meningioma, Paget's).
- Fractures.
- Evidence of neurosurgical procedures.
- Intracranial air.
- Post-nasal space.
- Craniocervical junction.

Indications for SXR after head injury

(But see  Computed tomography, pp. 598–600 for cranial CT; in general, CT is the preferred imaging modality.)

In an orientated adult patient

- Loss of consciousness or amnesia.
- Fall of >60cm.
- Full-thickness scalp laceration.
- Scalp haematoma.

If a skull fracture is detected, proceed to CT.

Ultrasound

US may be used in a variety of modes.

Mostly commonly used in neuroradiology

- *B mode*: gives 2D images.
- *Doppler effect* is used to assess alterations in the pattern (especially velocity) of flow in vessels.
- *Duplex scanning* combines *B mode* and *Doppler*.

Extracranial vessels

B mode

- Can image from the clavicle (common carotid bifurcation) and internal and external carotids to the angle of the jaw.
- Can image the proximal and distal subclavian and vertebral arteries.
- Supraorbital artery (anterior circulation).
- Fibrofatty plaques and thrombus on plaques not very echogenic, therefore missable.
- Fibrous plaques more echogenic.
- Calcification in plaques is highly echogenic.
- Can sometimes detect intraplaque haemorrhage or ulceration.

Note: requires patient co-operation and considerable operator skill. High-grade stenosis can appear as total occlusion.

Doppler mode

- Stenosis alters the normal pattern of velocities recorded.

Duplex

- Combination of anatomic and flow imaging more sensitive and specific for clinically significant stenoses.

Carotid duplex studies in cerebrovascular disease

- Use of carotid US: most commonly in the assessment of patients with carotid territory ischaemic strokes or TIAs, who might be candidates for carotid endarterectomy. Such surgery should be performed as soon as possible, so carotid Doppler studies should be arranged promptly after the first event. If a patient has neurological signs or symptoms suggestive of posterior circulation events, there is little point in organizing carotid (i.e. anterior circulation) studies. Both the degree of stenosis and the morphology of the plaque (irregular plaques are more pathogenic) are important.

Duplex studies in suspected giant cell arteritis

- Making, or excluding, the diagnosis of GCA can be difficult. Most guidelines suggest a temporal artery biopsy, but timely access to a biopsy may be difficult, GCA may occur without temporal artery involvement, and some patients with GCA have a -ve biopsy. Duplex studies of the temporal arteries have been shown to have high specificity and sensitivity.

Intracranial vessels

Transcranial Doppler

- 2MHz to penetrate thinner bone.
- Flow velocity in anterior, middle, and posterior cerebral, ophthalmic, and basilar arteries; carotid siphon.

What it shows

- Intracranial haemodynamics.
- Vasospasm in SAH.
- Monitoring of microemboli.
- This is an area of active research, with new clinical indications being described frequently.

Further reading

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Angiography

Indications

- Strongly suspected or confirmed SAH.
- Suspected cerebral vasculitis.
- Detection and delineation of other vascular abnormalities (e.g. AVM) of the brain or spinal cord.
- Delineation of tumour blood supply (occasionally).

Procedure

1. Catheter passed via the femoral artery to the carotid or vertebral artery under image intensification.
2. Contrast is given.
3. In digital subtraction angiography (DSA), subtraction of pre- from post-contrast images (pixel by pixel) is used to help remove signals from bone density.

Arch angiography (aortography)

- Visualizes the aorta, major neck vessels, and sometimes the circle of Willis.
- No venous imaging.

Selective intra-arterial angiography

- Later images show venous system.

Carotid artery

- Anteroposterior (AP), lateral, and oblique views—anterior and middle cerebral, and internal carotid arteries.

Vertebral artery

- Towne's (half-axial) and lateral views—vertebral, basilar, posterior cerebral arteries.

What can be demonstrated?

- Occlusion, stenosis, plaques.
- Aneurysms.
- Arteriovenous and other blood vessel abnormalities.
- Abnormal tumour circulation.*
- Displacement or compression of vessels.*
- Experimental role in acute stroke analysis.

Note: (*) although CT and MRI give finer spatial details, angiography is still useful, e.g. delineating blood supply of a tumour.

Complications

- Sensitivity to the contrast medium.
- Cerebral ischaemia, e.g. 2° to dislodgement of embolic fragments by catheter tip or thrombus in the catheter lumen.
- The rate of transient or permanent neurological defect following angiography depends on the operator.

Further reading

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- Osborne AG. *Diagnostic Cerebral Angiography*, 2nd edn. New York: Lippincott, Williams & Wilkins, 1999.

Myelography

Indications

- Largely superseded by CT and especially MRI.
- Still used in subjects in whom MRI is contraindicated (e.g. cardiac pacemaker, metallic implants, claustrophobia).
- Can screen whole spinal cord and cauda equina for compressive or expanding lesions.
- Can visualize roots.
- Spinal vasculature abnormalities.

Procedure

A total of 5–25mL of (usually water-soluble) radio-opaque contrast medium is injected via an LP needle in the usual location (occasionally a cisternal puncture is used). By tipping the patient on a tilt table, the whole spinal subarachnoid space may be visualized.

Complications

- Those of LP.
- Spinal arachnoiditis (after months or years), now rare with water-soluble contrast.
- Acute deterioration if there is cord/root compression.
- Direct neurotoxicity (3 in 10,000):
 - Seizures, encephalopathy.
 - Usually resolves in 48h.
- Allergic reaction to contrast. Give dexamethasone 4mg 12 and 2h prior to investigation if known allergy.

Note: send CSF for usual investigations (➔ Lumbar puncture, pp. 584–9).

Radionuclide scans

Positron emission tomography

Unstable positron-emitting isotopes (produced locally by a cyclotron or linear accelerator) are incorporated into biologically active compounds. The distribution of isotope shortly after IV administration is plotted. A range of compounds may be labelled such as ligands for specific neurotransmitter receptors or ¹⁸F-fluorodeoxyglucose (FDG). Commonly, PET is used to determine regional cerebral blood flow.

Single photon emission computed tomography (SPECT)

- Stable radioactive isotopes are incorporated into biologically active compounds.
- Their distribution after IV administration is plotted.
- These images often lack fine spatial detail.

Although the range of ligands available is limited, SPECT has certain advantages over PET:

- Isotopes are stable, and therefore a cyclotron or linear accelerator need not be on site.
- A labelled ligand can be given after a clinically important event, e.g. can give agent and scan within 20min of the occurrence of a seizure.

Uses of PET and SPECT

PET is not widely available as a clinical tool. With the ↑ availability of functional MRI (fMRI), the uses of PET in both clinical practice and neuroscience research have lessened. SPECT is more widely available in clinical centres.

Clinical applications of PET

PET is mainly used in neurological practice as whole-body FDG-PET to look for systemic malignancy, especially in paraneoplastic syndromes.

Clinical applications of SPECT

- An epileptogenic focus may show interictal hypometabolism (ictal hypermetabolism may be demonstrated with SPECT).
- Regional hypometabolism may be seen in neurodegenerative conditions such as Alzheimer's disease and frontotemporal dementia. This may aid in diagnosis and differential diagnosis.
- 'Pseudo-dementia' 2° to psychiatric disease such as depression (with normal SPECT scans) may sometimes be differentiated from dementia due to 'organic' neurological disease (with regional hypoperfusion), although psychiatric diseases may themselves be associated with regional hypoperfusion.
- Assessment of Parkinsonism.

The functional integrity of the nigrostriatal system can be assessed, e.g. by the use of SPECT ligands for the dopamine transporter (e.g. FP-CIT). Such DAT scans can be used to differentiate true Parkinsonism from other causes of movement disorders.

Clinical/research applications of PET and SPECT include

- Determination of regional cerebral blood flow, glucose metabolism, and oxygen utilization
 - Hypometabolism may be seen following a stroke. The affected area may exceed that with a demonstrable lesion on conventional CT or MR imaging.
 - *In vivo pharmacology* (e.g. distribution of neurotransmitter receptors).
- ⇒ Positron emission tomography, pp. 894–7.

Further reading

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Computed tomography

Cranial computed tomography

Look for:

- Disturbances in the normal anatomy of the ventricular system.
- Skull base and vault.
- Width of cortical fissures/sulci.
- Midline shift.
- Areas of abnormal tissue density.
- Opacity or lucency of sinuses.
- Normal flow voids.

High-density ('white') signal

- Fresh blood.
- Calcification:
 - Slow-growing tumour.
 - AVM/aneurysm.
 - Hamartoma.
 - In pineal/choroid plexus/basal ganglia, may be normal.

Low-density ('black') signal

- Infarction.
- Tumour.
- Abscess.
- Oedema.
- Encephalitis.
- Resolving haematoma.

Mixed density

- Tumour.
- Abscess.
- AVM.
- Contusion.
- Haemorrhagic infarct.

After administration of IV contrast medium, areas with a breakdown in the blood–brain barrier may 'enhance' (appear 'white'). This may reveal previously 'invisible' lesions (isodense with the surrounding tissue). Especially useful for tumour and infection.

Common patterns of enhancement include

- Ring enhancement of tumours and abscesses.
- Solid enhancement of meningiomas.
- Meningeal enhancement with meningeal disease involvement.

Indications for head CT after head injury

CT imaging of the head in adults

Request a CT brain scan immediately for adult patients with any of the following risk factors:

- GCS score <13 on initial assessment in the emergency department.
- GCS <15 2 h after the injury on assessment in the emergency department.

- Suspected open or depressed skull fracture.
- Any sign of basal skull fracture.
- Post-traumatic seizure.
- Focal neurological deficit.
- One or more episodes of vomiting.
- Amnesia for events >30min before impact.

CT imaging of the head in children

Request CT of the brain immediately for children with any one of the following risk factors:

- Age over 1 year: GCS <14 on assessment in the emergency department.
- Age under 1 year: GCS paediatric <15 on assessment in the emergency department.
- Age under 1 year and presence of bruise, swelling, or laceration (>5cm) on the head.
- Dangerous mechanism of injury.
- Clinical suspicion of non-accidental injury.
- Loss of consciousness lasting >5min (witnessed).
- Post-traumatic seizure but no history of epilepsy.
- Abnormal drowsiness.
- Suspected open or depressed skull injury, or tense fontanelle.
- Any sign of basal skull fracture.
- Focal neurological deficit.
- Three or more discrete episodes of vomiting.
- Amnesia (antergrade or retrograde) lasting >5 min.^{1,2}

CT angiography and venography

CT, especially rapid image acquisition with helical CT, can allow imaging of the intracranial vasculature. CT angiography is particularly used in the detection of aneurysms and is ↑ widely available.³ CT venography is important in the assessment of possible intracerebral venous thrombosis.⁴

CT of spine

MRI is usually preferable, but plain CT can give information about the discs and bony architecture. CT may be helpful in suspected bony abnormalities. Some patients cannot have MRI because of, e.g. metallic implants. CT myelography can be used to demonstrate compressive lesions.

¹ National Institute for Health and Care Excellence (2014). *Head injury: assessment and early management*. Clinical guideline CG176. <https://www.nice.org.uk/guidance/cg176>.

² Yates D, Aktar R, Hill J; Guideline Development Group. Assessment, investigation, and early management of head injury: summary of NICE guidance. *BMJ* 2007; 335: 719–20.

³ Karamessini MT, Kagadis GC, Petsas T, et al. CT angiography with three-dimensional techniques for the early diagnosis of intracranial aneurysms. Comparison with intra-arterial DSA and the surgical findings. *Eur J Radiol* 2004; 49: 212–23.

⁴ Smith R, Hourihan MD. Investigating suspected cerebral venous thrombosis. *BMJ* 2007; 334: 794–5.

Indications for cervical spine imaging after trauma

Plain radiograph is the initial investigation, but CT preferred when:

- Age >9 years:
 - GCS <13 on initial assessment.
 - Intubated patient.
 - Technically inadequate plain radiographs.
 - Clinical suspicion of injury despite normal radiograph.
 - Patient being scanned for multi-region trauma.
- Age <10 years:
 - GCS <9.
 - Strong clinical suspicion of injury despite normal radiograph.
 - Technically inadequate plain radiographs.^{1,2}

② OHCM 10e, p. 730, p. 746.

Magnetic resonance imaging

For most neurological indications, MRI is preferred to CT. It gives superior anatomical detail, and the range of sequences available allows superior determination of pathology. Unlike CT, there is no radiation exposure.

Note: MRI is not safe in the presence of ferromagnetic materials (e.g. certain prostheses, metal filings in the eye).

Some people find MRI difficult to tolerate because of claustrophobia. Counselling and experience of 'dummy' scanners prior to MRI may be helpful. Upright and 'open' scanners are increasingly available in specialist centres.

MRI sequences

There is a variety of sequences available, which offer various advantages in delineating anatomy and pathology. This is an area of active research, with new sequences continuing to enter clinical practice. It is important that the requesting clinician provides appropriate clinical details, so that the radiologist may select the appropriate imaging sequences (and planes) to be used.

The commonest sequences are T1 and T2 (see Table 9.2).

- T1 CSF is hypointense ('black'); fat and mature blood clot white.
- T2 CSF is hyperintense ('white').

MRI with enhancement

Intravenously administered gadolinium leaks through areas of damaged blood–brain barrier to give a marked enhancement. It may be helpful in delineating:

- Ischaemia.
- Infection.
- Tumour (may help differentiate from surrounding oedema).
- Active demyelination.

Table 9.2 Comparison of T1 and T2 MRI

T1	T2	Tissue or lesion
Good anatomical detail	Reveals most pathology better than T1	
Hypointense	Hyperintense	CSF
Hyperintense	Iso-intense	Fat, e.g. dermoid, lipoma, some metastases (melanoma), atheroma
Very hypointense	Very hyperintense	Cyst, hygroma
Hypointense	Hyperintense	Ischaemia, oedema, demyelination, many malignant tumours
Hyperintense	Moderately hyperintense	Subacute or chronic haemorrhage
Iso-intense	Hypointense	Acute haemorrhage
Iso-intense	Iso-intense	Meningioma

Magnetic resonance venography and angiography

MR may be used to obtain non-invasive images of blood vessels by using special MRI sequences and image reconstruction. Whilst standard angiography remains a 'gold standard' for many purposes, MRA has the advantage of being non-invasive and therefore 'safe'. MRA images flow, rather than structure, and therefore may fail to 'pick up' low flow abnormalities such as cavernous angiomas. ► *Caution:* congenital abnormalities in the venous sinuses may be misinterpreted as thrombosis on MR venography (MRV).

Uses

- Assessment of patency of major arterial and venous vessels.
- Visualization of large (~3mm diameter) aneurysms.

Functional magnetic resonance imaging

Certain (indirect) indices of neural activity (most commonly changes reflecting regional perfusion) may be imaged with sufficient temporal and spatial resolution to be useful for both research and clinical applications (although fMRI has been largely a research tool to date). As a conventional MRI machine, albeit with special software, is required, fMRI is being ↑ used in the clinical setting.

Clinical and research applications have included

- Demonstration of the language areas prior to epilepsy surgery.
- Demonstration of the functional anatomy of cognitive, sensory, and motor processes.

Diffusion tensor imaging

Diffusion tensor imaging (DTI) allows anatomical tract tracing *in vivo*, using specialized software on standard MRI machines. As with fMRI, DTI was initially a research tool but has now entered clinical practice in some neuroscience centres. Major tracts, such as the corticospinal tract and the optic radiation, can be identified; these can then be spared during resective surgery.

Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) can be used to measure the concentration of certain neurochemicals *in vivo* (see Fig. 9.1). Because of the low concentrations of the neurochemicals, measurements are taken from large volumes of interest (typically several cm³), therefore giving MRS a much lower spatial resolution than standard structural MRI (spatial resolution typically of 1–2mm³). Metabolites commonly measured include the following (although it is often the *ratio* of different metabolites that is particularly useful):

- N-acetyl-aspartate (NAA) is present in high concentration in neurons and axons. Areas of neuronal or axonal loss show reduced levels of NAA.
- Choline (Cho) is associated with turnover in cell membranes and ↑ cell division. Areas of demyelination and malignant tumours can cause raised Cho. ↑ Cho can help distinguish recurrent tumours from post-radiotherapy changes (this can be a difficult distinction to make using standard MRI).

- Creatine (Cr) (and phosphocreatine) is a marker of metabolism. Reduced levels may indicate cell death.
- Myo-inositol (m-In) may be ↑ in certain diseases such as Alzheimer's dementia.
- Lactate (Lac) is typically present in concentrations too low to be detected, except in areas of ischaemia, hypoxia, and certain tumours.

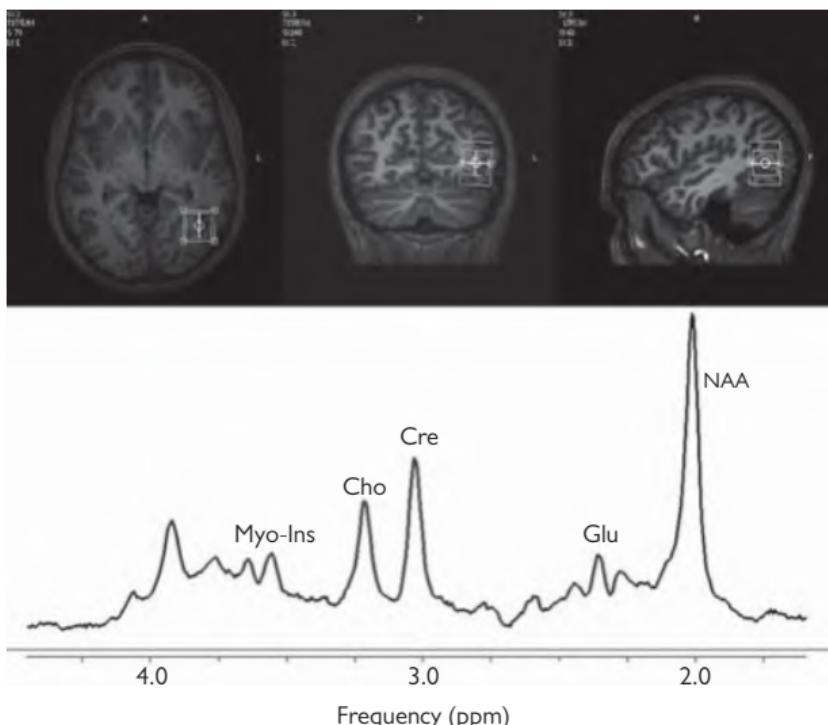


Fig. 9.1 The upper panels show the position of the volume of interest; the lower panel shows the MRS spectrum from that volume.

Further reading

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Nerve conduction studies

Please give your neurophysiologists as much information as possible about your case and, if necessary, discuss it with them. They will then be in the position to organize the most appropriate neurophysiological investigations. In certain circumstances, you may need to specifically ask for unusual investigations such as repetitive stimulation in suspected LEMS (➡ Repetitive stimulation, p. 608).

Sensory nerve action potential and sensory conduction velocity

Procedure

- Orthodromic conduction velocity: electrically stimulates distal sensory branches (e.g. index finger) and records the evoked sensory nerve action potential (SNAP) proximally (e.g. over the median nerve at the wrist). The distance between the two sites (D) and the latency (L) of the onset of the SNAP determine the sensory conduction velocity (D/L). The SNAP amplitude is also useful.
- Antidromic conduction velocity: supramaximal electrical stimulation proximally; records distally (e.g. by a ring electrode on the little finger). By varying the position of the stimulating electrode, the conduction velocity in various portions of the nerve may be ascertained.

What does it mean?

- ↓ SNAP amplitude, or SNAP absence altogether, implies a lesion distal to the dorsal root ganglion.
- ↓ velocity/↑ latency (see Table 9.3). Motor velocities are more commonly measured.

Motor conduction velocity

Procedure

Supramaximally stimulate a peripheral nerve trunk at a proximal (p) and a more distal (d) site. Record the time to the onset of the evoked muscle response (compound motor action potential (CMAP)) from each (Tp and Td) and the distance between them (D). The motor conduction velocity between p and d is therefore $D/(T_p - T_d)$.

Table 9.3 Typical values

	Latency	Amplitude
Median nerve (index finger to wrist)	2–3ms	9–40mV
Ulnar nerve (little finger to wrist)	2–2.6ms	6–30mV
Sural nerve (mid-calf to below med. mall)	2–4ms	5–40mV

med. mall, medial malleolus.

Typical values

- Median nerve in forearm (to abductor pollicis brevis) >48m/s.
- Ulnar nerve in forearm (to abductor digiti minimi) >48m/s.
- Common peroneal nerve (to extensor digitorum brevis) >40m/s.

What does it mean?

(See Table 9.4.)

Distal motor latency

- Latency from stimulation of most distal site on nerve to CMAP.

Typical values

- Median nerve (wrist to abductor pollicis brevis) <4.1m/s.
- Ulnar nerve (wrist to abductor digiti minimi) <3.8m/s.
- Radial nerve (spiral groove to brachioradialis) <5m/s.

Note: these latencies include time taken for impulses to pass along the most distal (unmyelinated) portion of the nerve and for transmission at the neuromuscular junction (therefore, they may not be used to calculate nerve conduction velocities). Compare with velocities elsewhere in the nerve being studied.

What does it mean?*Increased distal motor latency seen in*

- Conditions in which the very distal segment of a nerve is compromised (most commonly carpal tunnel syndrome).
- Early demyelinating neuropathy (e.g. GBS).
- Chronic demyelinating neuropathy.

Table 9.4 Typical patterns

	Conduction velocity	AP amplitude	AP dispersion
Axonal neuropathy	Late stage: ↓ distally ↑ proximally (loss of fastest conducting axons)	Late stage: ↓	Not seen
Demyelinating neuropathy	Marked slowing		Greater dispersion, perhaps especially in acquired, not inherited, demyelination
Ganglionopathies	Slowing proportional to loss of large fibres; often not marked	↓ proportional to loss of large fibres; often not marked	Not seen

Note: limbs should be warm; look for asymmetries. What are your laboratory's current values?

Compound motor action potential

The waveform, amplitude, and area under the curve of the CMAP reflect the number of depolarized muscle fibres (e.g. reduced in axonal neuropathy and denervated muscle) and the temporal dispersion of conduction velocities in the motor neurones to them (e.g. ↑ in demyelinating neuropathy).

Late responses

F wave

- If a motor nerve is stimulated, there are orthodromically directed action potentials (APs) that may cause a response in the muscle (CMAP). However, antidromically directed APs will also pass proximally towards the cell body. If these result in sufficient depolarization of the axon hillock, then a second orthodromic volley will pass down the nerve. This may cause a second motor AP (the F wave). Therefore, the F wave: (i) does not involve synapses (other than the neuromuscular junction, of course) and (ii) depends on the integrity of the whole axon.
- It may be difficult to elicit.
- Delay or absence of the F wave may reflect a lesion proximal to the site of stimulation, in parts of the nerve that may be inaccessible to electrodes, e.g. brachial plexopathy or thoracic outlet syndrome. May also be an early feature in GBS.

H wave

- This is 'an electrical ankle jerk': submaximal stimulation of the posterior tibial nerve in the popliteal fossa causes trans-synaptic activation of the soleus, recorded as a CMAP.
- Amplitude may be ↓ by afferent or efferent problems, e.g. neuropathy or radiculopathy.

Repetitive stimulation

- Procedure: stimulate a motor nerve with 3–5 supramaximal stimuli at 2–4Hz, whilst recording evoked CMAPs.
- *Normal response:* no change in CMAP amplitude.
- *In MG:* >10% decrement in CMAP amplitude after two stimuli.
- *In LEMS:*
 - After voluntary contraction or after rapid stimulation (20–50Hz) for 2–10s, the CMAP amplitude, often initially small, ↑ by 25% (suggestive) or 100% (diagnostic).
 - At a slow (3Hz) rate of stimulation, there is a response decrement.

Electromyogram

Procedure

- A concentric needle electrode is usually used.
- It is inserted into the muscle to be studied.
- The difference in potential between the inner part of the electrode and the outer core is amplified and displayed on an oscilloscope or computer screen.
- It is also 'displayed' as an auditory signal, and experienced electromyographers as much listen to as watch the pattern of electrical activity.

Normal muscle is 'silent' (electrically inactive) at rest (there is no 'spontaneous activity'), although there will be a brief burst of activity when the electrode is first inserted (the 'insertional activity').

The electrode can pick up electrical activity from muscle fibres within about 0.5mm of its tip; therefore, muscle fibres from several motor units (each innervated by a different motor neurone) in this volume can contribute to the signal. However, with care, potentials from a single motor unit may be recorded when a co-operative subject tries to exert the muscle a little (the 'motor unit potential'). With ↑ muscular effort, more muscle fibres are recruited, giving rise to the 'interference pattern'.

Various nerve and muscle problems cause characteristic alterations to these four patterns of activity

1. Insertional.
2. Spontaneous.
3. Motor unit potential.
4. Recruitment.
5. In addition, certain other patterns may be observed in certain diseases (in particular, myotonia).

1. Insertional activity

- Usually there is a brief burst of potentials which lasts <1s.
- Insertional activity is normal in upper motor neurone (UMN) lesions and most non-inflammatory myopathies.
- It may be longer-lasting in lower motor neurone (LMN) lesions, inflammatory myopathies, and acid maltase deficiency.
- In myotonia, myotonic discharges occur (→ Electromyogram, p. 611).

2. Spontaneous activity

- Normal muscles at rest are silent.
- This is also the case in UMN lesions, non-inflammatory myopathies (unless 2° denervation has set in), and myotonia.
- Fibrillation potentials and +ve sharp waves are seen in LMN lesions and inflammatory myopathies. They occur in regular bursts of constant amplitude (unlike activity related to voluntary contraction).
- Fibrillation potentials are spontaneous APs in irritable, acutely denervated muscle fibres. They are low-amplitude, brief -ve potentials.
- Positive sharp waves are brief +ve potentials, followed by a -ve wave. Typically, they can be seen for 2–3 weeks after denervation but may persist.

3. Motor unit potentials

- If the electrode is positioned quite close to the fibres of a motor unit which is active during slight voluntary contraction, then a motor unit potential (MUP) may be recorded. In normal muscle (and in UMN lesions), this waveform is triphasic, 5–10ms, and has an amplitude of 0.5–1mV (larger muscles have larger motor units).
- In myopathies and muscular dystrophies, the motor units are smaller and polyphasic. They tend to be briefer but, in some cases, last longer than usual.
- In denervated and then reinnervated muscles (typically LMN lesions), the size of individual motor units ↑ (as the surviving motor neurones 'take over' the muscle fibres previously innervated by the now absent other motor neurones). MUPs therefore are of greater amplitude and duration and are polyphasic.
- In myotonia, myotonic discharges are seen.

Note: up to 15–20% of MUPs in 'normal' muscle may be polyphasic.

4. Recruitment

- Normally, as the strength of voluntary contraction ↑, ↑ numbers of motor units are recruited, and these units tend to be larger (Heinneman's size principle). The potentials due to these active units overlap and become difficult, and finally impossible, to tell apart—a full 'interference pattern', usually well below the maximum voluntary contraction.
- In muscle diseases, a full interference pattern may be produced, but it is of low amplitude. In weak muscles, there may be 'early recruitment' (i.e. recruitment of many motor units at low levels of voluntary contraction).
- In denervated muscles, a full interference pattern may not be achieved, because of the ↓ number of motor units.
- In UNM lesions, there is a lower frequency of 'normal' MUPs.

5. Myotonia

- High-frequency repetitive discharges occurring after voluntary movement or provoked by moving the electrode. The amplitude and frequency wax and wane, giving the auditory signature likened to the sound of a Second World War dive bomber (or a motorcycle).
- Note: following the onset of a neuropathy, it may take at least 10–14 days for evidence of denervation to appear in the EMG. Therefore, a repeat study after this time is often useful.

Single-fibre electromyogram

A recording electrode with a smaller recording surface than usually used samples a few muscle fibres from a single motor unit (supplied by a single motor neurone). The variability ('jitter') in the timing of APs from different muscles should be <20–25ms. Conduction block during voluntary contraction may also be shown. These techniques are used to investigate neuro-muscular disorders and reinnervation in neuropathies.

Further reading

Blum AS, Rutkove SB (eds.). *The Clinical Neurophysiology Primer*. Totowa: Humana Press, 2007.

Electroencephalogram

The standard EEG is non-invasive. Electrodes are attached to the scalp with collodion adhesive. Stable recordings may be made for days. Usually they are arranged according to the international 10–20 system. This is a method for positioning electrodes over the scalp in an orderly and reproducible fashion. Additional electrodes can be applied to the scalp, depending on the region of interest.

Standard recording conditions

- Rest.
- Hyperventilation for 3–5min can activate generalized epileptiform changes (and precipitate absence seizures):
 - Can ↑ frequency of focal discharge.
 - Can ↑ slow-wave abnormalities.
- Photic stimulation (a strobe light at 30cm with a frequency of 1–50Hz); this can produce several patterns of activity:
 - *Photoparoxysmal response*—bilateral spike or spike and wave discharges not time-locked to the visual stimulus, which may outlast the visual stimulus by hundreds of milliseconds. Generalized, but may have frontal or occipital predominance; commonly seen in idiopathic generalized epilepsies; high-voltage occipital spikes, time-locked to the stimulus; weakly associated with epilepsy.
 - *Photomyogenic (photomyoclonic) responses*—non-specific, mostly frontal spikes due to muscle activity; associated with alcohol and some other drug withdrawal states.
- Sleep studies:
 - Subject either stays awake the night before the recording or is given a small dose of choral prior to the recording (sometimes both).
 - Subjects tend to show the earlier stages of non-REM sleep.
 - These studies ↑ the yield of EEG abnormalities, including epileptiform ones.
 - By capture of 'natural sleep': certain seizure types are commoner in sleep (e.g. juvenile myoclonic epilepsy (JME)).
 - Sleep deprivation itself ↑ the number of seizures and epileptiform changes.

➲ Polysomnography, p. 621.

Electroencephalogram: abnormalities

The normal electroencephalogram

There is a wide range of normal EEG phenomena. Some of the common patterns in the awake adult are listed in Table 9.5.

EEG abnormalities (not peri-ictal or ictal)

- A variety of EEG abnormalities may be seen outside the peri- or per-seizure period.
- Abnormalities in the EEG are not restricted to the appearance of abnormal waveforms.
- The loss, or redistribution in the scalp location, of normal background activities is abnormal.

The classification of EEG abnormalities is complex. Below is a highly simplified guide

1. General excess of slow waves—commonly seen in:
 - Metabolic encephalopathy.
 - Encephalitis.
 - Post-ictal states.
2. Focal slow waves—commonly seen in:
 - Large cerebral lesions (e.g. tumour, haematoma).
 - Post-ictal states.
 - Migraineurs.
3. Localized, intermittent, rhythmic slow waves—may be seen in idiopathic generalized and localization-related epilepsies.

Table 9.5 EEG rhythms

Activity	Frequency (Hz)	Amplitude (mV)	Scalp location	Behavioural state
Alpha	8–12	20–60	Usually occipital	Maximum relaxed, awake, eyes closed
Beta	>13	10–20	Frontocentral	Wakeful, drowsy; REM and SWS 1 and 2
Theta	4–8	Variable diffuse	Frontocentral, temporal	Minimally awake, drowsy, SWS
Delta	<4	Variable	Diffuse	Awake; drowsy
Mu	8–10	20–60	Central	Awake, suppressed in voluntary movements

SWS, slow-wave sleep.

The terms alpha, beta, theta, and delta are often used to describe the background activity but are also used to describe the frequency of EEG activity.

Sharp activity may be a normal phenomenon.

4. Epileptiform abnormalities:

- Spikes (if last <80ms) or sharp waves (80–200ms) may be associated with slow waves.
- Consistently focal spikes suggest epilepsy with a focal seizure onset.

Note: 2–4% of non-epileptics have occasional spikes or sharps.

5. Repetitive stereotyped ‘periodic’ complexes.

EEG patterns may show periodicity. These patterns may be epileptiform or not, and may be focal or generalized. They are an abnormal EEG feature, the interpretation of which depends on the clinical context.

Examples include

- **Burst suppression:** bursts of generalized high-voltage mixed waveforms, alternating with generalized voltage suppression:
 - Coma.
 - Late-stage status epilepticus (both convulsive and non-convulsive).
- **Triphasic waves over one or both temporal lobes:** common in herpes simplex encephalitis.
- **Periodic lateralized epileptiform discharges (PLEDs)** are localized sharp or slow-wave complexes 0.2–1s long, every 1–5s:
 - Non-specific but suggest localized cerebral insult (stroke, haematooma, tumour).
 - Occasionally seen in migraine and focal epilepsies.
- **BIPLEDs are bihemispheric PLEDs:** suggest more widespread insults, e.g. anoxia, encephalitis.
- **Bilateral or generalized high-voltage complexes for 0.5–2s every 4–15s:** characteristic of subacute sclerosing panencephalitis (SSPE).
- **Triangular waves:**
 - Characteristic of CJD.
 - Not seen in vCJD (may see a ‘disorganized’ EEG without repetitive complexes).
- **Runs of broad triphasic waves (1.5–3Hz):** severe metabolic encephalopathy (e.g. renal or hepatic failure).
- **Periodic spikes or sharp waves:** bi- or multiphasic morphology (0.5–2Hz); usually generalized—suggest severe encephalopathy, e.g.
 - Herpes encephalitis.
 - CJD (in the setting of rapid dementia and myoclonus).
 - Lithium intoxication.
 - Post-anoxic brain injury.
 - Tricyclic antidepressant overdose.

Electroencephalogram: in epilepsy

Idiopathic (genetic) generalized epilepsy (IGE)

- Generalized, bilaterally synchronous epileptiform discharges with virtually normal background.
- Absence epilepsy: 3Hz spike and wave.
- JME: 6Hz multiple spike and wave.

Symptomatic (secondary) generalized epilepsy

- More variable.
- *Inter-ictal background activity*: excess slow.
- *Inter-ictal epileptiform activity*: irregular spikes or sharp and slow waves 1.5–4Hz. Usually generalized, but may show asymmetry or (multi-) focal features.

Localization-related partial focal epilepsy

- Inter-ictal EEG is often normal, particularly if the focus is located deeply (especially common with frontal foci).
- There may be lateralized or localized spikes or sharp waves.

Electroencephalogram: how to use

In suspected epilepsy

- Routine EEG with photic stimulation and hyperventilation gives about up to a 50% detection rate for inter-ictal epileptiform abnormalities in a subject with epilepsy (higher 'yield' in 1° generalized epilepsies than in localization-related epilepsies).
- Sleep-deprived or choral-induced sleep recording may ↑ the yield of EEG abnormalities to up to 60–70%.
- Consider 24h or longer ambulatory EEG, ideally with audio/video monitoring. Most useful in helping to determine the nature of the seizure in a subject with frequent (e.g. daily) attacks.

In general, avoid reduction in anti-epileptic drugs or drugs such as pentylenetetrazole to induce seizures, except in exceptional circumstances, e.g. videotelemetry as part of work-up for epilepsy surgery.

Note:

- No inter-ictal spikes does not imply no epilepsy.
- Similarly, inter-ictal spikes do not always imply epilepsy.
- A –ve ictal EEG does not necessarily imply a non-epileptic ('pseudo-') seizure, especially in simple partial and some brief complex partial seizures (CPS). Scalp electrodes may fail to record deep, especially frontal, activity.
- However, a tonic–clonic seizure with loss of consciousness should be associated with an epileptiform EEG during the ictus. This EEG activity may be obscured by muscle artefact, but post-ictal slowing may be seen.
- The EEG may be slow after a tonic–clonic seizure for many tens of minutes.

Note: the diagnosis of epilepsy is mainly clinical! Remember that most episodes of altered consciousness are not epileptic in origin. In many cases, cardiological investigations are appropriate. Have a low threshold for ordering a 12-lead ECG. Ambulatory ECG monitoring, particularly with cardiac memo devices, and ambulatory BP monitoring can be very useful.

In established epilepsy

- Classification (e.g. CPS vs absence).
- Assessment of frequency of seizures (e.g. ambulatory EEG to assess frequency of absence seizures).
- Reduction in inter-ictal discharges in some syndromes (e.g. absence, photosensitive epilepsy) correlates with anti-epileptic drug efficacy.

In focal cerebral dysfunction

Often not particularly helpful. Modern imaging studies usually provide more information.

- Small, deep, or slow-growing lesions often cause no effects.
- Asymmetric voltage attenuation may be caused by a subdural haematoma (or other fluid collection) overlying the cortex.
- Direct grey matter involvement may cause alteration/loss of normal EEG or cause epileptiform discharges.
- Subcortical white matter changes can cause localized polymorphic slow waves.
- Deeper subcortical lesions tend to produce more widespread slow-wave disturbances.

In central nervous system infections

- CJD and SSPE have relatively characteristic EEG associations.
- Meningitis and encephalitis cases may show diffuse background disturbances and polymorphic or bilateral intermittent slow wave abnormalities.
- Encephalitis usually causes more changes than meningitis.
- Focal changes may be seen over abscesses and in cases of herpes simplex encephalitis.

In dementia

- To exclude some conditions such as toxic encephalopathy, non-convulsive status epilepticus (NCSE).
- A few dementing conditions have characteristic EEGs (CJD, SSPE).
- Slowing of background frequency occurs in Alzheimer's disease, but values may overlap with those of the normal aged, therefore not very helpful clinically.

In confusional states

- Helpful in diagnosing NCSE (absence and complex partial status).
- To exclude cerebral dysfunction.
- Not very useful in psychiatric diagnosis per se, but an abnormal EEG in a confusional state may help exclude psychogenic causes for an apparent reduction in level of consciousness.

In toxic metabolic encephalopathies

- EEG always abnormal.
- Diffuse slowing in mild cases.
- Other abnormalities may develop in later stages.
- Specific patterns may be seen in certain aetiologies.
- *Excess fast activity*: barbiturate and benzodiazepine toxicity.
- *Triphasic waves*: hepatic and renal failure, anoxia, hypoglycaemia, hyperosmolality, lithium toxicity.
- *Periodic spikes or sharp waves*: anoxia, renal failure, lithium and tricyclic antidepressant toxicity.

In coma

- EEG, especially serial EEGs, provides an indication of the degree of cerebral dysfunction.
- In general, any 'normal'-looking EEG, spontaneous variability, sleep-wake changes, and reaction to external stimuli are relatively good prognostic signs.
- An invariant, unreactive EEG is a poor prognostic sign; the pattern, however, is not uniform; it may include periodic spikes or sharp waves, episodic voltage attenuation, alpha coma, and burst suppression.
- May give some diagnostic clues, e.g. localized abnormality—supratentorial mass lesion; persistent epileptiform discharges—status epilepticus.
- 'Alpha coma': monotonous unresponsive alpha with anterior distribution seen after a cardio/respiratory arrest is a poor prognostic feature.
- Monotonous, but partially reactive, alpha may follow brainstem infarcts.

Electroencephalogram: invasive techniques

These are generally restricted to specialist centres, most commonly used in the pre-surgical work-up of patients.

Foramen ovale electrodes, corticography (usually done by laying strips of electrodes on the surface of the brain), and depth EEG (electrodes implanted into the parenchyma of the brain) may be used, depending on the region of interest. Sphenoidal electrodes are rarely used today but can give useful EEG information about the medial temporal structures.

Further reading

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Polysomnography

- This is a multimodal recording used in the analysis of sleep-related disorders.
- There is concurrent recording of EMG, EEG, and electro-oculography (EOG—eye movements), often with audiovisual channels. Other physiological parameters may also be recorded, e.g. nasal airflow, chest expansion.

Sleep is divided into three stages (N1–N3) of progressively ‘deeper’ non-REM (NREM) sleep and a stage of REM sleep (see Table 9.6), characterized physiologically by bursts of rapid eye movements (saccades). NREM sleep was previously divided into four stages: 1 (now N1), 2 (now N2), and 3 and 4 (now N3).

Multiple sleep latency test

This is a diagnostic test for narcolepsy. Following a good night’s sleep, normal subjects typically enter REM sleep with a latency of $>>10\text{min}$ (usually $\sim 90\text{min}$). In narcolepsy, the latency is $<10\text{min}$.

Table 9.6 Sleep stages

Stage	Behaviour	Main EEG pattern	Comments
N1	Drowsy	Diffuse alpha and theta	Diminished EMG; rolling eye movement
N2	Light sleep	High theta; K complexes; sleep spindles	Further diminished EMG; may have rolling eye movements
N3	Deep sleep	Delta	Diminished EMG; no eye movements
REM	No body movements, but vivid dreams	Low amplitude, mixed frequency	Muscular atonia; rapid eye movements

REM: rapid eye movements.

There is progression through stages N1–N3, and several episodes of REM during a typical night’s sleep. Polysomnography can be important in understanding the pathophysiology of the insomnias, parasomnias and other sleep patterns.

Further reading

Abad VC, Guilleminault C. Polysomnographic evaluation of sleep disorders. In: MJ Aminoff (ed.). *Aminoff's Electrodiagnosis in Clinical Neurology*, 6th edn. Oxford: Elsevier Saunders, 2012; p. 727.

Sensory evoked potentials or responses

Whilst many techniques and protocols have been developed in research laboratories, there are only a few techniques in widespread clinical use. A stimulus is delivered to the periphery, thus activating a sensory system and evoking an electrical response over a more central, often cortical, area. Multiple surface electrode recordings time-locked to the peripheral stimulus are recorded and averaged, to help eliminate ongoing random background 'noise' from the sensory stimulus-evoked 'signal'. Deviations of this evoked potential (EP) or response (ER) from the norm (especially in latency and waveform) suggest pathology in the sensory pathway tested.

Visual evoked potentials

Pattern-evoked visual evoked potentials

An alternating chequerboard pattern (temporal frequency 1–2Hz) is presented to each eye individually (see Fig. 9.2). The EP is recorded over the occipital (1° visual) cortex. Most commonly, the first large +ve wave, called P1 or P100 (as it typically occurs at about 100ms), is studied.

A delayed, smaller, or dispersed VEP indicates disease in the retino-geniculo-striate pathway (if severe refractive errors or cataracts have been excluded), but most commonly affecting the optic nerve (a unocular deficit implies a lesion anterior to the optic chiasm) or at the chiasm.

Flash-evoked visual evoked potentials

In subjects with very poor vision or fixation and in the very young, a bright flash may be used as the stimulus. This gives less reproducible results, particularly in the P100 latency.

Common uses

The VEP is used in general to document intrinsic, inflammatory, or compressive lesions of the optic nerve (or chiasm).

1. Suspected optic or retrobulbar neuritis.
2. In a patient with suspected MS, evidence of a VEP abnormality in an asymptomatic eye would suggest a previous episode of optic neuritis.
3. Evaluation of hysterical blindness (may need to use a strobe light stimulus if patient non-co-operative).
4. Evaluation of optic nerve function in compressive lesions such as dysthyroid eye disease, optic nerve glioma.
5. Follow-up after surgery to decompress the optic nerve or chiasm.
6. Assessment of poor visual acuity in patients unable to co-operate with usual testing. Vary the size of the chequerboard squares; subjects with poor acuity will only have a VEP to the coarser patterns.

Somatosensory evoked potentials

- Stimulation site over a peripheral nerve, e.g. ulnar or median at the wrist, common peroneal at the knee, posterior tibial at the ankle (see Fig. 9.3).
- Record over Erb's point (above the medial end of the clavicle), C7 or C2 vertebra, or the parietal cortex for arm stimulation; L1, C7, C2, or vertex for leg stimulation.
- Calculate absolute and interpeak latencies.

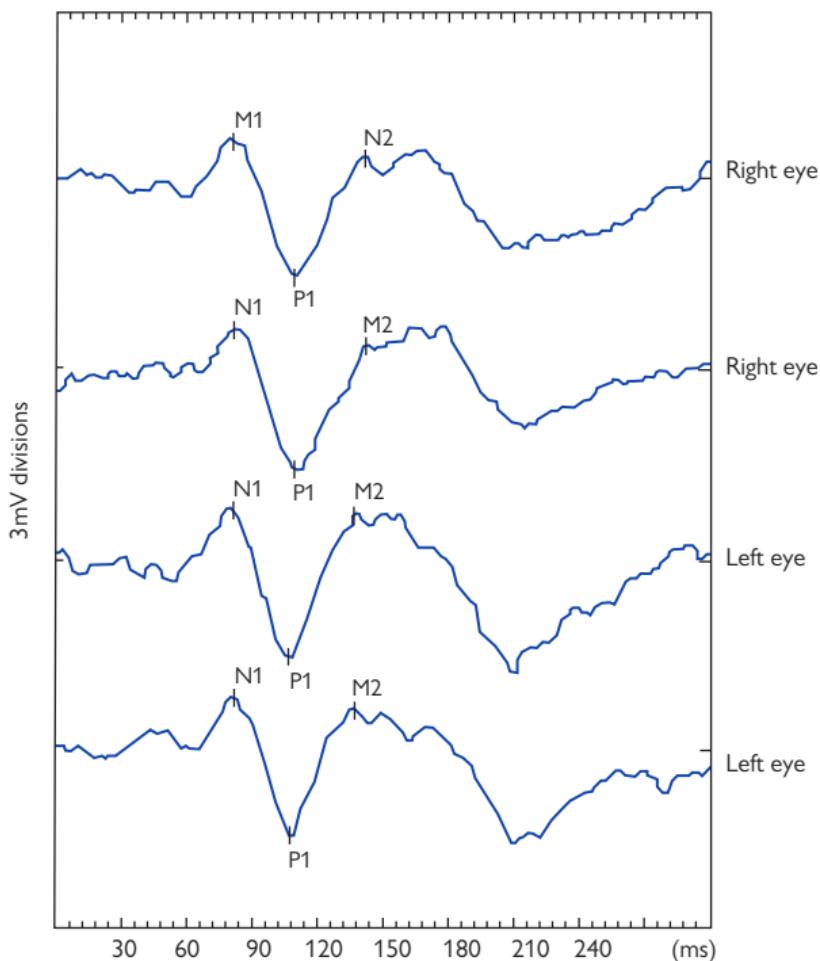


Fig. 9.2 Visual evoked potential (to chequerboard stimulus).

- Need to show with NCS that distal parts of the somatosensory pathways are conducting normally.
- Assesses the dorsal column, not anterolateral (spinothalamic) tract pathways:
 - For example, stimulate the median nerve at the wrist; prolonged latency to Erb's point suggests a brachial plexus (or more distal) lesion.
 - Prolonged Erb's point to C2 latency suggests a spinal cord lesion.

Uses

- Diagnosis of plexopathies.
- Evaluation of subclinical myelopathy in possible MS.
- Evaluation of hysterical sensory loss.
- Per-operative monitoring (e.g. during scoliosis surgery).

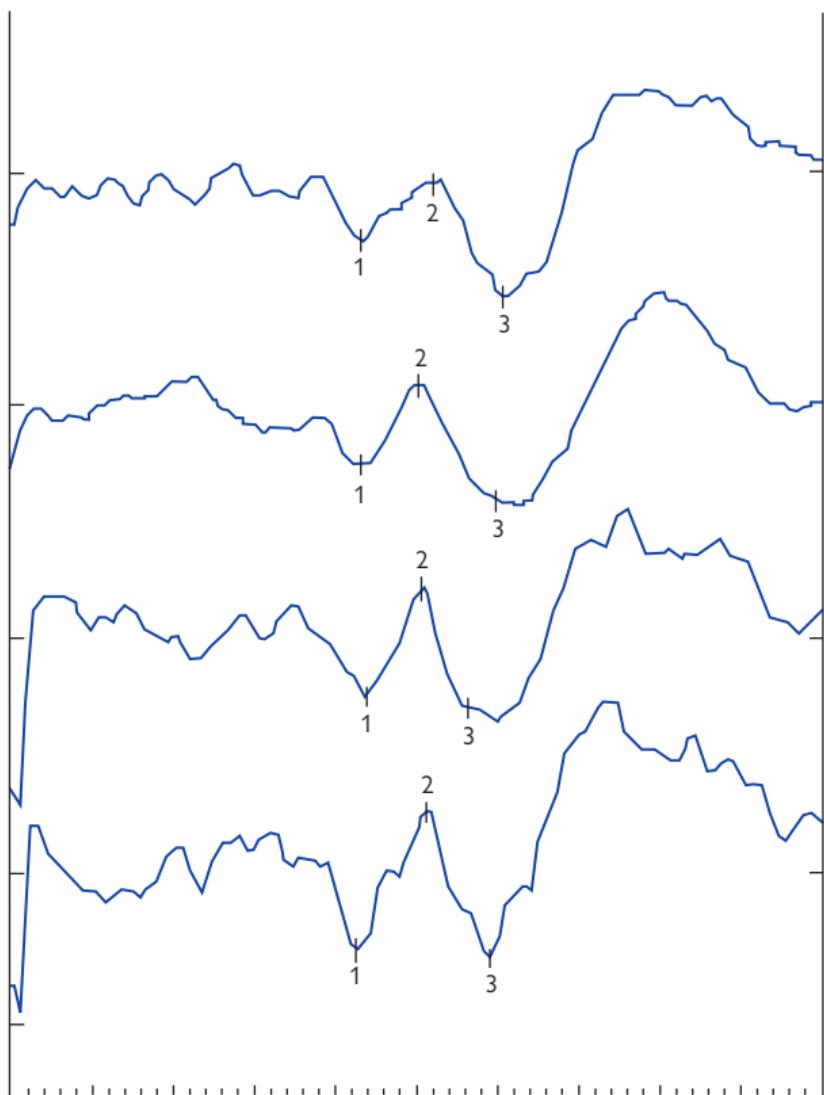


Fig. 9.3 Leg somatosensory potentials.

Brainstem auditory evoked potentials (BAEPs, BAERs, BSAEPs)

(See Fig. 9.4.)

- Stimulus: rarefaction clicks of 50 or 100ms duration, presented monaurally at 10Hz at 60–70dB above threshold (masking the noise to the other ear).
- Record over the mastoid and vertex of the skull.
- Classic waveform has seven peaks, said to be generated by sequential auditory nuclei:

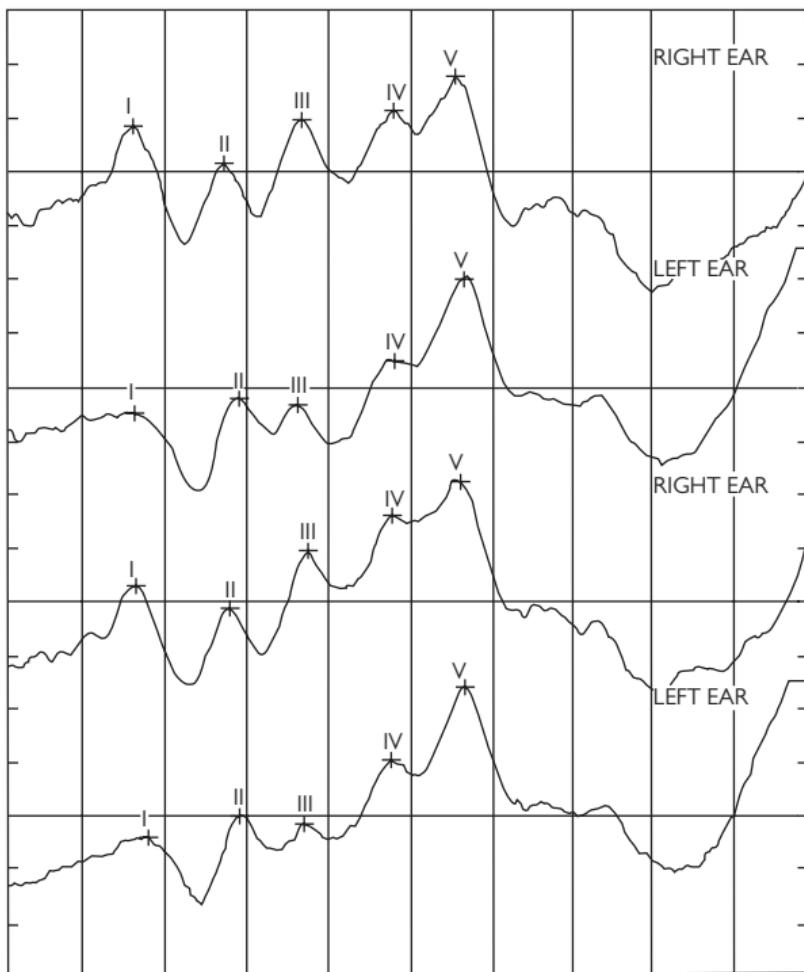


Fig. 9.4 Brainstem auditory evoked potentials.

- I: VIIIth nerve (must be present to interpret subsequent waves).
- II: cochlear nucleus (may be absent in normals).
- III: superior olive.
- IV: lateral lemniscus (may be absent in normals).
- V: inferior colliculus (should be 50% or more of wave I's amplitude).
- VI: medial geniculate (too variable for regular clinical use).
- VII: auditory thalamocortical radiation (too variable for regular clinical use).

Latency I-V (central conduction time) should be no more than 4.75ms. The difference between left and right central conduction times should be <0.4ms.

Uses

- Hearing assessment, especially in children.
- Evaluation in suspected MS and other myelinopathies (e.g. adrenoleukodystrophy; MRI more important now).
- Evaluation and detection of posterior fossa lesions (e.g. acoustic neuromas; MRI more important now).
- Evaluation of brainstem function (e.g. tumour, CVAs).
- Evaluation of brainstem function in coma and brain death.
- Per-operative, e.g. acoustic neuroma excision.

Use of evoked potentials in the diagnosis of multiple sclerosis

Traditionally, trimodal EPs (VEPs, SSEPs, and BAEPs) have been requested to look for evidence of a disturbed conduction in multiple sensory systems. Modern practice, however, is to request only VEPs, if any at all. MRI is much more useful in demonstrated dissemination of CNS lesions.⁵

Further reading

Blum AS, Rutkove SB (eds.). *The clinical neurophysiology primer*. Totowa: Humana Press, 2007.

⁵ Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: revisions to the 'McDonald criteria'. *Ann Neurol* 2005; **58**: 840–6.

Transcranial magnetic stimulation

- Brief, high-current pulse produced in a circular or figure-of-eight-shaped coil held over the scalp.
- This induces a magnetic field with flux perpendicular to the coil.
- This, in turn, produces an electric field perpendicular to the magnetic field.

The result is excitation or inhibition of the subjacent cortex (depending on stimulus parameters).

- Transcranial magnetic stimulation (TMS) has been used for diagnostic purposes in a number of ways, although as yet it is not in widespread clinical use.

Measurement of central motor conduction time

- TMS over the motor cortex indirectly (presumably via synaptic activation of corticospinal neurones) causes a volley of activity in the corticospinal tracts. The latency of the EMG in, say, the abductor digiti minimi may be measured.
- May be used in cervical myelopathy and MS to show ↑ latency of EMG in hand muscles evoked by TMS over the motor cortex. If the EMG latency to more distal stimulation (e.g. at C7 over the spinal cord and in the ulnar nerve) is normal, then an ↑ central motor conduction time may be inferred.
- Latency may also be ↑ in other neurodegenerative conditions.

Motor evoked potentials

Abnormalities in the amplitude of motor evoked potentials (MEP) may reflect abnormalities anywhere in the pathway from the motor cortex to the muscles.

Silent period

- If a subject maintains muscle contraction and a single suprathreshold TMS pulse is applied to the contralateral motor cortex, ongoing EMG activity ceases for a few hundred milliseconds after the MEP (the 'silent period').
- Silent period may be long in, e.g. stroke, MS, spinal cord injury.
- Silent period may be short in, e.g. MND, PD.

Interhemispheric conduction

- Interhemispheric inhibition may be ↓ in MS or MND.
- It may be absent following lesions to the corpus callosum.

Motor cortex excitability

- High thresholds may be seen in stroke or MS.
- Low thresholds and ↑ intracortical inhibition may be seen in MND.
- ↓ intracortical inhibition may be seen in PD.

Psychogenic limb weakness

Some authorities have used TMS to evoke muscle activity in 'paralysed' limbs in patients with psychogenic paralysis. This needs to be done in the context of a 'holistic' approach to the patient, aimed at dealing with any psychological pathology.

Potential clinical applications

There have been many TMS studies, some that may prove useful as clinical tests, e.g.

- Determination of lateralization of language function by repetitive TMS (rTMS) prior to surgery for epilepsy.
- Assessment of cortical excitability in certain epilepsy syndromes.
- Assessment of ↓ intracortical inhibition in dystonia.
- Assessment of recovery from stroke.⁶

Further reading

Currà A, Modugno N, Inghilleri M, Manfredi M, Hallett M, Berardelli A. Transcranial magnetic stimulation techniques in clinical investigation. *Neurology* 2002; **59**: 1851–9.

Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. *Lancet Neurol* 2003; **2**: 145–56.

⁶ Devlin JT, Watkins KE. Stimulating language: insights from TMS. *Brain* 2007; **130**: 610–22.

Neurological investigation of sphincter disturbance

Electromyogram

- Of pelvic floor muscles may be helpful in faecal incontinence, stress urinary incontinence, and cauda equina syndrome.
- Pelvic floor and sphincter muscle EMGs may reflect pudendal nerve damage.
- Anal sphincter EMG abnormalities may reflect damage to Onuf's nucleus, e.g. in multi-system atrophy. It is characteristically unaffected in MND.

Magnetic resonance imaging

- In suspected sacral spinal cord, conus medullaris, and equina lesions.

Urodynamics

Flowmetry

- Measurement of rate and amount of urine flow over time.
- Allows calculation of parameters such as time to maximal flow, maximum and mean flow rate, and volume voided.
- Post-micturition US can determine residual volume.

Cystometry (needs urinary catheterization)

- Measurement of intravesicular pressure during filling (usually at 50mL/min) or emptying. Typically, bladder filling sensation starts at about 100mL and the bladder is full at 400–600mL (with no more than 15cmH₂O rise in pressure). Detrusor instability may cause sharp rises in pressure during filling.
- During voiding, flow rate should be >15mL/min (♂) or >20mL/min (♀) with pressures of <50cmH₂O (♂) or 30cmH₂O (♀).

Further reading

Fowler CJ. Investigational techniques. *Eur Urol* 1998; **34**(Suppl): 10–12.

Panicker JN, Fowler CJ. The bare essentials: uro-neurology. *Pract Neurol* 2010; **10**: 178–85.

Edrophonium (Tensilon®) test

Procedure

- Explain the test to the patient.
- Select weak and/or fatiguable muscles to be assessed.
- Attach an ECG monitor.
- Draw up 0.6mg of atropine (for use if extreme bradycardia develops), 10mg of edrophonium in 5mL of normal saline (A), 5mL of normal saline (B), and saline flush.
- Administer 1mL of the test solution (A or B, ideally patient and administering physician should be blinded to the nature of the solution).
- If no adverse reaction, administer the remaining 4mL.
- Repeat with the other solution (B or A).

Note: if the diagnosis of MG is clinically obvious, and the patient has responded to pyridostigmine given empirically, there is little point in stopping this and performing an edrophonium test.

Interpretation

- In MG, there should be a response within 30–60s, which should wear off in 2–4min.
- There may be a response in LEMS, polymyositis, and MND.

Amobarbital (Wada) test

Amobarbital is injected into the right or left internal carotid artery. It is a short-acting barbiturate that temporarily causes hemispheric dysfunction on the injected side. If injected into the left in most right-handers, the ability to speak and continue to hold up the right arm is temporally impaired. If speech is preserved following right-sided injection, it suggests normal left lateralization for language function. More complex testing may also be undertaken during the period of hemispheric dysfunction, but it is usually used to determine language dominance prior to certain neurosurgical procedures. Increasingly, non-invasive techniques, such as fMRI, are being used in place of the Wada test.

Further reading

Wagner K, Hader C, Metternich B, Buschmann F, Schwarzwald R, Schulze-Bonhage A. Who needs a Wada test? Present clinical indications for amobarbital procedures. *J Neurol Neurosurg Psychiatry* 2012; **83**: 503–9.

Biopsies

- Always liaise with those taking the biopsy and those processing it!
- A biopsy should be undertaken to answer specific questions, in light of a differential diagnosis formulated following history, examination, and other investigations.

Skeletal muscle

Indications

- 1° muscle disease, e.g. metabolic myopathy, polymyositis, muscular dystrophy.
- Neurogenic atrophy.
- Mitochondrial cytopathies (even in the absence of clinical muscle involvement).
- Multi-organ disease, e.g. vasculitides.

Which muscle to biopsy?

- An involved, but not end-stage, muscle.
- One that has not been used for EMG recording or had an injection for >1 month.
- Quadriceps and deltoid often used.

Open or needle biopsy?

Open biopsy

- Larger specimen.
- Can fix specimen at *in situ* length.
- Especially for inflammatory myopathy and in vasculitis.

Needle biopsy

- Smaller scar.
- Multiple biopsies possible.
- However:
 - Smaller biopsies.
 - Difficulties in orientating the sample.

What may be done to the tissue?

- Routine histology.
- Examination of small blood vessels.
- Histochemistry.
- EM.
- Tests of muscle metabolism.
- Mitochondrial DNA studies.

Nerve

Indications

- Distinction between segmental demyelination and axonal degeneration (if not already determined).
- Certain neuropathies with characteristic histological features, e.g. due to amyloid deposition, sarcoid, vasculitis, neoplastic involvement.
- Certain myelinopathies (e.g. leukodystrophies) with peripheral nervous system (PNS) and CNS involvement.

Which nerve to biopsy?

- The cutaneous branch of the sural nerve at the ankle (usually).
- Superficial peroneal (sometimes).
- Superficial radial (occasionally).
- Occasionally, small motor nerve twigs are obtained in muscle biopsy.
- Overlying skin may be co-biopsied.

What is done?

- 2–3cm of full-thickness nerve or fascicle.

What may be done to the tissue?

- Routine light microscopy (morphometry, structural survey; amyloidosis).
- Frozen section light microscopy (immunochemistry).
- EM (ultrastructure).
- Teased out single fibres (to examine sequential myelin internodes).

Brain/meningeal biopsy**Indications**

- Diagnosis and management of suspected 1° and some metastatic brain tumours.
- Differential diagnosis of other mass lesions (inflammatory and infective).
- Differentiation of radiation necrosis and tumour regrowth.
- Differentiation of neoplastic and non-neoplastic cysts (and their drainage).
- Diagnostic biopsy of a suspected infectious lesion that has not responded to a trial of therapy.
- Diagnosis of cerebral vasculitis or vasculopathy.

What is done?

- High-quality cranial CT/MRI, possibly with contrast, to delineate the lesion.
- If no discrete lesion, generally an area of non-dominant, non-eloquent cerebrum is taken.
- Stereotactic needle biopsy with image guidance:
 - Deep, small lesions in 'eloquent' areas.
 - Multiple biopsies along the needle track (useful in heterogeneous lesions such as some gliomas).
- And/or open biopsy:
 - Accessible lesions.
 - When resection considered during procedure.
- Intra-operative evaluation of frozen samples:
 - For example, can a biopsy be made?
 - For example, is the sample adequate?

Note: caution in suspected CJD!!

Skin

- Some storage diseases:
 - Lafora body.
 - Batten's disease.
- Mitochondrial cytopathies.

Bone marrow

- Niemann–Pick type C.
- Haematological and other malignancies.

Rectal and appendicectomy

- Most neuronal storage diseases affect the autonomic nervous system, so evidence can be sought in neurones of the gut's intrinsic plexi.
- Amyloid in rectal biopsy.

Tonsillar biopsy

- Research tool in vCJD.

Oligoclonal bands

- Electrophoresis of serum and CSF separates protein components by size and charge.
- OCBs may be present in serum and CSF. Bands in the CSF not seen in the serum suggest intrathecal-specific synthesis of IgG.
- This pattern is seen in most (95%) cases of established MS but may also occur in other conditions such as chronic meningitis, neurosyphilis, SSPE, and neurosarcoid (although uncommonly).

Further reading

Thompson EJ. Cerebrospinal fluid. In: RAC Hughes (ed.). *Neurological Investigations*. London: BMJ Publications, 1997; pp. 443–66.

Diagnostic and prognostic antibodies, and other markers in blood and urine

Multi-system disorders

PNS and CNS are affected in many multi-system disorders; markers in blood and other fluids and tissues for these are therefore commonly requested in neurology patients.

Vasculitides, e.g.

- ENAs in SLE.
- ANCA in Wegener's.
- RF in RA.

Enteropathies, e.g.

- Gliadin and endomysial antibodies in coeliac disease.

Systemic infections, e.g.

- Serology for many diseases, e.g. *Borrelia* in Lyme disease, HIV.
- PCR for TB.

Disorders of coagulation: thrombophilia screen currently commonly includes

- Protein S and C levels.
- Antithrombin III levels.
- Screening for the Leiden mutation in factor V.
- Lupus anticoagulant.
- Tumour markers, e.g.
 - carcinoembryonic antigen (CEA) for gut neoplasia.
 - Serum and urinary paraproteins in haematological disorders like myeloma.

Sarcoid

- ACE and ACE genotype.

Endocrinopathies, e.g.

- TSH, FT4, and FT3, thyroid autoantibodies in thyroid dysfunction.

Other metabolic disorders, e.g.

- *Wilson's disease*: blood copper and caeruloplasmin; some authorities also request 24h urinary copper excretion. Note: slit lamp examination performed by an experienced ophthalmologist reveals Kayser–Fleischer rings in most cases of Wilson's disease with neurological involvement.
- *Phaeochromocytoma*: catecholamine metabolites in three 24h urine collections.

Disease-specific markers

- *MG*: anti-ACh receptor and muscle-specific kinase (MuSK) antibodies.
- *Neuromyelitis optica*: aquaporin 4 and MOG antibodies

Paraneoplastic antibodies

Certain neurological syndromes are ‘paraneoplastic’, i.e. due to remote, but non-metastatic, effects of non-nervous system cancers. These paraneoplastic syndromes are rare, but important to recognize. In perhaps 50% of cases, the neurological symptoms may predate those of the cancer. This is an area of intensive research. Antibody tests that are well described include those in Table 9.7.

Most of these paraneoplastic antibodies target intracellular antigens and are not thought to be pathogenic in themselves. Increasingly, autoantibodies to antigens on the surface of neurones or glia are being recognized and associated with clinical syndromes (see Table 9.8). Such antibodies may be directly pathogenic. Recognizing clinical syndromes associated with these cell surface-directed antibodies is important, as many respond to immunotherapies.

Table 9.7 Paraneoplastic antibodies

Antibody	Paraneoplastic syndrome	Associated tumour
Anti-Hu (ANNA1)	Encephalomyelitis Sensory neuropathy Cerebellar degeneration Chronic GI pseudo-obstruction Limbic encephalitis	Small-cell lung cancer (SCLC)
Anti-Yo (PCA1)	Cerebellar degeneration	Breast, ovary
Anti-Ri (ANNA2)	Brainstem encephalitis	Breast, SCLC
CV2 (CRMP5)	Encephalomyelitis Chorea Sensory neuropathy Sensorimotor neuropathy Cerebellar degeneration Limbic encephalitis Chronic GI pseudo-obstruction	Thymoma, SCLC
Anti-Ma2 (Ta)	Limbic/diencephalic encephalitis Cerebellar degeneration Brainstem encephalitis	Testis, lung
Anti-amphiphysin	Stiff person syndrome Other syndromes	Breast, SCLC
CAR	Retinopathy	Breast, SCLC
Tr	Cerebellar ataxia	Hodgkin's

Table 9.8 Neuronal surface antibody antibodies

Antibody	Syndrome	Associated tumour
NMDAR	Encephalitis; dyskinesia; psychiatric presentation; epilepsy	Ovarian teratoma
LGI1	Limbic encephalitis	Rare
CASPR2	Limbic encephalitis Morvan's syndrome	Lung, breast, thymus
GABA _A R	Limbic encephalomyelitis Prominent seizures	SCLC
mGluR5	Limbic encephalitis Orphelia syndrome	Hodgkin's lymphoma
GlyR	Progressive encephalomyelitis with rigidity and myoclonus	Thymoma
VGCC	Cerebellar ataxia	SCLC
mGluR1	Cerebellar ataxia	Hodgkin's lymphoma

Further reading

- Graus F, Dalmau J. Paraneoplastic neurological syndromes. *Curr Opin Neurol* 2012; **25**: 795–801.
- Graus F, Delattre JY, Antoine JC, et al. Recommended diagnostic criteria for paraneoplastic neurological syndromes. *J Neurol Neurosurg Psychiatr* 2004; **75**: 1135–40.
- Honorat J, Antoine J-C. Paraneoplastic neurological syndromes. *Orphanet J Rare Dis* 2007; **2**: 22.
- Zuliani L, Graus F, Giometto B, Bien C, Vincent A. Central nervous system neuronal surface antibody associated syndromes: review and guidelines for recognition. *J Neurol Neurosurg Psychiatry* 2012; **83**: 638–45.

Biochemical markers

Many 'inborn errors of metabolism' cause neurological disease. A variety of investigations, including tests on blood, urine, and CSF, biopsies, and genetic analyses, are used in their diagnosis (Diagnostic and prognostic antibodies, and other markers in blood and urine, pp. 636–8; Genetic tests, pp. 642–5; for an accessible review, see Gray *et al.*).⁷

Biochemical tests

Some basic principles

Many autosomal recessive and X-linked metabolic diseases are caused by reduced or absent activity of a specific enzyme, in turn due to a single gene defect.

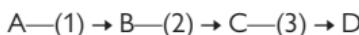
In some, there is a tissue-specific deficit

For example, McArdle's (glycogen storage disease V) demonstrates the absence of phosphorylase activity in muscle biopsy (as only the myophosphorylase isozyme is affected).

In other conditions, notably the lipidoses, the enzyme is deficient in many tissues

For example, in Niemann–Pick diseases A and B, sphingomyelinase is deficient in the brain and spinal cord, but also in the GIT, liver, spleen, and BM. Abnormal lipid metabolism can therefore be demonstrated in relatively easily accessible tissue such as fibroblasts.

Not only may the absence or lower activity of an enzyme reduce the amount of product of the reaction it catalyses, but it may also lead to the accumulation of precursors in the metabolic pathway:



If enzyme (3) is reduced, A, B, and C may accumulate, with lower levels of D than usual being produced

- For example, in acute intermittent porphyria, there is ↑ urinary excretion of ↓ heme aminolevulinic acid and porphobilinogen (intermediates in the haem synthetic pathway) during an acute attack.
- ↓ levels of porphobilinogen deaminase may be demonstrated in erythrocytes, leucocytes, and cultured fibroblasts.

⁷ Gray RG, Preece MA, Green SH, Whitehouse W, Winer J, Green A. Inborn errors of metabolism as a cause of neurological disease in adults: an approach to investigation. *J Neurol Neurosurg Psychiatr* 2000; **69**: 5–12.

Ischaemic forearm exercise test (ischaemic lactate test)

Procedure

1. Rest the patient supine for 30min.
2. Draw a baseline lactate sample from a catheter in an antecubital vein.
3. Inflate the sphygmomanometer cuff on that arm to above arterial pressure.
4. Subject squeezes a rubber ball in that hand until exhaustion.
5. Rapidly deflate the cuff.
6. Take further venous samples at 30, 60, and 240s.

Results

Normally the venous lactate will rise by 2-, 3-, or even 4-fold; if it fails to rise by 1.5-fold, then there is likely to be a glycogenolysis or glycolysis defect (or the patient has not exercised sufficiently!).⁷

Genetic tests

The list of diseases for which we have specific genetic tests grows each month. Rather than give a necessarily incomplete compendium, we discuss some general principles.

Several important neurological conditions may today be diagnosed by (relatively) simple genetic tests, whereas in the past biopsy was necessary. For example, Duchenne, Becker, and oculopharyngeal muscular dystrophies are associated with well-defined genetic abnormalities. Similarly, several mitochondrial cytopathies (such as myoclonic epilepsy and ragged red fibres (MERRF) and mitochondrial myopathy, lactic acidosis, and stroke-like episodes (MELAS)) may now often be diagnosed by finding common mutations or deletions in mitochondrial DNA.

When might a neurologist refer to a clinical geneticist?

- Genetic counselling of an index patient and his family.
- Cytogenetic or molecular diagnosis.
- Long-term follow-up of family:
 - Notification of advances.
 - Counselling family members as they become adult.
 - Coordinating care with paediatric and adult neurologists.

Cytogenetics: when to do it

- ♀ with an X-linked disorder.
- Unexplained developmental delay.
- Unexplained major CNS malformation.
- The coexistence of two genetic diseases in a patient.

What is done?

- Conventional karyotype.
- FISH for suspected submicroscopic chromosomal aberrations, e.g. a p13.3 deletion may cause lissencephaly.

Molecular genetics: when to do it

- Confirming a clinical diagnosis.
- Identify carriers in the family.

What is done?

An ever ↑ range of diseases may be tested for. Some of these tests may be routinely available at your local clinical genetics laboratory, others at regional, national, or even supranational centres. Other tests may be available on a 'research' basis. It is clear, however, that tests for genetic 'lesions' or risk factors will become increasingly available. Rather than give an, at best, partial list of readily available tests, we give a few examples below of the kinds of tests that are available. The astute reader will spot that different mutations within a given gene can give rise to different clinical phenotypes. Indeed, recent work has shown that the same mutation in some genes can give rise to >1 phenotype—we clearly have a great deal yet to learn about the genetics of neurological diseases!

Table 9.9 Some trinucleotide repeat diseases

Disease	Gene	Triplet repeats	Transmission
Fragile X	<i>FMR1</i>	CGG	X-linked
Myotonic dystrophy	<i>DM</i>	CTG	AD
Friedreich's ataxia	<i>FRDA</i>	GAA	AR
Spinobulbar muscular atrophy	Androgen receptor	CAG	X-linked
Huntington's disease	<i>IT15</i>	CAG	AD
Spinocerebellar atrophy			
• SCA 1	<i>SCA 1</i>	CAG	AD
• SCA 2	<i>SCA 2</i>	CAG	AD
• SCA 3	<i>SCA 3</i>	CAG	AD
• SCA 6	<i>SCA 6</i>	CAG	AD
• SCA 7	<i>SCA 7</i>	CAG	AD
Dentarubropallidolusian atrophy	<i>DRPLA</i>	CAG	AD

AD, autosomal dominant; AR, autosomal recessive.

Note: SCA 6 is a CAG triplet expansion in the *CACNL1A4* calcium channel gene. Other (non-triplet repeat) mutations in the gene cause other conditions—episodic ataxia type 2 and familial hemiplegic migraine.

Detection of deletions

- For example, in mitochondrial (mt)DNA in MELAS and MERRF.
- For example, of dystrophin gene in Duchenne and Becker muscular dystrophies.

Detection of DNA rearrangement

- For example, *PMP22* gene duplication in some cases of Charcot–Marie–Tooth disease type 1 (or hereditary motor sensory neuropathy type 1 (HMSN1); deletions within this gene cause hereditary neuropathy with liability to pressure palsies (HNPP).

Detection of trinucleotide repeats

- Found in >10 neurological diseases (see Table 9.9).
- So far, there is no overlap in the number of repeats in controls and affected patients (except rarely in Huntington's, in the region of 33–36 repeats).
- Anticipation (more severe phenotype and earlier onset) often reflects in ↑ number of repeats in the most recent generations (especially myotonic dystrophy).

Detection of single base mutations

This involves fragmenting the DNA of the gene into manageable pieces, then amplifying these so that there are multiple copies. Subsequently, various methods may be used to detect fragments with abnormal sequences, even if only differing at a single base from the 'wild-type'. There are several such techniques, constantly being refined, and many are restricted to research laboratories.

- However, molecular genetics is proceeding at a tremendous pace, both in terms of the number of conditions with identified genetic lesions and the laboratory techniques for analysis.
- High-speed DNA sequencing will facilitate sequencing large pieces of DNA.
- For example, point mutations in the *MPZ* gene, which encodes P0, a component of the myelin sheath, have been found in some families with Charcot–Marie–Tooth disease type 1B.

Genetic risk factors

Another area of clinical genetics which is likely to become more important is the detection of genetic 'risk factors' for diseases. Certain allelic variants, whilst not 'causing' a disease in the traditional sense, may predispose an individual to exhibiting a certain clinical phenotype or alter the age at which it might become apparent.

For example, there are three allelic variants in the apolipoprotein E (*APOE4*) gene e2, e3, and e4. Homozygosity for e4 is likely to be a risk factor for developing Alzheimer's disease and for developing it at an earlier age. However, the majority of e4 homozygotes do not develop the condition (therefore, it is not 'causative').

Detection of the presence of abnormal protein or altered levels of normal protein

Immunocytochemistry and immunoblotting (western blots) on tissue samples from the patient allow direct visualization of the presence of abnormal protein or the absence or reduced levels of normal protein, in a variety of conditions. (These techniques are not genetic in the strictest sense but are often useful in 'genetic' conditions.)

For example, Duchenne and Becker muscular dystrophies have absent or reduced levels of dystrophin in muscle biopsy samples.

Whole genome sequencing

With developments in technology and informatics, it is becoming increasingly feasible (in terms of both cost and time) to sequence the entire genome of individuals. Whole genome sequencing has resulted in the detection of rare pathogenic mutations and the determination of genetic risk factors.

The 100,000 Genomes Project was established in 2012 and aims to sequence 100,000 whole genomes from NHS (England) patients with rare diseases, their families, and patients with cancer (<http://www.genomicsengland.co.uk/the-100000-genomes-project/>).

Useful website

Online Mendelian Inheritance in Man (OMIM) is a continually updated catalogue of 'genetic' diseases in man, giving data about the genotype, mode of inheritance, and clinical phenotype of thousands of disorders (not just neurological).

Further reading

- Manolio TA. Genome-wide association studies and the risk of disease. *N Engl J Med* 2010; 363: 166–76.
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- Young AB. Huntington's disease and other trinucleotide repeat disorders. In: JB Martin (ed.). *Scientific American Molecular Neurology*. New York: Scientific American, 1998; pp. 35–54.

Neuro-otology

Pure tone audiometry

- Measures the threshold for air (AC) and bone conduction (BC) at frequencies from 250 to 8000Hz.

Typical patterns

- Conduction deafness: BC > AC at all frequencies.
- Sensorineural deafness: AC = BC at all frequencies, but ↑ deafness as the frequency rises.

More specialized tests

- Tone decay.
- Loudness discomfort.
- Speech audiometry.
- Acoustic impedance.

Caloric testing

Procedure

1. Inspect the eardrum; if intact, proceed.
2. Place the patient supine with the neck flexed 30° (on pillow).
3. Irrigate the external auditory meatus with 30°C water (ice water if testing for brain death).
4. Observe for (or record*) nystagmus.
5. Repeat after 5min with 44°C water.

Note: (*) there are various techniques for recording eye movements that are available in specialized clinical and research laboratories.

What should happen

1. Cold water induces convection of fluid in the ipsilateral lateral semicircular canal (LSCC).
2. There is less output from the ipsilateral LSCC.
3. Imbalance of signals from the two LSCCs results in eye drift towards the irrigated ear.
4. Fast-phase contraversive movements correct for eye drift (hence, nystagmus with the fast phase away from the irrigated ear).
5. This nystagmus starts in about 20s and persists for 1min.
6. Warm water reverses the nystagmus.

Common pathological responses

Canal paresis

1. Reduced duration of nystagmus following irrigation on one side (with cold or warm water).
2. Suggests ipsilateral peripheral or central lesion.

Directional preponderance

1. Prolonged nystagmus in one direction.
2. Suggests a central lesion on the side of preponderance or contralateral peripheral lesion.

Combination of clinical examination, audiometry, and caloric testing of the vestibulo-ocular reflex will help localize a lesion (peripheral vs central; left vs right).

Brainstem auditory evoked responses

- Sensory evoked potentials or responses, Brainstem auditory evoked potentials (BAEPs, BAERs, BSAEPs), pp. 624–6.

Further reading

Brandt T, Dieterich M, Strupp M. *Vertigo and Dizziness*, 2nd edn. London: Springer, 2014.

Renal medicine

- Estimation of kidney function 650
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- Assessment of proteinuria 658
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Estimation of kidney function

Estimates based on serum creatinine

Measurement of serum creatinine concentration is the most commonly used way of assessing the excretory function of the kidneys, which is mostly dependent on the glomerular filtration rate (GFR). Creatinine is the non-enzymatic breakdown product of creatine and phosphocreatine (almost exclusively found in skeletal muscle). Its production rate is proportional to muscle mass—the average individual produces around 10mmol/day. Endogenous production of creatinine is usually constant, but ingestion of cooked meat and severe exercise cause a rapid, temporary rise in production of creatinine, and thus in creatinine concentration. It is excreted mainly by glomerular filtration, but tubular secretion of creatinine also occurs and contributes a significant proportion of overall excretion when GFR falls, resulting in overestimation of the GFR at low GFR. Drugs, e.g. cimetidine, trimethoprim, can block the secretory component and elevate serum creatinine without any change in true GFR.

Because of the reciprocal relationship between clearance and serum creatinine, serum creatinine does not rise outside the normal range until there has been a substantial fall in GFR, particularly in patients with low muscle mass (see Fig. 10.1). However, in an individual patient, a progressive ↑ in serum creatinine over time, even within the normal range, indicates declining GFR. Wide variation between individuals based on muscle mass, sex, and age makes serum creatinine an imperfect screening test for renal failure. Estimation of 24h urine creatinine excretion allows measurement of CrC but is beset with difficulties largely related to the timing and completeness of urine collections. For these reasons, use of CrC in clinical practice has been superseded by the use of prediction formulae.

The 4-variable Modification of Diet in Renal Disease formula

The GFR may be estimated by the 4-variable Modification of Diet in Renal Disease (MDRD) formula. This is the simplest of several formulae derived from the MDRD data set and gives an estimate of the GFR normalized to a body surface area of 1.73m^2 :

$$\text{eGFR } (\text{mL/min}/1.73\text{m}^2)$$

$$= 175 \times \{[\text{serum creatinine } (\text{mmol/L})/88.4]^{-1.154}\} \times \text{age } (\text{years})^{-0.203}$$

× 0.742 if ♀ and

× 1.21 if Afro-Caribbean

This formula does not give an accurate estimate of the GFR at extremes of muscle mass, including amputees, and is not validated in the paediatric population. Most laboratories in the UK now measure creatinine with an assay calibrated to an isotope dilution mass spectrometry (IDMS) standard and are able to report an eGFR, alongside creatinine results, using this formula.

► Where the laboratory reports a value for the eGFR, this should be used, rather than any value subsequently derived.

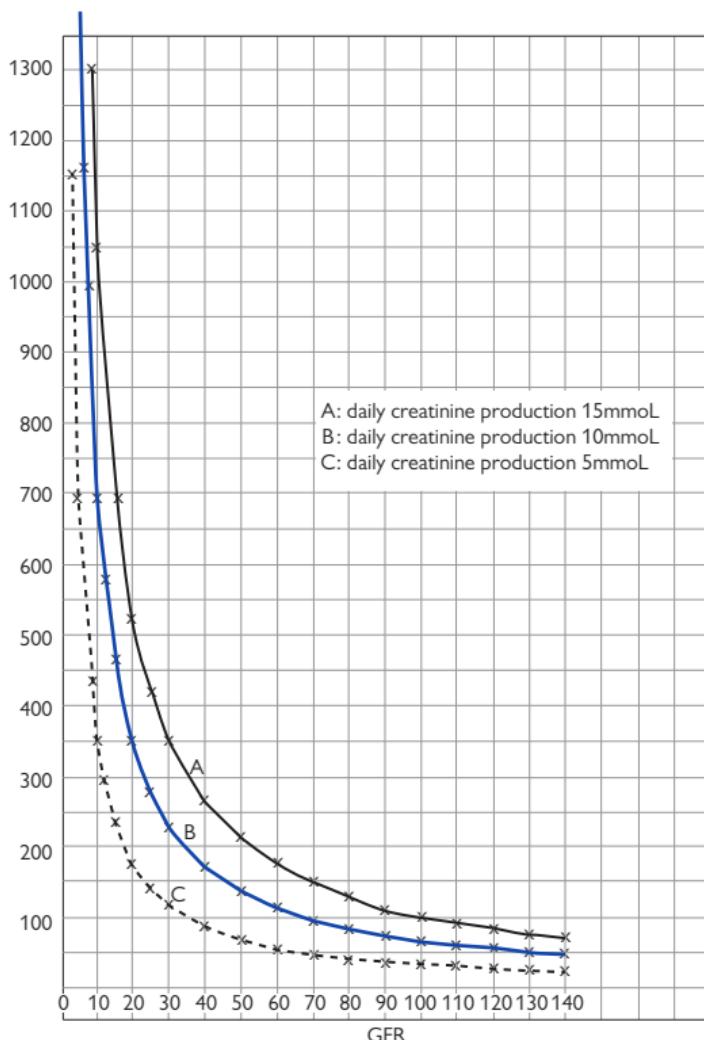


Fig. 10.1 Creatinine production is dependent on muscle mass, which varies widely. Graph illustrates the theoretical relationship between the GFR and plasma creatinine, ignoring the effects of tubular secretion of creatinine, which results in overestimation of the GFR from plasma creatinine or measurement of CrC. Note that in a patient with low muscle mass, creatinine does not rise outside the normal range until the GFR has fallen to <30mL/min, whereas a patient with higher muscle mass will reach the same level of creatinine at a GFR of 90mL/min.

The formula previously used a constant of 186, rather than 175, as it was developed using a creatinine assay that gave higher values than the IDMS standard; it remains important to check which assay is being used before using this or any other formula to estimate the GFR.

The Cockcroft and Gault formula and other estimates of eGFR

The Cockcroft and Gault formula gives an estimate of CrC, using age, weight, and serum creatinine as input variables. For simplicity of reporting, because weight is not required and because the MDRD formula gives an estimate of the GFR, which is normalized to the body surface area (and thus gives an estimate of how well kidney function is matched to body size), the MDRD formula is now preferred.

$$\text{CrC} = (\{[140 - \text{age (years)}] \times \text{weight (kg)}\} / \text{serum creatinine (mmol/L)})$$

× 1.23 if ♂ or
× 1.04 if ♀

 See <http://ckdepis.org/equations/creatinine-based-equations/> for a discussion of these and other formulae.

Where estimation of the GFR is made, the formula used should be stated. There are important differences between the two estimates, particularly as the GFR declines at extremes of body size, but either formula is a major advance on the use of serum creatinine alone.

CKD-EPI

The CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation gives a more accurate estimation of the GFR in patients with normal or near-normal renal function than the MDRD. The result is that fewer people with near-normal GFR are misclassified as having CKD and, as a result, treated as 'high risk' for cardiovascular outcomes, based on their eGFR.

$$\text{eGFR} = 141 \times \min(\text{SCr}/\kappa, 1)^\alpha \times \max(\text{SCr}/\kappa, 1)^{-1.209} \times 0.993^{\text{Age}}$$

× 1.018 if ♂
× 1.159 if black

where SCr (standardized serum creatinine) = mg/dL

$\kappa = 0.7$ if ♂ or 0.9 if ♀, and

$\alpha = -0.329$ if ♂ or -0.411 if ♀

min indicates the minimum of SCr/κ or 1, and

max indicates the maximum of SCr/κ or 1.

Classification of kidney disease

The following classification of chronic kidney disease (CKD) is now commonly used in the UK and is based on the Kidney Disease Improving Global Outcomes (KDIGO) classification.¹ Independently of the eGFR, the degree of albuminuria has been shown to be a strong predictor of cardiovascular mortality, progression of CKD, and acute kidney injury (AKI), and for these reasons, a measure of albuminuria was added to the original CKD classification in 2012.

- CKD G1: normal GFR; GFR >90mL/min/1.73m² with other evidence of chronic kidney damage.*
- CKD G2: mild ↓ in GFR; GFR 60–89mL/min/1.73m² with other evidence of chronic kidney damage.*

Note: (*) other evidence of CKD may include persistent microalbuminuria, proteinuria, or glomerular haematuria, structural abnormalities, including renal scarring and polycystic disease, and biopsy-proven chronic glomerulonephritis. An eGFR of >60 in the absence of other evidence of kidney damage should be considered normal.

- CKD G3a: moderate ↓ in GFR 45–59mL/min/1.73m².
- CKD G3b: moderate ↓ in GFR 30–44mL/min/1.73m².
- CKD G4: severe ↓ in GFR 15–29mL/min/1.73m².
- CKD G5: established renal failure (ERF) GFR <15mL/min/1.73m² or on dialysis.

The staging for albuminuria quantification is then added as follows:

- A1—albumin:creatinine ratio (ACR) <3mg/mmol.
- A2—ACR 3–30mg/mmol.
- A3—ACR ≥30mg/mmol.

Using this staging system, a person with an eGFR of 48mL/min/1.73m² and an ACR of 25mg/mmol has CKD stage G3a, A2.

Classification of CKD in this way allows identification of those with severe disease in need of specialist assessment and can be used to guide BP targets and frequency of monitoring in those with less severe disease.²

Serum urea

Urea is the product of protein catabolism in the liver. Production is ↑ by high protein intake, catabolic states, breakdown of blood in the gut lumen in GI bleeding, and tetracycline treatment, and may ↓ in liver disease. Urea is freely filtered at the glomerulus, with variable reabsorption, which is influenced by extracellular volume status. Intravascular volume depletion, diuretics, CCF, GI bleeding, tetracyclines, and renal failure cause elevated levels. Disproportionate rise in serum urea, compared to creatinine, occurs in hypovolaemia and GI bleeding. Reduced levels are seen in chronic liver disease and alcohol abuse. By itself, serum urea is a very poor marker of excretory kidney function.

1 KDIGO. Guidelines. <http://kdigo.org/guidelines/>.

2 National Institute for Health and Care Excellence (2014). *Chronic kidney disease in adults: assessment and management*. Clinical guideline CG182. <https://www.nice.org.uk/guidance/cg182>.

Cystatin C

Cystatin C, a 13kDa protein of the cystatin superfamily of cysteine protease inhibitors, is produced by all nucleated cells at a relatively constant rate and excreted nearly exclusively by glomerular filtration. It can be assayed using efficient enzyme-linked immunoassays. Multiple studies have shown that cystatin C is probably a more sensitive and specific marker than creatinine for assessing impaired excretory renal function. Minor reductions in GFR cause cystatin C concentrations to rise above normal, even when serum creatinine is still within normal range. Cystatin C-based estimation of the GFR is now recommended by NICE for confirmatory testing in patients with an eGFR_{creatinine} of 45–59mL/min/1.73/m² with no other evidence of CKD.

Measurement of glomerular filtration rate

Occasionally, it is necessary to measure renal excretory function accurately, e.g. in clinical research:

- When using drugs with a narrow therapeutic index and which are excreted by the kidney.
- When accurate measurement of kidney function is required in patients with abnormal muscle mass, e.g. paraplegics with bilateral lower limb muscle wasting.

The 'gold standard' for measurement of the GFR is measurement of inulin clearance (see Fig. 10.2); inulin is freely filtered, not protein-bound, and not reabsorbed or secreted. However, measurement of inulin is not widely available as a routine laboratory test.

Radionuclide studies

Radionuclide studies are contraindicated during pregnancy; women of childbearing age should have a –ve pregnancy test before proceeding with the test.

A variety of radioisotope markers are available for estimating the GFR. An ideal marker should be safe, not be extensively protein-bound, be freely filtered, not be secreted or reabsorbed in the renal tubule, and be excreted only by the kidney.

- The commonly used markers are ⁵¹Cr EDTA, ^{99m}Tc DTPA (diethylenetriaminepentaacetic acid), iohexol, and ¹²⁵I iothalamate. Iothalamate is also available without radiolabelling and can be measured by fluorimetry.
- These substances are injected IV and after allowing for equilibration, plasma levels are measured at predetermined intervals. Plasma clearance, and hence renal elimination, is calculated from the rate of fall of the concentration of the substance in the bloodstream.
- ⁵¹Cr EDTA has been the most extensively studied marker and is extensively used in Europe as a single injection technique followed by plasma sampling at 0, 90, 120, 150, and 240min. ⁵¹Cr EDTA is reliable, even at low levels of renal function. Studies in humans suggest renal clearance estimated by this method is ~10% lower than that of inulin.

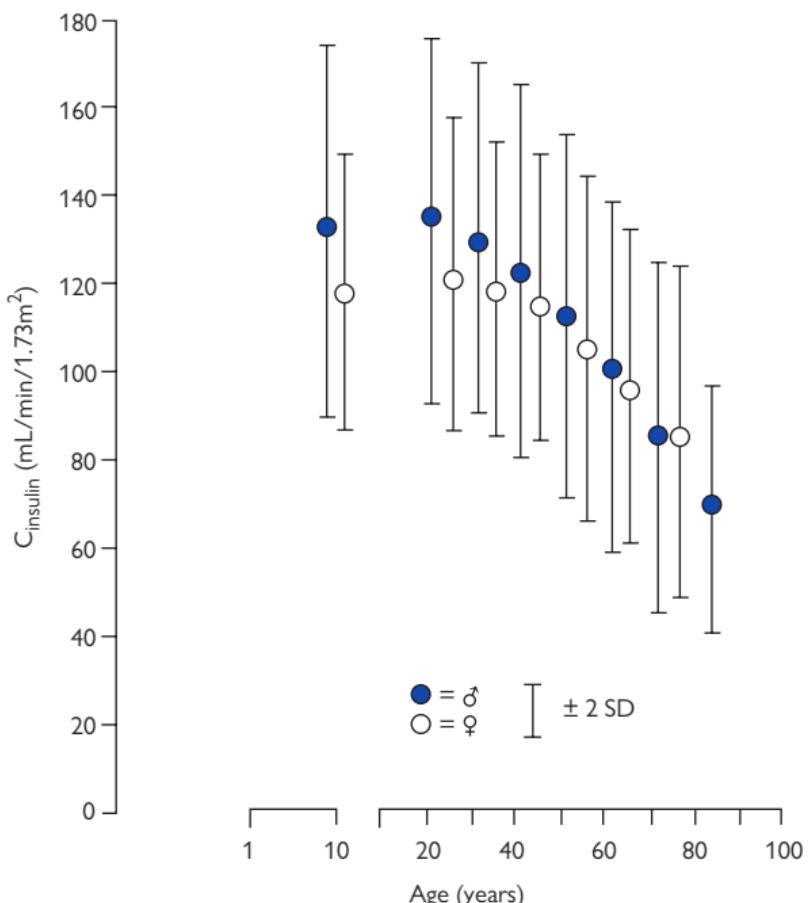


Fig. 10.2 GFR, measured by inulin clearance (C_{inulin}), in apparently healthy individuals according to age.

- ^{125}I iothalamate is only slightly protein-bound, and studies suggest clearance values similar to that of inulin. Unlike other markers, it can also be administered SC, and this allows for slow equilibration with stable plasma concentrations. It is considered safe, but potential problems of thyroid uptake necessitate pre-treatment with oral iodine.
- $^{99\text{m}}\text{Tc}$ DTPA is used in renal isotope scanning, which allows anatomical correlation to renal function such as information on relative uptake by each kidney. $^{99\text{m}}\text{Tc}$ has a very short half-life, and radiation exposure is minimized. Protein binding can result in diminished renal clearance.

Assessment of proteinuria

Proteinuria may result from ↑ glomerular permeability or tubular disease, causing ↓ reabsorption of filtered protein or ↑ excretion of tubular enzymes. Glomerular proteinuria is commoner and more likely to signal potentially progressive kidney damage. Disease states influence the absolute amount of protein excreted, so protein excretion should be assessed either by measurement of excretion over 24h (the 'gold standard', but highly inconvenient) or after correction for the degree of urine concentration. Because total daily urine creatinine excretion is constant, the ACR ratio or protein:creatinine ratio (PCrR) in the urine can allow correction for urine concentration. Although dipstick tests are useful, they can be misleading, with false +ve (concentrated urine) and false -ve (dilute urine) results. Assuming an average creatinine production of 10mmol/day, a PCrR of mg/mmol allows estimation of the daily protein excretion as $10 \times \text{mg}/24\text{h}$. Greater precision can be gained by the use of prediction equations for creatinine excretion (see  <http://ckdepot.org/equations/creatinine-excretion/>), but this is not yet standard practice.

Indications for quantitation of proteinuria

Diagnosis of nephrotic syndrome

Nephrotic syndrome is defined as triad of *oedema*, *hypoalbuminaemia*, and *proteinuria* $>3\text{g}/24\text{h}$ (or urine PCrR $>3\text{mg}/\text{mmol}$). Hyperlipidaemia is also commonly present.

Prognosis of progressive renal disease

Proteinuria is one of the most potent risk markers for progressive loss of renal function in renal disease, e.g. diabetic nephropathy, chronic glomerulonephritis, and reflux nephropathy. In addition, treatments that reduce proteinuria (e.g. antihypertensive drugs, particularly ACE inhibitors and angiotensin II receptor blockers (ARBs)) ↓ the rate of progression. Presence of significant proteinuria should result in adoption of lower BP targets and preferential use of ACE inhibitors or ARB drugs. Because reduction of proteinuria is an important therapeutic aim, regular assessment of the severity of proteinuria is also important in monitoring the effects of treatment. Annual measurement of the ACR is now recommended in the UK for all patients with CKD stages 3–5.

Diagnosis of early diabetic nephropathy

Diabetic nephropathy is mostly treatable in its early stages—characterized by an ↑ in GFR, ↑ albumin excretion, and then hypertension. 'Microalbuminuria' is the term for pathologically ↑ albumin excretion below the limit of detection of standard dipstick tests for proteinuria. Microalbuminuria without diabetic retinopathy should raise suspicion of non-diabetic kidney disease.

Quantitation of proteinuria

Urine protein and creatinine concentrations should be measured in an EMU sample (because protein excretion ↑ with activity).

Detection of proteinuria in glomerular disease should be by ACR. This allows for detection of proteinuria, and thus CKD, at lower levels of protein excretion than either reagent strips or PCrR. Microalbuminuria is present if urine ACR is 2.5mg/mmol (in ♂) or >3.5mg/mmol (in ♀) in repeated samples. Reagent strip testing for albuminuria is not recommended, although strips that detect, and even quantify, microalbuminuria are now available.³

Measurement of total protein in urine is cheap but does not differentiate between the various proteins present in urine. Proteinuria with >30mg/mmol creatinine is usually defined as pathological, but patients with early diabetic nephropathy have total protein excretions below this limit, hence the preferred use of ACR in the identification of early renal disease. Although less sensitive than ACR at detection of low levels of proteinuria and less precise at all degrees of proteinuria, PCrR can still be used for quantification and monitoring of established proteinuric renal disease if ACR cannot be used for reasons of cost.

Diagnosis of postural proteinuria

Protein excretion ↑ with activity and upright posture. For reasons that are not completely understood, this ↑ is exaggerated in some individuals, resulting in +ve dipstick tests for proteinuria and even ↑ 24h urine protein excretion. This 'postural proteinuria' has a nearly completely benign prognosis. In patients with proteinuria who have no other evidence of renal disease, it is worth quantifying proteinuria separately in urine collected whilst the patient has been recumbent overnight and in a daytime specimen. This can be done by measuring ACR on both EMU and a sample taken after a period of activity. Normal protein excretion during the night with ↑ protein excretion during the day indicates postural proteinuria.

Assessment of tubular proteinuria

This is occasionally of value to detect the relatively low-grade proteinuria that results from tubular disease, e.g. Dent's disease (a rare genetic disorder caused by mutation in a tubular chloride channel), which causes calcium stone formation and tubular proteinuria. Other examples include screening for generalized tubular dysfunction and for drug toxicity, e.g. during treatment with platinum derivatives. Tubular proteinuria is best diagnosed by measurement of specific proteins whose presence in the urine results from tubular disease, e.g. retinol-binding protein (RBP), N-acetyl-D-glucosaminidase (NAG) or β2-microglobulin, either in 24h urine specimens or as ratios between the protein concentration and creatinine concentration.

³ National Institute for Health and Care Excellence (2008). *Chronic kidney disease: early identification and management of chronic kidney disease in adults in primary and secondary care*. Clinical guideline CG73.  <http://www.nice.org.uk/Guidance/CG73>.

Assessment of selectivity of proteinuria

The more severe the damage to glomerular permeability, the larger the protein molecules that pass through the glomerulus in glomerular disease. Measurement of the ratio of clearance of transferrin or albumin (a small molecule) to IgG (a large molecule) can therefore be used as a measure of selectivity and is calculated as follows:

$$\text{Albumin/IgG clearance} = \left\{ \frac{(\text{urine [IgG]} \times \text{serum [albu-min]})}{(\text{serum IgG} \times \text{urine [albumin]})} \right\} \times 100\%$$

Transferrin/IgG clearance is calculated similarly.

A ratio of <0.16 indicates highly selective proteinuria.

In children, minimal change nephropathy causes selective proteinuria. Non-selective proteinuria raises the possibility of an alternative type of renal disease and might lead to a recommendation of renal biopsy to avoid steroid treatment when this would be unlikely to be of benefit. Measurement of selectivity in adults rarely influences clinical decision-making.

Detection and quantitation of urinary light chains (Bence–Jones protein)

Detection of urinary light chains (BJP) is useful in the initial diagnosis of multiple myeloma. Quantification of Bence–Jones proteinuria as a marker of disease activity during treatment has been superseded by measurement of serum free light chains.

Assessment of renal tubular function

There are two main types of renal tubular diseases: those due to a single defect, usually genetic, in solute secretion or reabsorption, and those due to generalized tubular damage.

Screening tests for generalized tubular dysfunction: test for

- **Renal glycosuria:** dipstick or laboratory test for glucose in urine plus normal plasma glucose.
- **Hypophosphataemia:** can be followed by estimation of phosphate reabsorption (→ Assessment of phosphate reabsorption, pp. 662–3).
- **Low-molecular-weight proteinuria:** due to failure of tubular reabsorption plus ↑ release of proteins derived from tubular cells.
- **Normal anion gap metabolic acidosis:** serum bicarbonate, plus sodium, potassium, and chloride to permit calculation of the anion gap (followed by tests to confirm RTA; → Assessment of urinary acidification, pp. 668–9).
- **Aminoaciduria:** detected by amino acid electrophoresis on a random urine sample.
- **Hypouricaemia:** plasma urate may be low due to ↓ tubular reabsorption. (This can be followed by measurement of fractional urate excretion; → Assessment of renal urate handling, p. 663.)

Assessment of phosphate reabsorption

This is occasionally useful in the differential diagnosis of hypophosphataemia, e.g. in confirming the diagnosis of X-linked hypophosphataemic rickets. This can be calculated using a 24h urine collection for phosphate or more easily using a spot fasting urine sample and serum test.

Procedure

- The patient is asked to fast overnight.
- The overnight urine is discarded.
- The next urine sample is obtained for phosphate and creatinine, together with a blood sample for urea, creatinine, and phosphate.
- Both are analysed for phosphate and creatinine.

Fractional phosphate excretion is calculated as

$$\text{FE}_{\text{PO}_4} = \frac{C_p / C_{\text{Cr}}}{[\text{serum creatinine} \times \text{urine phosphate}] / [\text{urine creatinine} \times \text{serum phosphate}]} = \frac{\text{serum creatinine} \times \text{urine phosphate}}{\text{urine creatinine} \times \text{serum phosphate}}$$

This is the fraction of filtered phosphate, which appears in the urine.

Fractional tubular reabsorption of phosphate (TRP) is calculated as

$$1 - \text{FE}_{\text{PO}_4}$$

TmP/GFR, the tubular maximum for phosphate reabsorption, can be read off a nomogram⁴ or can be calculated as follows:

⁴ Walton RJ, Bijvoet OLM. Nomogram for derivation of renal threshold phosphate concentration. *Lancet* 1975; ii: 309–10.

If TRP <0.86,

$$\text{TmP/GFR} = \text{TRP} \times \text{plasma phosphate}$$

If TRP >0.86,

$$\text{TmP} = \{0.3 \times \text{TRP}/[1 - (0.8 \times \text{TRP})]\} \times \text{plasma phosphate}$$

Interpretation

The adult reference range for TmP/GFR is 0.80–1.35 mmol/L with defined age- and sex-specific values. Higher values of normal are seen in infancy and childhood.⁵ Low values are seen in X-linked hypophosphataemic rickets and osteogenic osteomalacia, both of which are thought to be due to overproduction or failure of inactivation of phosphatonins (a group of phosphaturic hormones, including FGF-23 and FRP4). TmP/GFR is raised in hypoparathyroidism and reduced in hyperparathyroidism and by PTH-related peptide secretion.

Reduced phosphate reabsorption may also be seen in hypercalciuric stone formers, but it remains difficult to be certain whether this is the 1° disorder, causing ↑ production of 1,25-(OH)₂ vitamin D, or 2° to tubular damage as a result of renal stones.

Reduced phosphate reabsorption is also seen in a number of 1° and 2° disorders of renal tubular function.

Assessment of tubular urate handling

The relative contributions of production rate, glomerular filtration, pre-secretory reabsorption, secretion, and post-secretory reabsorption to control plasma urate concentration cannot be dissected out without complex tests involving selective pharmacological blockade of some of these processes. However, it is possible to determine whether an abnormal plasma urate concentration is due to abnormal production or abnormal renal handling.

The 24h urinary urate excretion is ↑ in overproduction, but normal in patients whose hyperuricaemia is due to ↓ excretion. In the latter case, urate excretion is normal, not ↓, because in under-excretion, the steady state is maintained at the expense of a raised plasma level. If 24h urinary urate is raised, the collection should be repeated on a low purine diet.

Fractional excretion of urate is calculated as

$$\{(\text{urinary [urate]} \times \text{plasma [creatinine]}) / (\text{plasma [urate]} \times \text{urinary [creatinine]})\} \times 100\%$$

Normal values are dependent on age and sex, but in adults, they are of the order of 10%. High fractional excretion is a cause of hypouricaemia in SIADH and several other conditions; low fractional excretion occurs in 1° gout, but also in a familial syndrome called uromodulin-associated kidney disease or autosomal dominant tubulointerstitial kidney disease, comprising hyperuricaemia with early-onset gout and progressive renal failure.

⁵ Payne RB. Renal tubular reabsorption of phosphate (TmP/GFR): indications and interpretation. *Ann Clin Biochem* 1998; 35: 201–6.

Assessment of acid–base balance

Plasma HCO_3^- and Cl^- are the two major anions in extracellular fluid. The major reason for measuring them is to assess acid–base status. Changes in serum $[\text{HCO}_3^-]$ concentration reflect changes in acid–base balance, with a ↓ in $[\text{HCO}_3^-]$ reflecting metabolic acidosis and an ↑ reflecting alkalosis. Plasma Cl^- is helpful in assessing the cause of acidosis or alkalosis.

There is no justification at all for performing an arterial puncture to measure arterial pH as part of the assessment of metabolic acidosis or alkalosis—it can be adequately assessed from serum $[\text{HCO}_3^-]$. Arterial samples are needed when it is unclear whether the acid–base disturbance is respiratory or metabolic in origin or in mixed disturbances.

Plasma bicarbonate

The most reliable way to interpret plasma HCO_3^- is to use the acid–base diagram (see Fig. 10.3), which allows assessment of how much the change in $[\text{HCO}_3^-]$ concentration is due to changes in CO_2 excretion via the lungs and how much to changes in $[\text{H}^+]$ or HCO_3^- wasting. In the absence of significant respiratory disease, it can often safely be assumed that any change is due to metabolic causes, in which case low plasma HCO_3^- indicates ↑ H^+ production (or occasionally ↑ HCO_3^- loss) and vice versa. If ABGs are obtained, the ‘standard bicarbonate’ is a calculated value which indicates what the plasma HCO_3^- would be if CO_2 excretion were normal and is thus a way of allowing assessment of whether there is a metabolic component to an abnormal HCO_3^- concentration or whether it is solely due to the respiratory disturbance.

Remember that the kidneys compensate for respiratory disease and the lungs for metabolic disease; for instance, metabolic acidosis causes hyperventilation, resulting in lower PCO_2 and lessening the acidosis seen. However, overcompensation does not occur.

Plasma chloride

Many laboratories omit plasma Cl^- assays from ‘routine’ serum chemistry measurements, but this measurement is helpful if a systemic acid–base disturbance is suspected. As a useful over-simplification, low HCO_3^- with high Cl^- can be seen as accumulation of hydrochloric acid, which can only result from altered renal handling of acid, as in RTA. If HCO_3^- is low with a normal or low Cl^- , some other acid must be accumulating. More precision in deciding the cause of metabolic acidosis can be obtained by calculating the anion gap.

The anion gap

The anion gap is the difference between the sum of the concentrations of the positively charged ions routinely measured in plasma and the negatively charged ions:

$$\text{Anion gap} = ([\text{Na}^+] + [\text{K}^+]) - ([\text{Cl}^-] + [\text{HCO}_3^-])$$

Obviously, the total +ve charges in plasma must be balanced by the same number of -ve charges. The normal anion gap is caused by the fact that there are more unmeasured anions in plasma (mostly albumin, but including

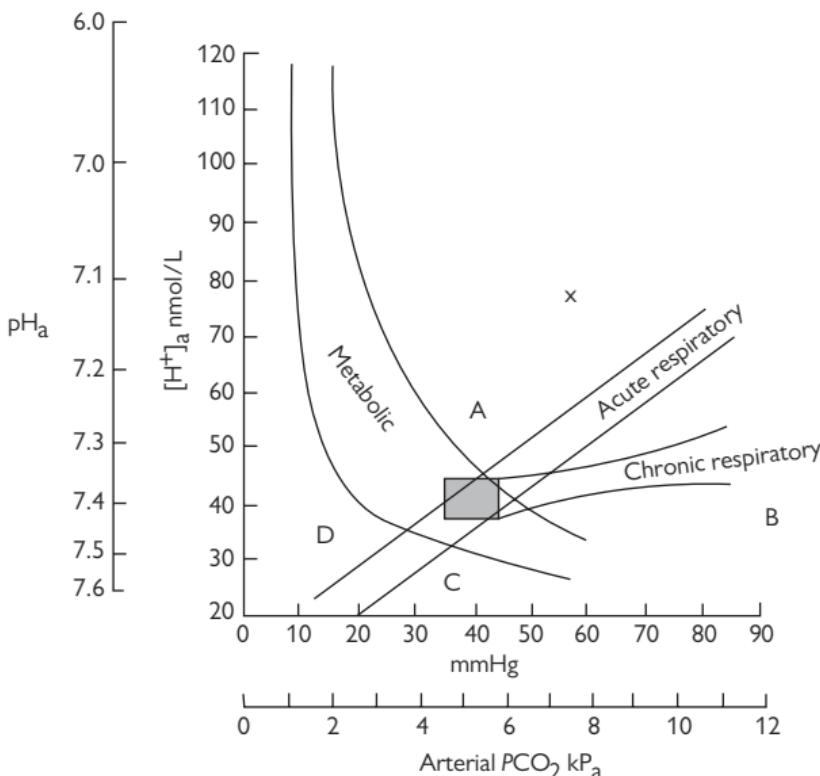


Fig. 10.3 Acid-base diagram.

lactate, sulfate, and others) than cations (including calcium and magnesium). The concentrations of all of these unmeasured ions can vary, so the normal range for the anion gap is wide, e.g. hypoalbuminaemia reduces the anion gap by 2.5mEq per 1g/dL fall in serum albumin.

The main use of calculation of the anion gap is in the differential diagnosis of metabolic acidosis.

A high anion gap acidosis is caused by an abnormally high concentration of an unmeasured anion, such as

- Acute or chronic renal dysfunction.
- L-lactate (reflecting anaerobic metabolism or hepatic dysfunction).
- Salicylate (in aspirin poisoning).
- Chronic paracetamol use at therapeutic doses in malnourished patients through the accumulation of pyroglutamic acid (also called 5-oxoproline).
- β -hydroxybutyrate (in DKA).
- Glycolate and oxalate (in methanol poisoning).
- Hippurate (in toluene poisoning, e.g. glue sniffing).
- D-lactate (from gut bacterial fermentation in blind loop syndrome).

A normal anion gap acidosis may be caused by loss of bicarbonate or failure of renal H⁺ excretion, for instance

- RTA.
- High ileostomy losses (bicarbonate wasting).
- Carbonic anhydrase inhibitors.
- Urinary diversions, e.g. uretersigmoidostomy (Cl⁻/HCO₃⁻) exchange and NH₄⁺ reabsorption in the colonic segment).

► Beware: in North America, the anion gap is usually calculated as [Na⁺] – ([Cl⁻] + [HCO₃⁻]), not including [K⁺] in the measured cations. This results in a lower reference range for the anion gap. In addition, different laboratories use different assays for Cl⁻. For these reasons, the local laboratory reference range for the anion gap should be used.

In general, the anion gap is only useful when very high, confirming high concentrations of an unmeasured anion. If the diagnosis is not already obvious, this then justifies further investigation, including assay of plasma lactate concentration.

Assessment of urinary acidification

Indications

- Unexplained hyperchloraemic metabolic acidosis.

Defects in the kidneys' ability to excrete acid in the urine may lead to permanent systemic acidosis, or to systemic acidosis at times of ↑ acid generation, depending on the severity of the defect. Acidification defects may occur as part of generalized tubular disease or as isolated, often genetically determined, alterations in function, most commonly of cell surface ion pumps.

Ammonium chloride loading test

This test is regarded as the 'gold standard' for the diagnosis of distal ('type 1') RTA where there is impaired excretion of 'fixed acid' into the distal tubule.

Procedure

- The patient attends after an overnight fast but is allowed to drink water.
- At the start of the test, a urine sample is sent to the laboratory for measurement of pH, and a plasma or serum sample is sent for measurement of HCO_3^- . Because pH changes rapidly in urine exposed to air, the urine container should be filled to the top or the urine sent in a stoppered syringe and sent to the laboratory without any delay.
- If the urine pH is <5.4, this indicates normal acidifying ability, and there is no need to continue with the test.
- If the venous blood HCO_3^- is low, with a urine pH of >5.4, the diagnosis of RTA is confirmed.
- If neither of these conditions is met, then proceed to give the patient ammonium chloride, 0.1g/kg body weight. Ammonium chloride is given as capsules, is unpalatable, and frequently causes nausea and vomiting, but this can be reduced if the capsules are taken slowly or with bread and honey. Even if the patient vomits, it is worth proceeding with the test, as acidosis is often achieved; no more ammonium chloride should be given.
- Urine samples are then collected hourly for the next 6–8h and sent, protected from the air (as above), for pH analysis in the laboratory. If any sample has a pH of <5.4, the test can be stopped, as this indicates normal acidifying ability of the distal tubule.
- At 3h after ingestion of ammonium chloride, a venous sample should be sent for plasma HCO_3^- measurement to ensure that acidaemia has occurred.

Alternative tests of distal acidification

Rationale

The distal tubule reabsorbs Na^+ in exchange for H^+ . Furosemide, by ↑ delivery of Na^+ to the distal tubule, therefore causes a fall in urine pH, particularly in 'salt-avid' states produced by Na^+ restriction or fludrocortisone.⁶

⁶ Walsh SB, Shirley DG, Wrong OM, Unwin RJ. Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. *Kidney Int* 2007; 71: 1310–16.

Procedure

- No need to fast or fluid-restrict.
- Collect a baseline urine sample for pH.
- Administer furosemide 40mg and fludrocortisone 1mg orally.
- Collect urine for pH measurement hourly for 4h or until urine pH <5.3.
- Urine pH persistently >5.3 at 4h implies distal RTA.

Bicarbonate infusion test

This is the 'gold standard' for the diagnosis of proximal ('type 2') RTA, which is characterized by impaired HCO_3^- reabsorption. In this condition, urine pH may be <5.5 in untreated patients, because at steady state, serum HCO_3^- levels fall to the point at which filtered HCO_3^- is reabsorbed and distal acidification mechanisms are intact.

Procedure

- Sodium bicarbonate is infused IV at 0.5–1.0mmol/kg/h. After 60min, plasma bicarbonate is measured to confirm that this has risen to >20mmol/L. Urine pH is measured hourly and urine HCO_3^- measured to allow calculation of the fractional excretion of HCO_3^- :

$$\text{FE}_{\text{HCO}_3} = \{(\text{urine } [\text{HCO}_3^-]) \times \text{plasma } [\text{creatinine}] \} / (\text{plasma } [\text{HCO}_3^-] \times \text{urine } [\text{creatinine}]) \times 100\%$$

- Fractional excretion of HCO_3^- is normally <15%.
- A level of >20% confirms type 2 RTA.

Plasma potassium

Although most of the body's K⁺ is intracellular, small changes in extracellular K⁺ concentration can cause major changes in membrane excitability. Hypokalaemia causes ↑ excitability, causing atrial and ventricular cardiac arrhythmias; hyperkalaemia ↓ excitability, causing a characteristic pattern of ECG changes and eventually causing asystole. Both hypokalaemia and hyperkalaemia may be associated with skeletal muscle paralysis.

Plasma K⁺ concentration is influenced both by distribution across cell membranes and by the balance between intake and excretion. Renal excretion is dependent on renal function, urine flow rate, and aldosterone.

Pseudohyperkalaemia

Caused by excessive release of K⁺ from cells after venepuncture and should be considered when hyperkalaemia 'does not fit' with the clinical picture. The laboratory should report the presence of visible haemolysis (usually due to RBC trauma during difficult venepuncture), but pseudohyperkalaemia can also occur in the absence of visible haemolysis, e.g.:

- Haematological malignancies causing a high white cell or platelet count, e.g. chronic lymphatic leukaemia.
- Other causes of leucocytosis and thrombocytosis, e.g. leukaemoid reactions, RhA.
- Familial pseudohyperkalaemia: rare disorder of RBC cation transport leading to an ↑ rate of release of K⁺ from red cells at low temperatures, associated with stomatocytosis.

Diagnosis can be confirmed by showing that plasma [K⁺] is normal in a heparinized sample analysed immediately and then by demonstrating that delayed separation results in higher values being obtained. Pseudohyperkalaemia with a normal WBC and platelet count can be further investigated by measuring the rate of rise of plasma [K⁺] in samples incubated at 37°C and 22°C, and studying the effects of drugs that affect cation exchange, e.g. thiazide diuretics and quinine. Artefactual hyperkalaemia can be caused by fist clenching plus a venous tourniquet during phlebotomy; plasma K⁺ can rise by as much as 2mmol/L.

Hyperkalaemia due to redistribution across cell membranes

Hyperkalaemic periodic paralysis is an autosomal dominant genetic muscle disorder caused by mutations in the voltage-gated sodium channel SCN4A. It presents in early infancy with attacks of paralysis associated with hyperkalaemia.

Other causes of release of potassium from tissues (including muscle) include

- Exercise.
- Acidosis (particularly inorganic acidosis).
- Muscle damage (rhabdomyolysis), e.g. crush injury, revascularization of ischaemic limb, prolonged unconsciousness following drug intoxication.
- Burns.

- Tumour lysis, e.g. after initiation of chemotherapy for haematological malignancy.
- Drugs, e.g. digoxin, depolarizing muscle relaxants, β -blockers.
- Malignant hyperthermia.

Hyperkalaemia due to altered external balance

- ↑ ingestion is seldom able to cause hyperkalaemia on its own but can contribute to hyperkalaemia when combined with impaired excretion of a K⁺ load.
- ↓ excretion may be due to ↓ GFR, ↓ urine flow rate, ↓ aldosterone production, drugs which inhibit renal tubular K⁺ excretion, or genetic defects in renal K⁺ excretion (pseudohypoaldosteronism, Liddle's syndrome).
- In most cases, the cause is obvious.

Investigation of unexplained hyperkalaemia

- Serum creatinine, CK, HCO₃⁻.
- Urine K⁺ is of limited utility because urinary K⁺ excretion is primarily determined by, and roughly equal to, K⁺ intake.
- Tests for type IV RTA:
 - Normal Synacthen® test (to exclude Addison's disease).
 - 24h urinary aldosterone (low in type IV RTA).
 - Plasma renin and aldosterone response to upright posture and 40mg furosemide (subnormal levels of both suggest hyporeninaemic hypoaldosteronism).
 - Correction of hyperkalaemia with oral fludrocortisone 0.1mg/day.

Pseudohypokalaemia

Can be caused by delayed separation of samples kept at warm ambient temperatures and is caused by continued uptake of K⁺ into cells. This occurs more in heparinized samples than in those allowed to clot.

Hypokalaemia due to redistribution across cell membranes

- Alkalosis.
- Insulin treatment.
- β 2-adrenergic stimulation (e.g. high-dose nebulizers).
- B₁₂ therapy of PA.
- Rapid cell division, e.g. acute leukaemia.
- Hypokalaemic periodic paralysis. Mutations in the CANCL1A3 and SCN4A voltage-gated ion channels can lead to this rare condition. Carbohydrate intake or rest after exercise typically precipitates hypokalaemia.
 - Confirm diagnosis (under strict supervision) by infusing 2g/kg glucose and 0.1U/kg insulin; consider referral for mutation analysis of relevant ion channels.
 - Consider thyrotoxic hypokalaemic periodic paralysis in non-familial patients, particularly of oriental background; check TFTs.

Hypokalaemia due to increased renal loss

- Chapter 2.

Urine potassium, chloride, and magnesium measurements

Urine potassium

Measurement of urine K^+ concentration is occasionally useful in the differential diagnosis of hyperkalaemia. The proportion of K^+ filtered at the glomerulus which is excreted in the urine is extremely variable and is modulated by the distal tubule in response to aldosterone, plasma K^+ concentration, acid–base balance, urine flow rate, Na^+ status, and other factors. The final concentration of K^+ in the urine also depends on urine dilution, controlled independently by factors (e.g. ADH) controlling water excretion.

Low urinary K^+ (<20mmol/L) with hypokalaemia is seen in

- GI K^+ loss, e.g. diarrhoea, laxative abuse, villous adenoma, high ileostomy output, enterocutaneous fistula, uretersigmoidostomy.
- Dietary deficiency
- Skin losses, e.g. burns, severe eczema.

High urinary K^+ (>20mmol/L) with hypokalaemia and normal blood pressure is seen in

- Vomiting (K^+ is exchanged for hydrogen ions: acid–base preservation takes precedence). Note: urinary Cl^- will be low.
- Diuretic use, abuse, and conditions which mimic diuretic use, e.g. Bartter's syndrome, Gitelman's syndrome.
- Tubular damage causing K^+ wasting, e.g. RTA types 1 and 2.
- DKA.

High urinary K^+ (>20mmol/L) with hypokalaemia and high blood pressure is seen in

- Hyperaldosteronism: adrenal adenomas, bilateral adrenal hyperplasia.
- Apparent mineralocorticoid excess.
- Liddle's syndrome.

Urine chloride

This measurement is helpful in the differential diagnosis of otherwise unexplained normotensive hypokalaemia. Urine Cl^- is low if hypokalaemia is being caused by extrarenal sodium chloride or hydrogen chloride losses, as seen in diarrhoea or vomiting, respectively. In these conditions, K^+ is exchanged in the distal tubule for Na^+ or hydrogen, respectively, but Cl^- is conserved. Urine Cl^- is high when the cause of hypokalaemia is inappropriate loss of potassium chloride, as in diuretic use and in Bartter's syndrome (the genetic equivalent of being on permanent high-dose loop diuretics) and Gitelman's syndrome (the genetic equivalent of being on permanent high-dose thiazide diuretics).

The distinction between drug-induced and genetic causes can be very difficult to make, but temporary withdrawal from diuretics causes intense Cl^- retention and a very low urine Cl^- concentration, which is never seen in Bartter's or Gitelman's syndromes. Repeated measurements of urine Cl^- are therefore helpful in this situation, together with screens for the presence of diuretics in the urine when urine Cl^- is high.

Urine magnesium

This measurement can be helpful in identifying the cause of hypomagnesaemia by distinguishing inappropriate tubular Mg²⁺ loss (e.g. from diuretics, aminoglycosides, Gitelman's syndrome) from GI Mg²⁺ loss (e.g. from diarrhoea, proton pump inhibitor use).

$$\text{FE}_{\text{Mg}} = \{(\text{urine } [\text{Mg}^{2+}] \times \text{plasma } [\text{creatinine}]) / ((0.7 \times (\text{plasma } [\text{Mg}^{2+}] \\ \times \text{urine } [\text{creatinine}]))\} \times 100\%$$

Plasma Mg²⁺ is multiplied by 0.7, as only 70% of Mg²⁺ is non-albumin-bound and thus freely filtered.

Fractional Mg²⁺ excretion of >2% in a subject with normal renal function indicates renal Mg²⁺ loss.

Urine sodium concentration

In health, serum electrolyte concentrations are kept constant because intake of electrolytes is balanced by excretion in the faeces and urine. Renal excretion is tightly regulated to achieve this balance. These basic principles imply that the urinary excretion of, for instance, Na^+ , is nearly totally dependent on dietary intake of Na^+ . Because this is very variable, there is no 'normal range' of urine Na^+ , or any other urinary electrolyte. Measurements of urinary electrolytes therefore have to be interpreted with great caution.

The 24h urine Na^+ excretion is a good marker at steady state for dietary intake and has been used in epidemiological studies of the relationship of salt intake to BP. Dietary Na^+ intake varies from as little as 10mmol/day in the Amazon rainforest to >400mmol/day in Westerners living on processed foods. Current UK advice is to restrict Na^+ intake to around 100mmol/day.

In clinical practice, there are several reasons for measuring Na^+ output, including

- *Calcium stone formers:* Na^+ and Ca^{2+} excretion are linked, and reduction of excessive salt intake results in a reduction in Ca^{2+} excretion.
- *Cystine stone formers:* similarly, cystine excretion is reduced by reduction of dietary salt intake.
- *During antihypertensive and antiproteinuric treatment:* salt restriction amplifies the effects of ACE inhibitors in reducing not only systemic BP, but also protein excretion in renal disease, and may be more tolerable than diuretic treatment.

The 24h urine Na^+ is usually measured on a sample collected in a plain container. However, it can also be measured, by flame photometry, in a sample collected into an acid container, and this is useful if Ca^{2+} and oxalate excretion are also being measured, for instance in stone formers.

Spot urine Na^+ concentration is of very limited value, because Na^+ excretion varies considerably through the day and because it is normally influenced by urine dilution, and hence by recent water intake. However, there are two situations in which it may be of value.

Acute kidney injury

The normal response of the kidneys to underperfusion from hypovolaemia or hypotension is to retain salt avidly, urine Na^+ concentration dropping to <10mmol/L. If urinary Na^+ concentration is this low in AKI, this indicates normal ability of the renal tubules to retain salt. Low urine Na^+ concentration is seen in 'pre-renal' renal failure; ATN results in loss of tubular salt reabsorption and a higher urine Na^+ concentration. The problem is that some conditions other than underperfusion can cause low urine Na^+ (e.g. contrast nephropathy, rhabdomyolysis). High urine Na^+ does not necessarily indicate ATN; indeed, it is seen in normal people. In any case, the measurement seldom has a useful impact on management, which both in pre-renal failure and in ATN is to restore renal perfusion by correcting hypovolaemia, hypotension, and sepsis as quickly as possible.

Syndrome of inappropriate antidiuretic hormone

This diagnosis cannot be made in a hypovolaemic patient, because hypovolaemia is a physiological stimulus to ADH secretion. For this reason, the diagnosis cannot be made if the urine Na^+ concentration is low (Hyponatraemia (including syndrome of inappropriate antidiuretic hormone), pp. 139–40).

Fractional excretion of sodium is calculated as

$$\left\{ \frac{(\text{urine } [\text{Na}^+] \times \text{plasma } [\text{creatinine}])}{(\text{plasma } [\text{sodium}] \times \text{urine } [\text{creatinine}])} \right\} \times 100\%$$

This gives an index of avidity of Na^+ reabsorption independent of changes in overall renal function. An FE_{Na} of <1% is seen in pre-renal failure and of >1% in ATN. However, this measurement is prone to some of the same criticisms as that of urine Na^+ excretion.

Sodium-wasting and sodium-retaining states

Na^+ wasting is caused by diuretics, Bartter's syndrome, Gitelman's syndrome, and occasionally renal tubular disease. It cannot be diagnosed by measurement of urine Na^+ excretion alone, as at steady state, this equals Na^+ intake, but is diagnosed by finding clinical evidence of hypovolaemia without avid renal Na^+ retention.

Na^+ retention is caused by diseases causing effective hypovolaemia (e.g. CCF), in which case the diagnosis is suggested by oedema and the clinical signs of the underlying disease. However, Na^+ retention can also cause hypertension without oedema, as in hyperaldosteronism, pseudohyperaldosteronism, chronic renal failure, and inherited disorders of renal tubular Na^+ excretion (e.g. Liddle's syndrome). Again, measurement of Na^+ excretion alone is not helpful in the diagnosis of these conditions.

Urine dipstick testing

Urine analysis has been used for many years for screening patients with potential renal disease and for serial assessment of patients with known renal pathology. However, the results of dipstick testing are dependent on urine dilution and, for this reason, laboratory measurement of the ACR is the preferred screening test for proteinuria. Reagent strips should only be used for the detection of microalbuminuria if they are specifically capable of detecting low concentrations of albumin and expressing the result as an ACR.

Many commercially available dipsticks rapidly test the urine for multiple chemical contents. The sticks use reagent strips, which change colour following a chemical reaction with an active constituent, depending on the presence (or absence) of a particular component.

Depending on the type of dipstick used, urine can be tested for

- pH.
- Protein.
- Albumin.
- Hb.
- Glucose.
- Leucocyte esterases and nitrites.
- Specific gravity.
- Ketones.
- Urobilinogen.

The reagent strip is fully immersed in urine obtained by voiding or, if a 1% risk of iatrogenic urine infection is warranted, by urethral catheterization, and the excess shaken off. The change in colour, if any, is read after the time specified by the manufacturer—usually 30s.

pH

Dipstick testing only gives a rough estimate of the pH, because of the effects of storage and reaction on exposure to atmospheric air on urine pH *in vitro*. The dipstick contains a polyionic polymer bound with H⁺, which is released on reaction with the cations in urine. Release of H⁺ causes a change in colour of a pH-sensitive dye. Normal pH varies between 4.5 and 8.0, depending on the diet; vegetarians, in whom fixed acid ingestion is low, commonly have alkaline urine. Urine infection with urease-producing organisms also causes alkaline urine.

Urine pH >5.5 in spite of metabolic acidosis is seen in RTA. Urine pH is important in some recurrent stone formers. For instance, uric acid solubility in urine is critically dependent on urine pH, and many uric acid stone formers are found to have normal 24h urinary urate, but highly acidic and concentrated urine (e.g. as a result of high losses from an ileostomy). In patients with triple phosphate stones, alkaline urine is commonly seen due to infection with urea-splitting organisms.

Protein

Binding of proteins to the dye indicators is highly pH-dependent, and the indicators undergo a sequential colour change based on the concentration of protein in the sample. Albumin binds at a pH of 5–8 and has the highest affinity, so most commercially available dipsticks almost exclusively detect albumin. Dipsticks are thus cheap and reliable, and give rapid, semi-quantitative assessment of proteinuric renal disease. However, these tests measure the concentration of protein, rather than absolute excretion; false –ve tests are therefore possible in dilute urine caused by a high fluid intake, and false +ve tests may be obtained in highly concentrated urine. At pH <5 or >8, results obtained by dipsticks are not accurate. Ig light chains (BJP) do not result in +ve dipstick tests for proteinuria, even when present in high concentrations. Sticks able to detect low concentrations of albumin and give a ‘near patient’ quantification of ACR are available, but not in universal use.

Haemoglobin

Reagent strips use peroxidase-like activity of Hb to induce a colour change in a dye linked to organic peroxide. This reaction does not distinguish haemoglobinuria from erythrocyturia or from myoglobinuria. False +ve results are obtained with myoglobin, contamination with menstrual blood, semen, and iodine. Positive dipsticks for blood with absence of RBC on microscopy suggest lysis of RBCs due to prolonged storage, myoglobinuria, or haemoglobinuria. False –ve results are seen with high-dose vitamin C and captopril.

Glucose

Most strips use the glucose oxidase/peroxidase method and can estimate levels as low as 50mg/dL. Ketones, salicylate, and ascorbic acid can interfere with results. These strips estimate all reducing sugars, including fructose and lactose. In the absence of concomitant hyperglycaemia, glycosuria is suggestive of proximal tubular disorders or, rarely, reduced renal threshold for glucose. When associated with glomerular disease, glycosuria with a normal serum glucose may be a marker of worse prognosis. Drugs that inhibit Na⁺-dependent glucose transport (the SGLT-2 inhibitors) are now available as novel hypoglycaemic drugs and act by causing renal glycosuria.

Leucocyte esterases and nitrites

The esterase method relies on esterases released from lysed WBCs. Esterases release pyrroles, which react with a diazonium salt on the dipstick, resulting in a colour change. False +ve results are seen in vaginal contamination and excessively dilute urine which favours cell lysis. Presence of glucose, albumin, ketones, tetracyclines, and cephalosporins in the urine can give false –ve results, along with a concentrated urine, which impedes the release of esterases.

Most, but not all, uropathogenic bacteria convert nitrates to nitrites, which react with a diazonium compound, resulting in a colour change. False –ve results are due to frequent bladder emptying, prolonged external storage, and ascorbic acid. Some bacteria, including *Neisseria gonorrhoeae* and *Mycobacterium tuberculosis*, do not convert nitrates.

Sensitivity and specificity of the above tests vary and are not useful for screening low-risk populations. However, a –ve test is useful in excluding a UTI in a patient with a high pretest probability of infection. Further guidance on the diagnosis of UTIs is available at:  <https://www.gov.uk/government/publications/urinary-tract-infection-diagnosis>.

Specific gravity

Dipstick testing for specific gravity (SG) is not accurate; non-ionic constituents, including albumin, glucose, and urea, are also estimated. Normal values are between 1003 and 1030 but vary with the patient's hydration status, and hence urinary concentration. SG ↓ with age, as the kidney loses its concentrating ability. Fixed SG of 1010 is a variable feature of advanced CKD.

Ketones

Acetoacetic acid is detected by the nitroprusside test. Ascorbic acid results in false +ve results. Dipsticks do not detect β -hydroxybutyrate, which comprises the largest ketone fraction in blood.

Urine culture

There are numerous situations in which an accurate diagnosis of UTI is important. ‘Sending an MSU’ is not, however, quite as simple as it sounds and is not always the most appropriate test.

Obtaining a midstream urine sample

The aim is to obtain a sample of bladder urine, avoiding contamination by cells or organisms on the perineal skin. Men should retract the foreskin prior to micturition; women should hold the labia well apart with the parted fingers of one hand, to allow the urine to exit directly from the urethral meatus. The patient should be asked to begin to pass urine and then, without stopping passing urine, pass a sterile container into the path of the urinary stream and collect a sample, before finishing passing urine normally. If a sterile foil container has been used to catch the specimen, the specimen is then transferred into a specimen container and sent to the laboratory.

Suprapubic aspiration of urine

In patients suspected of having bladder infection, but in whom the results of culture of MSUs are equivocal, it may be necessary to proceed to suprapubic aspiration (widely performed in paediatrics, but not in adults). After skin preparation, a fine needle (e.g. an LP needle) is introduced into the bladder by direct puncture just above the symphysis pubis and urine is aspirated. US can be used to confirm that the bladder is full prior to the procedure.

‘In-out’ catheter urine specimens

Although bladder catheterization carries a small (1–2%) risk of introducing new infection into the bladder, this risk is sometimes justified by the importance of obtaining urine direct from the bladder. A urethral catheter is passed into the bladder, the first few millilitres discarded, and a sample collected.

Obtaining urine specimens from ileal conduits

Urine in ileal conduit bags is always contaminated by skin organisms, and the culture of ‘bag urine’ is not a useful way of diagnosing upper UTIs in patients with conduits. In patients suspected of having an ascending infection, a urine specimen should be obtained by passing a catheter as far into the conduit as it will go.

‘Two glass test’

This is a test for urethritis and is performed when a patient presents with dysuria or urethral discharge and a sexual history suggesting a possible recent infection. Culture of a urethral swab or of the urethral discharge should also be obtained and sent for gonorrhoea testing (requires attendance at a sexual health clinic). Two urine samples are collected—the first 10mL passed and a midstream sample. Each is sent for culture; urethritis is diagnosed when the bacterial count is highest in the first sample. The first sample should also be sent for *Chlamydia* testing.

'Stamey-Meares test'

This test is performed for the diagnosis of prostatitis. An MSU sample is obtained, and then the patient is asked to stop passing urine. The prostate gland is massaged per rectum, and 'expressed prostatic secretions' collected, followed by a final urine sample. In prostatitis, bacterial counts are higher in the expressed prostatic secretions or the post-massage urine sample than in the midstream sample.

Indwelling catheter urine specimens

Colonization of the bladder is nearly inevitable within a fortnight of insertion of an indwelling urethral or suprapubic catheter. Unnecessary antibiotic treatment ↑ the selective pressure for the emergence of antibiotic-resistant organisms and ↑ the risk of antibiotic-associated diarrhoea and hospital-acquired infection. Antibiotic therapy must therefore be reserved for symptomatic infection. There is no point in sending catheter specimens, unless there is a suspicion of symptomatic infection at the time. 'Surveillance' samples sent to predict which antibiotics should be used if the patient becomes symptomatic at a later time are unjustified, because the colonizing organisms may change over time. A fresh specimen of urine is obtained from the collection port into the collection pot. Samples should NOT be collected from the reservoir into which the catheter drains.

Localization tests

- On rare occasions, it is justified to attempt to localize the site of infection to the bladder or to one or other kidney.
- The 'gold standard' is to obtain samples from each ureter and from the bladder during rigid cystoscopy under general anaesthesia.
- The 'Fairley test' requires passage of a urethral catheter followed by a bladder washout with a wide-spectrum antibacterial and a fibrinolytic enzyme. Sequential samples of urine are then obtained. If infection is present in the upper tracts, this will not have been affected by the bladder washout, and organisms will be detected in the first specimen obtained after the washout, whereas if infection was confined to the bladder, subsequent samples will be sterile. This is now rarely used in practice, as it involves inserting a catheter into an already infected system and is labour-intensive.
- Infection may be confined to one or other kidney as a result of ureteric obstruction or may be present within a renal cyst. In these situations, direct aspiration of urine under US control in the radiology department is necessary.

Microscopy and culture of urine

Once a sample has been obtained, it is sent to a microbiology laboratory for microscopy and culture.

Microscopy is required to assess pyuria (WBCs in the urine) and contamination.

- Significant pyuria indicates inflammation within the urinary tract; if this persists, despite -ve urine cultures, the patient has 'sterile pyuria', for which there are a number of causes, including infection with an organism which does not grow on conventional culture media, e.g. *Chlamydia*.
- Pyuria plus a +ve culture confirms the diagnosis of UTI.
- The absence of pyuria makes a UTI less likely but can occur in the early stages of infection or in the presence of a very high fluid intake.
- Contamination (in the ♀) is indicated by the presence of large numbers of squamous cells, which usually come from the vaginal wall; however, squamous cells can occasionally come from the bladder.

Culture and sensitivity are necessary to decide what treatment is necessary and to differentiate contamination of the urine sample by organisms outside the bladder from true infection.

- A 'pure growth' of a single organism to $>10^5$ CFU/mL is the conventional criterion for UTI.
- However, low counts of 10^2 – 10^4 CFU/mL can be associated with early infection and should be taken seriously in the presence of suggestive symptoms in women.
- Low counts in men are likely to represent true infection, because contamination is uncommon.
- Genuine mixed growth may occur, in the presence of impaired urinary drainage or a foreign body within the urinary tract.

Urine microscopy

Urine microscopy is a useful, quick, reliable, cheap, and underused investigation—the ‘liquid renal biopsy’! Far more information can be obtained by careful microscopy than is usually obtained in the microbiology laboratory where the priority is detection of significant urine infection.

Indications

- Suspected UTI.
- Suspected acute glomerulonephritis.
- Suspected acute interstitial nephritis (requires staining for eosinophils).
- Unexplained acute or chronic renal failure.
- Haematuria (with or without proteinuria) on urine dipstick test.
- Suspected urinary tract malignancy.

Procedure

A freshly voided, clean-catch, midstream early morning specimen is ideal. The sample should be centrifuged and resuspended in a small volume. Although bright field microscopy will allow identification of most formed elements in the urine sediment, phase contrast microscopy is useful for detection of red cell ghosts, ‘glomerular’ red cells, and some other constituents. Staining of the urine sediment is not necessary for most purposes but is useful for identification of eosinophils and malignant cells—this is usually performed in the cytology laboratory.

Haematuria

RBCs appear as non-nucleated, biconcave discs. Even when urine is red in colour or dipsticks +ve for blood, it should be examined for the presence of red cells. The differential diagnosis of haematuria is broad, but it is broadly classified into glomerular (renal) and infrarenal causes. Transit of red cells through the renal tubules causes osmotic changes in their shape and size; ‘dysmorphic’ or ‘crenated’ red cells are best seen using phase contrast microscopy and may be missed altogether if bright field microscopy is used. In experienced hands, detection of these glomerular red cells strongly suggests a glomerular origin for haematuria, although failure to detect these changes does not reliably indicate a lower urinary tract cause of bleeding—heavy haematuria in IgA nephropathy, for instance, can result in large numbers of normal red cells in the urine. Urine pH, concentration, and storage can affect red cell morphology.

Leukocyturia

The presence of significant numbers of polymorphs (pyuria) in urine is highly suggestive of UTI but can also occur in glomerulonephritis, interstitial nephritis, and peri-ureteric inflammation, for instance in acute appendicitis. The presence of leucocyte casts is diagnostic of renal parenchymal infection ('acute pyelonephritis'). Eosinophiluria is associated with acute allergic interstitial nephritis and athero-embolic renal disease.

Other cells

Squamous epithelial cells are usually taken as indicative of vaginal contamination but may also derive from the bladder and urethra. Occasionally, malignant cells arising from the lower urinary tract are picked up on routine microscopy. Spermatozoa are also rarely seen.

Microorganisms

Identification of bacteriuria, in association with leukocyturia, is very suggestive of an infection. Organisms may be in chains or clusters, and some are motile. Fungi, including yeast, and protozoans, including *Trichomonas*, can also be readily identified.

Casts

Casts are cylindrical bodies, which usually form in the distal tubule and collecting duct. They consist of cells or cell debris held together by Tamm–Horsfall protein. Staining and phase contrast microscopy improve identification and characterization of casts, but results are operator-dependent. Extreme shaking or agitation can disintegrate casts.

- *Hyaline casts* appear translucent and homogeneous and are present in normal urine. Number may be ↑ in dehydration and proteinuria.
- *Cellular casts*, especially red cell casts, always indicate significant parenchymal renal disease. Red cell casts are strongly suggestive of acute glomerulonephritis but may occur in interstitial nephritis and ATN as well.
- *White cell casts* are seen in acute pyelonephritis and acute interstitial nephritis.
- *Granular casts* are formed from cell debris and are seen in a wide variety of renal diseases.
- *Waxy broad casts* form in atrophic renal tubules and are seen in chronic renal failure.

Crystals

A variety of crystals can be visualized and are of importance in stone formers. A freshly voided sample should be examined, as storage and temperature changes can affect the type and number of crystals found. Calcium oxalate crystals may be seen in hypercalciuria, hyperoxaluria, and ethylene glycol poisoning. Presence of even a single crystal of cystine is diagnostic of cystinuria, as cystine is not a constituent of normal urine. Calcium phosphate crystals can form in normal urine as it cools and are of no pathological significance.

Investigations in patients with renal or bladder stones

Not all renal tract stones are formed because of abnormal urine chemistry. They may also be formed because of stasis, e.g. in calyceal or bladder diverticula. Infection ('struvite') stones are the result of chronic infection in the urinary tract with urease-producing organisms, which metabolize urea to form an alkaline urine in which struvite readily precipitates.

Indications

Although up to 75% of patients who present with renal stones eventually form a second stone, this may not be for 20 years. Most urologists therefore only refer patients for metabolic evaluation if there is a heightened suspicion of an underlying metabolic cause.

Situations in which evaluation is definitely indicated include

- Formation of stones in childhood or adolescence.
- Recurrent stone formation.
- Nephrocalcinosis (calcification in the renal parenchyma) as well as stone formation in the collecting systems.

Radiology

IVU or unenhanced helical CT will usually have been performed during the patient's presentation with stone disease, but the films should be reviewed to look for evidence of any cause of stasis within the collecting systems, and in particular for medullary sponge kidney. Radiolucent stones can be detected using US, IVU, or CT scanning, and can be made of cystine, uric acid, xanthine, or 2,8 dihydroxyadenine. 'Staghorn' calculi filling the collecting systems are most often struvite (infection) stones, but not always—calcium oxalate stones can grow to similar size and shape, particularly in hyperoxaluria, as can cystine stones.

Stone analysis

Depending on the facilities in the laboratory, this may be qualitative or semi-quantitative. The purpose of analysis is to distinguish calcium stones from cystine, urate, and struvite stones, to pick up the rare types of stone, and in addition to distinguish calcium oxalate from calcium phosphate stones. The result of stone analysis should be used to guide further investigation. Stones can be obtained for analysis either at surgery, including percutaneous nephrolithotomy, or by asking a patient to pass urine through a fine sieve.

'Spot' urine tests

Amino acid analysis on a random sample of urine shows ↑ excretion of cystine, ornithine, lysine, and arginine in cystinuria, and this finding is sufficient to confirm a suspected diagnosis. However, measurement of 24h urinary cystine excretion is necessary for optimal management of this condition.

Random urine calcium:creatinine and oxalate:creatinine ratios are used in children to diagnose hypercalciuria and hyperoxaluria but are not as reliable as 24h urine collections, which are preferred in adults.

24h urine collections

Collections must be made into an acidified container for measurement of calcium and oxalate, and into a plain container for measurement of urate (because acidification is necessary to prevent calcium from binding to the plastic surface of the urine container and to prevent *in vitro* generation of oxalate, and because acidification precipitates uric acid crystals). Measurement of sodium and citrate excretion can be made on either type of collection.

- *Calcium excretion* is not a good predictor of stone formation (calcium activity is less than concentration due to the presence in urine of anions that form soluble complexes with calcium). However, marked ↑ of urinary calcium is a risk factor for stone formation.
- *Oxalate excretion* correlates well with the risk of recurrent calcium oxalate stone formation, even within the normal range. Marked hyperoxaluria may result from enteric hyperoxaluria (↑ colonic oxalate absorption resulting from small bowel resection, jejunoleal bypass, or malabsorption), acute ethylene glycol poisoning, excess dietary oxalate, or as a result of 1° hyperoxaluria (one of several metabolic defects causing ↑ endogenous oxalate production).
- *Glycolate and L-glycerate* should be measured in patients suspected of having 1° hyperoxaluria to allow differentiation between type 1 and type 2 1° hyperoxaluria. Raised urine glycolate suggests type 1 disease, and raised L-glycerate suggests type 2 disease, but neither test is 100% sensitive or specific.
- *Liver biopsy* with assays to detect enzyme activity can be used to diagnose 1° hyperoxaluria
- *Genetic testing* is now widely used for the diagnosis of type 1, 2, and 3 1° hyperoxaluria.
- *Citrate excretion* should be measured because citrate is a potent inhibitor of calcium stone formation; correction of hypocitraturia with, for instance, oral potassium citrate, reduces stone recurrence rate.
- *Sodium excretion* (a good marker for dietary sodium intake) should be measured in calcium stone formers and in patients with cystinuria, because reduction of dietary sodium intake results in ↓ excretion of calcium and cystine, respectively.
- *Cystine excretion* should be measured in cystine stone formers. The aim of treatment is to maintain the cystine concentration well below the solubility limit for cystine (~1mmol/L at urine pH of 7). Rather than a single 24h collection, it is worth asking the patient to split the urine collection into daytime and night-time aliquots to ensure that this target is met at night, when urine tends to become more concentrated, as well as during the day.
- *Urinary phosphate measurement* is of no proven value in the management even of calcium phosphate stone formers.

Tests of urinary calcium excretion

Tests performed after calcium restriction and following a high-calcium test meal have been used widely in the USA to differentiate 'absorptive' from 'renal' hypercalciuria. These tests are necessary to define different phenotypes associated with hypercalciuria for research studies, but there is no evidence that management strategies based on them have any advantage over those based on simpler tests of urine chemistry.

Renal biopsy

Percutaneous renal biopsy is a valuable tool to establish diagnosis, suggest prognosis, and guide therapy in renal diseases. It also has a major role in the management of a renal transplant recipient.

Definite indications (result likely to change management)

- Nephrotic syndrome (in adults).
- Steroid-unresponsive nephrotic syndrome in children.
- Acute nephritic syndrome.
- Rapidly progressive glomerulonephritis.
- Unexplained renal failure with normal-sized kidneys relative to body size and age.
- Renal involvement in multi-system disorders.
- Diagnosis of renal transplant dysfunction.

Relative indications (result may change management or help to define prognosis)

- Non-nephrotic range proteinuria with or without haematuria.
- Isolated haematuria (only rarely does biopsy change management—sometimes justified for potential live kidney donors, or for employment or insurance purposes).
- Unexplained CKD.
- Diabetic patient with renal dysfunction, particularly with features not typical of diabetic nephropathy, or without retinopathy.

Absolute contraindications

- Uncontrolled severe hypertension.
- Bleeding diathesis, including platelets $<50 \times 10^9/L$, uncorrected bleeding/clotting disorders, and patients on anticoagulation with prolonged clotting times.

Relative contraindications

- Single kidney.
- Kidney size small, compared to patient's body size and age.
- Renal tumour/mass—risk of abdominal seeding.
- Uncooperative patient (can be done under sedation or under GAn).
- Multiple renal cysts.

Procedure

1. Recent imaging of kidneys to document the size and rule out obstruction is mandatory. A recent normal platelet count, clotting profile, and informed consent are necessary.
2. The procedure is performed where proper US facilities are available and is usually done under LAn. Sedation can be given to an uncooperative or tense patient.
3. An attending pathologist or technician at the time of sampling to comment on adequacy of tissue is very useful.
4. Biopsy can be performed with either a spring-loaded disposable device or a biopsy gun, depending on local practice.
5. The patient lies prone and the kidney is identified with US.
6. The skin over the target area is prepared and anaesthetized with lidocaine.

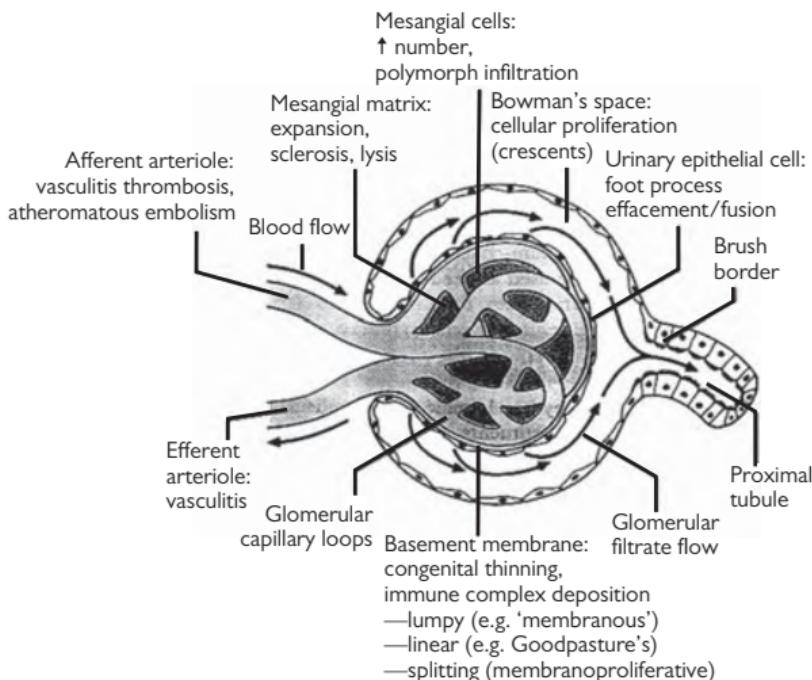


Fig. 10.4 The normal glomerulus and its repertoire of response to injury.

Reproduced from Tomson CRV. *Essential Medicine*, 2nd edn. London: Churchill Livingstone, 1998.

7. A small cut in the skin is made using a scalpel. The kidney is localized with a fine-bore 21G LP needle and LA infiltrated up to the level of the renal capsule. Either kidney can be biopsied—all parenchymal renal diseases are bilateral. After suitably protecting the US probe, the biopsy needle/gun is inserted along the anaesthetized track under real-time US guidance to the level of the renal capsule, aiming to obtain a sample from the cortex of the lower pole. The patient is asked to hold their breath whilst the biopsy is taken.
8. The needle/gun is fired and subsequently withdrawn. The patient is then allowed to breathe normally.
9. Two cores of tissue are usually taken; this may require three or four 'passes' with the biopsy needle. If an attending pathologist or technician is present, they can comment on the adequacy of tissue by examining the core for glomeruli (see Fig. 10.4) using a hand-held magnifying glass or a simple microscope. If immunofluorescence is to be performed, part of one core is placed in saline; the remainder is placed in formalin.
10. Following the biopsy, the patient is turned supine and strict bed rest enforced for a minimum of 6h. Vital signs are monitored every 15min for 2h, every 30min for 2h, and hourly thereafter. If no complications are encountered at 6h, the patient is allowed to mobilize. Most bleeding complications occur within the first 8h, but bleeding can start up to 72h after the biopsy. If macroscopic haematuria is present and does not resolve within the observation period, discharge should be delayed.

Renal imaging

Contrast nephropathy

Renal toxicity due to radiocontrast agents may cause or exacerbate renal impairment. The risk of nephropathy ↑ with ↑ contrast dose and is higher with intra-arterial injection. Nephrotoxicity is due to a combination of local vasoconstriction and direct tubular injury. There is an ↑ risk in patients with pre-existing renal impairment, diabetes, myeloma, hypovolaemia, or effective hypovolaemia (e.g. CCF), and concurrent administration of medications that can reduce renal blood flow, including NSAIDs, ACE inhibitors, and ARBs. The Royal College of Radiologists recommends discussion with the referring clinician before contrast is given to any patient taking metformin, given the theoretical risk of precipitating lactic acidosis, but it is no longer recommended that metformin is routinely discontinued in this setting.⁷

Although usually reversible, contrast nephropathy can precipitate the need for dialysis in patients whose renal function is already seriously impaired. Non-ionic media, use of the minimum possible dose, and IV pre-treatment with saline or sodium bicarbonate reduce the risk in high-risk patients.

Details of the radiological investigation of the urinary tract appear in
⑦ Renal imaging, pp. 690–2; ⑦ Radiology of the urinary tract, pp. 808–11;
⑦ Static cortical renography: DMSA imaging, p. 924; ⑦ Dynamic renography, pp. 926–7; ⑦ Captopril renography, p. 928.

Gadolinium-based contrast media

An association between exposure to gadolinium-based contrast media and nephrogenic systemic fibrosis (NSF) was established in 2006. NSF manifests as fibrosis of the skin and connective tissue, causing contractures and joint immobility, and can cause visceral fibrosis, in some cases leading to a fatal outcome. With relatively few cases reported so far, the risks are hard to quantify, but patients with CKD stages 4–5 can be considered high risk and CKD stage 3 low risk. No cases have been reported with eGFR of >60mL/min/1.73m². Post-exposure dialysis is recommended for patients already established on renal replacement therapy, but dialysis is not recommended for those not established on renal replacement; the risks of temporary vascular access and dialysis initiation outweigh the benefits of gadolinium removal. The risk of developing NSF seems to relate to the extent to which the contrast medium releases free gadolinium (Gd^{3+}) ions, with cyclic agents less likely to release Gd^{3+} than linear chelates.⁸

7 Royal College of Radiologists (2015). *Standards for intravascular contrast agent administration to adult patients*, 3rd edn. ⑧ https://www.rcr.ac.uk/sites/default/files/Intravasc_contrast_web.pdf.

8 Medicines and Healthcare Products Regulatory Agency (2007). *Gadolinium-containing MRI contrast agents: nephrogenic systemic fibrosis*. ⑧ <http://www.mhra.gov.uk/safety-public-assessment-reports/CON081759>.

Choice of investigation

Unexplained renal impairment

When a patient first presents with renal impairment it is important to decide whether this is acute—and therefore potentially reversible—or chronic. Although the history, examination, and blood tests may give some clues, considerable doubt may remain.

All patients presenting with renal impairment should therefore undergo

- *Ultrasound:*
 - Hydronephrosis suggests obstructive nephropathy.
 - Small, smooth kidneys with ↑ echogenicity and ↓ corticomedullary differentiation suggests chronic parenchymal renal disease, e.g. chronic glomerulonephritis.
 - Irregular cortical scarring can be caused by reflux nephropathy, previous obstructive nephropathy (e.g. complicating renal stones), and renal infarction from vascular disease or embolism.
 - Renal asymmetry, particularly in a patient with known atherosclerosis elsewhere, suggests RAS, although this can just as commonly be bilateral.
 - Renal enlargement can occur in ATN, renal vein thrombosis, and renal infiltration, e.g. in haematological malignancy.
- Plain abdominal film (KUB) if renal stones or nephrocalcinosis suspected—nephrocalcinosis and urinary tract stones, particularly if outside the renal pelvis, can be missed on US.

Further radiological investigations, including renal angiography and isotope scanning, are sometimes helpful.

Suspected nephrolithiasis

Unenhanced helical CT is increasingly the investigation of first choice and is more useful than IVU, particularly if the GFR is reduced or radiolucent stones present. IVU remains readily available and may be the preferred investigation in some centres.

Investigation of haematuria

In patients over 40 and possibly in some younger patients, it is important to exclude urinary tract malignancy. US is the investigation of choice for the detection of renal cell carcinoma but will miss some transitional cell carcinomas of the renal pelvis, which are best detected using CT or IVU. Cystoscopy or high-resolution US of the full bladder is required to rule out transitional cell carcinoma of the bladder.

Investigation of suspected renal artery stenosis

In younger patients in whom fibromuscular dysplasia is suspected, conventional angiography should be performed. Atherosclerotic RAS can be reasonably assessed by contrast CT angiography or by gadolinium-enhanced MRA, with direct angiography reserved for interventional procedures.

Reflux nephropathy

Confirming this diagnosis can be important in counselling patients, as reflux nephropathy is often inherited as an autosomal dominant trait. Cortical scarring is best detected using a static DMSA (dimercaptosuccinic acid)

renal scan. However, there are other causes of cortical scarring. The diagnosis is best confirmed by showing the combination of cortical scarring with underlying calyceal deformity on IVU. Demonstration of vesico-ureteric reflux on direct or indirect micturating cystourethrography is useful in infants and small children, but reflux commonly resolves with growth, so these tests are seldom used in adults.

Obstructive uropathy

Although hydronephrosis demonstrated on IVU or US is usually sufficient to confirm obstruction, it is possible to have obstruction without much dilatation (e.g. complicating encasement by tumour). More commonly, there is uncertainty over whether dilatation of the collecting system and pelvis is due to previous obstruction, now resolved, or continuing obstruction. Diuretic MAG3 (mercaptoacetyltriglycine) renography may be useful but gives less reliable results as the GFR falls. Insertion of a nephrostomy to determine whether direct drainage of urine improves renal function or retrograde insertion of ureteric stents may be useful for both diagnosis and treatment of obstruction. If doubt persists, a Whitaker test may be performed; this involves infusion of saline at a constant rate through a nephrostomy tube and measuring the relationship between pressure and flow down the ureter.

Renal transplant dysfunction

The differential diagnosis usually lies between obstruction, ureteric leak, rejection, ATN, nephrotoxicity, and renal vein thrombosis. Depending on the centre, US with Doppler assessment of renal blood flow (giving resistance index) or isotope renography may be the investigation of first choice.

Renal bone disease

Parathyroid hormone

Indications

Diagnosis of 1° hyperparathyroidism in patients with hypercalcaemia.

Diagnosis of 2° or tertiary hyperparathyroidism in patients with stage 4 or 5 CKD.

Procedure

A serum sample is sent and separated within 4h of venepuncture.

Alternatively, PTH remains stable for 24h if the sample is taken into EDTA, allowing samples to be sent from 1° care. Check with the local laboratory.

Other markers of bone biochemistry

Serum Ca^{2+} is often normal, even in patients with significant renal disease, because a fall in serum Ca^{2+} caused by reduced $1,25-(\text{OH})_2$ vitamin D production results in an ↑ in PTH secretion, returning serum Ca^{2+} towards normal. Hypocalcaemia occurs after parathyroidectomy or after treatment with bisphosphonates. Hypercalcaemia occurs when the PTH release loses sensitivity to serum Ca^{2+} in tertiary hyperparathyroidism.

If PTH is raised in the presence of CKD 3, check 25-(OH) vitamin D; vitamin D deficiency should be corrected before treatment of hyperparathyroidism.

- Serum phosphate is often ↑ in patients with renal impairment due to impaired renal excretion of phosphate.
- Serum total ALP rises in severe hyperparathyroidism and osteomalacia.
- Serum bone ALP is a more sensitive marker of bone turnover, but quantitative measurement is not widely available. If total ALP is raised, ALP isoenzymes can be measured as an indicator of whether the ↑ is of bone origin.
- FGF-23 (fibroblast growth factor) is a phosphaturic peptide produced primarily by osteocytes. Patients with CKD have ↑ FGF-23 concentrations due to phosphate retention and ↓ clearance. FGF-23 excess is a better predictor of adverse outcomes in CKD than serum phosphate levels and could represent a novel early biomarker for disordered bone mineral metabolism. However, the only current clinical indication for FGF-23 measurement is in the diagnosis of tumour-induced osteomalacia.

Serum aluminium and the desferrioxamine test

Patients with renal disease may be exposed to aluminium from contaminated water used for the preparation of dialysates or by ingesting aluminium hydroxide as an antacid or, rarely nowadays, as a phosphate binder taken with meals. Because of the effects of aluminium on the brain, BM, and bones, it is important to monitor patients at risk for evidence of aluminium accumulation.

Serum aluminium has to be taken into an aluminium-free glass tube. Serum aluminium levels reflect current exposure and do not give any information about cumulative exposure. Serum aluminium levels may be ↑ by iron

deficiency. Levels above 60 $\mu\text{g/L}$ (2.2 $\mu\text{mol/L}$) are considered indicative of a dangerous level of exposure and should lead to a review of treatment.

The increment in serum aluminium 24 or 48h after IV desferrioxamine is a marker of aluminium 'load'. The original protocol requires the use of 40mg/kg desferrioxamine; a rise in serum aluminium of >200 $\mu\text{g/L}$ correlates well with the presence of aluminium-related bone disease on bone biopsy. Low-dose protocols have also been described and validated.

Skeletal survey

Severe hyperparathyroidism causes erosion of the terminal phalanges, subperiosteal erosions, and, in rare cases, brown tumours and pathological fractures. Severe osteomalacia causes loss of bone density and Looser zones (pseudo-fractures). These radiological signs are not commonly seen in modern renal patients because biochemical monitoring allows earlier detection and treatment of bone disease.

Transiliac bone biopsy

This is the 'gold standard' for the diagnosis of renal bone disease but is not commonly used in clinical (as opposed to research) settings. However, it can be useful particularly for the confirmation of aluminium-related bone disease and low bone turnover states. For maximum information to be gained from this invasive test, double tetracycline labelling should be performed, allowing the pathologist to determine the rate of new bone formation.

Procedure

Fourteen and 13 days before the procedure, the patient takes a tetracycline antibiotic, e.g. oxytetracycline 250mg four times daily (qds), and 4 and 3 days before the procedure a different tetracycline, e.g. demeclocycline 300mg bd. Under GA, a transiliac core of bone, including both cortical surfaces, is taken and placed in absolute alcohol. The sample should be sent to a laboratory specializing in the interpretation of bone biopsies in patients with metabolic bone disease.

Immunological tests in renal medicine

Immune-mediated diseases can affect the kidney in isolation or as part of a systemic disorder. Immunological tests commonly used to diagnose or monitor progress of renal disease are discussed here.

Complement

Indications

Acute nephritic syndrome, renal failure with skin ± neurological involvement, and suspected SLE, endocarditis, or cryoglobulinaemia. The normal complement system, its activation pathways, and assay methods are discussed elsewhere (⇒ Serum complement components, p. 351). In relation to renal disease, hypocomplementaemia is important and relative deficiencies of various components can point to certain disorders. (See Table 10.1.)

Successful treatment normalizes complement levels in endocarditis and in SLE, except when SLE results from congenital complement deficiency.

C3 nephritic factor (C3Nef) is an IgG autoantibody that binds to, and stabilizes, alternative pathway C3 convertase—C3bBb. This results in continuous activation of the alternative pathway with C3 depletion. It is detected by ELISA. C3Nef is classically associated with the C3 glomerulopathies, dense deposit disease, and C3 glomerulonephritis (previously known as type 2 membranoproliferative glomerulonephritis).

Immunoglobulins and serum electrophoresis for paraproteins

Indications

- Suspected myeloma or other clonal B-cell disorders.
- Unexplained renal failure, with or without proteinuria, particularly in patients >50 years.
- Renal failure in association with hypercalcaemia.

Serum electrophoresis to identify a monoclonal Ig band may be useful but should always be combined with tests for urinary light chains (BJPs), as some types of myeloma cause light chain proteinuria without a monoclonal band in the serum. Serum free light chain assays are also now available and should be used to monitor myeloma in patients with kidney disease.

Table 10.1 Complement levels in selected diseases

	C3	C4
Post-streptococcal GN	Low	Normal
SLE	Low	Low
Cryoglobulinaemia	Low/normal	Very low
Membranoproliferative GN	Low	Low/normal
Subacute endocarditis	Normal/low	Low

GN, glomerulonephritis.

In patients with normal renal function, κ light chains are cleared more rapidly than λ light chains, so the $\kappa:\lambda$ ratio is lower than the production ratio; as the GFR falls, this difference becomes less. The normal range for the $\kappa:\lambda$ ratio is 0.26–1.65, but a normal range of 0.37–3.1 should be used if eGFR <60mL/min/1.73m². Routine serum and urine electrophoresis in the absence of clinical features to suggest a B-cell disorder will identify many patients with MGUS incidental to their renal disease and seldom identifies treatable disease.

Measurement of serum Ig concentrations is of value in patients with known myeloma but is otherwise not useful in the assessment of patients with renal disease. Polyclonal hypergammaglobulinaemia is seen in chronic infections, connective tissue disorders (e.g. RhA, Sjögren's syndrome), neoplasms, and chronic liver disease. Measurement of serum IgA concentration is of no value in the diagnosis or monitoring of IgA nephropathy, although a raised total IgA concentration is seen in some patients with this disease.

Paraproteins are products of abnormal B-cell clones and can be detected in serum as monoclonal bands on Ig electrophoresis or in urine as BJPs. Paraproteins may be whole Igs or heavy or light chains in isolation.

Light chains are sufficiently small to be filtered at the glomerulus, are not reabsorbed, and are not picked up on routine dipsticks. BJPs are light chains excreted in the urine; they precipitate on heating to 45°C and re-dissolve on boiling, but are now detected by electrophoretic techniques.

Paraproteins can cause a number of different renal lesions, including

- Myeloma cast nephropathy.
- Light chain nephropathy.
- AL amyloidosis.
- Fibrillary/immunotactoid glomerulopathy (although this appearance is more frequently not associated with a plasma cell dyscrasia).

Cryoglobulins

Cryoglobulins are Igs, which precipitate on cooling and re-dissolve on warming.

Cryoglobulinaemia should be suspected in

- Renal failure with otherwise unexplained hypocomplementaemia or +ve RF.
- Renal failure in association with skin and neurological involvement.
- Unexplained proteinuria/renal failure in patients with clonal B-cell disorders.

Meticulous attention to collection, transportation, and assessment of the sample is required; a serum sample must be kept at 37°C and sent to the laboratory for analysis immediately, having warned the laboratory that the sample is on the way. False –ve results are common due to improper handling of the specimen.

Once a cryoglobulin has been found, further electrophoresis and immunofixation allow identification of three distinct types

- Type 1 has a single monoclonal Ig (IgG, IgA, or IgM) and is associated with monoclonal B-cell disorders.

- Type 2 has a monoclonal IgM directed against the Fc portion of IgG, and the cryoprotein therefore consists of monoclonal IgM with polyclonal IgG. Tests for RF (i.e. anti-IgG antibodies) are +ve. This may be associated with haematological malignancy, chronic hepatitis C infection, or may be unexplained ('essential').
- Type 3 has polyclonal IgG and polyclonal IgM and occurs in chronic infections (e.g. bacterial endocarditis, viral hepatitis), autoimmune disorders (e.g. RhA, SLE), or may be unexplained ('essential').

Hypocomplementaemia, especially very low C4 levels due to classical complement pathway activation, is characteristic and helpful in diagnosing active cryoglobulinaemic disorder. Renal disease can present as an acute nephritic disorder or as nephrotic syndrome, and is usually seen in association with skin and systemic involvement.

Antineutrophil cytoplasmic antibody

These are autoantibodies directed against enzymes present in the cytoplasm of human neutrophils. They are present in nearly all patients with small-vessel vasculitis (including GPA, microscopic polyangiitis, renal-limited crescentic glomerulonephritis, and Churg–Strauss syndrome). ANCA and its pattern of distribution are conventionally detected by indirect immunofluorescence, with pANCA having a characteristic perinuclear staining pattern and cANCA a cytoplasmic staining pattern. Changing titres can reflect changing disease activity. ELISA is now readily available for the two commonest ANCA protein targets: proteinase 3 (PR3) which is the commonest target of cANCA and MPO, the commonest target of pANCA. Low-titre +ve ANCA is unlikely to reflect an underlying disease if the ELISA is –ve. However, a –ve ANCA does not rule out vasculitis, and false +ve tests occur, so the test is not a substitute for renal biopsy.

Anti-glomerular basement membrane antibody

Circulating anti-glomerular basement membrane (GBM) antibodies are present in Goodpasture's disease and may also be +ve in ANCA +ve vasculitis where 'double positivity' may confer a worse prognosis than ANCA positivity alone. ELISA and RIAs are available. Confirmatory renal biopsy in Goodpasture's disease shows linear deposition of anti-GBM IgG along the GBM, detectable by immunofluorescence or immunoperoxidase staining.

Poisoning and overdose

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General principles

Many poisoned patients recover without specific management other than good supportive care. A minority have life-threatening toxicity. In assessing the poisoned patient, it is important to ensure adequate airway, breathing, and circulation, take a thorough history, and undertake a full clinical examination. Tablets, bottles, syringes, aerosol containers, and other items found with or near the patient should be retained and any corroborative history obtained. It is usually best to analyse biological specimens (usually blood and/or urine) if analytical confirmation of toxin exposures is required.

The role of blood and urine tests in toxicology

Close collaboration between analytical staff and clinicians is required if anything other than the simplest toxicological analysis is to be useful.

Toxicological analysis using blood or urine is used to confirm:

- The diagnosis of poisoning, when this is in doubt or for medicolegal purposes.
- To help in the management, or in the diagnosis of brain death.
- To work out the time to restart chronic drug therapy e.g. anticonvulsants.

Few centres have full analytical toxicology services, and a 'toxicology screen' rarely influences acute inpatient management, with the exception of paracetamol, salicylate, lithium, digoxin, and iron poisoning, and on occasions a drugs of abuse screen. Toxicological analysis of blood plasma or serum is also of value if an extracorporeal method of elimination, such as haemodialysis or MARS[®] (Molecular Absorbance Recirculating Systems),¹ is being contemplated. Any toxicology analysis should be tailored to that patient's circumstances and the poisons commonly encountered in that country. In Western Europe and North America, most patients will have taken pharmaceutical agents (often in combination), but pesticide poisoning, for example, is common in less well-developed countries.

Plasma paracetamol, salicylate, lithium, digoxin, and iron measurements in blood are usually available on an urgent basis. For other patients, particularly those who present a complex clinical picture or who are unconscious, a 50mL sample of urine and a 10mL sample of heparinized blood should be collected on admission and stored at 4°C (refrigerated). This can be analysed later if it is felt the result will influence your management or if needed for medicolegal purposes (→ Samples of medicolegal importance, p. 702). Urine is useful for screening, especially for drugs of abuse, as it is often available in large volumes and often contains higher concentrations of poisons and their metabolites than blood samples. The samples should be obtained as soon as possible after admission, ideally before any therapeutic drugs are administered. Urine samples usually provide qualitative results, e.g. detect the presence of amphetamines or benzodiazepines. Quantitative measurements in urine are of little use because some compounds, such as benzodiazepines, are extensively metabolized prior to excretion in urine.

¹ Wittebole X, Hantson P. Use of the molecular adsorbent recirculating system (MARSTM) for the management of acute poisoning with or without liver failure. *Clin Tox* 2011; 49: 782–93.

Sample requirements

Plasma or serum is normally used for quantitative assays for drugs and drug metabolites, and in general there are no marked differences in concentration between these fluids. Evacuated blood tubes and containers containing gel separators or soft rubber stoppers are not recommended if a toxicological analysis is to be performed, as the plasticizers (phosphates and phthalates) used in many such tubes may interfere with chromatographic methods, and volatile compounds, such as CO or ethanol, may be lost.

EDTA tubes are preferred for COHb assays and for measurement of lead and some other metals, as these are concentrated in RBCs. A fluoride/oxalate tube should be used if ethanol, cocaine, or benzodiazepines are being assayed, although special tubes containing 1% (w/v) fluoride are needed if enzymic hydrolysis of these and other compounds is to be completely prevented.

The use of disinfectant swabs containing alcohols should be avoided, as should heparin, which contains phenolic preservatives (chlorbutol, cresol), and preservatives containing mercury salts (see Table 11.1).

Table 11.1 Sample requirements for metals/trace elements analysis

Metal	Sample needed
Aluminium	10mL whole blood in plastic (not glass) tube—no anticoagulant/beads*
Antimony	5mL heparinized whole blood; 20mL urine
Arsenic	5mL heparinized whole blood; 20mL urine
Bismuth	5mL heparinized whole blood
Cadmium	2mL EDTA whole blood*; 10mL urine*
Chromium	2mL heparinized whole blood*; 20mL urine*
Copper	2mL heparinized or clotted whole blood, or 1mL plasma; 10mL urine
Iron	5mL clotted blood or 2mL serum (avoid haemolysis)
Lead	2mL EDTA whole blood
Lithium	5mL clotted blood or 2mL serum (NOT lithium heparin tube!)
Manganese	1mL heparinized whole blood or 0.5mL plasma*
Mercury	5mL heparinized whole blood; 20mL urine
Selenium	2mL heparinized whole blood or 1mL plasma/serum
Thallium	5mL heparinized whole blood; 20mL urine
Zinc	2mL whole blood (not EDTA) or 1mL plasma/serum

* Send unused container from the same batch to check for possible contamination.

Samples of medicolegal importance

A toxicology screen is helpful if murder, assault, or child abuse is suspected. Samples collected in such cases are often so important that they should be kept securely at -20°C or below, until investigation of the incident is concluded. Legal requirements mean that all specimens should be clearly labelled with the patient's family or last name and any forenames, the date and time of collection, and the nature of the specimen, if this is not obvious. Strict chain of custody procedures should be implemented, and the doctor or nurse taking the sample should seal the bag with a tamper-proof device and sign and date the seal. A chain of custody form must accompany the sample and should be signed and dated by every person taking possession of the sample. The sample should be secured in a locked container or refrigerator if left unattended before arrival at the laboratory.

Post-mortem concentrations of some drugs may not necessarily represent antemortem levels and should therefore be interpreted with caution, e.g. in one case where a patient died $\sim 2\text{h}$ after admission to hospital, the post-mortem serum olanzapine concentration was elevated 5-fold over the antemortem level.² With passage of time after death, some drugs (especially basic drugs with a high volume of distribution) move along a concentration gradient from areas of high concentration in solid organs into the local blood, a phenomenon called 'post-mortem redistribution'. Therefore, the highest blood drug concentrations are found in central vessels such as the pulmonary artery or vein and the lowest levels in femoral veins e.g. morphine, amphetamines. Thus, the most appropriate post-mortem blood sample is a femoral blood sample.

The stability of the drug in post-mortem blood samples that are stored also needs to be considered. After a specimen has been collected, enzymes may remain active and continue to metabolize the drug *in vitro*. In general, drug instability occurs because functional groups are susceptible to transformation (e.g. 6-acetylmorphine) or readily oxidized or reduced. Olanzapine blood concentrations \downarrow with time by 23–84% during storage.³ However, conjugated drugs, such as the glucuronides, may deconjugate, which \uparrow the free drug concentration. In general, drug stability should be evaluated with consideration of long-term storage, the effect of freeze–thaw cycles, short-term stability (e.g. refrigerated samples), and bench-top (room temperature) stability. Thus, femoral blood sample drug concentrations should be measured as soon as possible after death and samples must be stored appropriately.

Further reading

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- Kerrigan S. Sampling, storage and stability. In: A Negrusz, G Cooper (eds.). *Clarke's Analytical Forensic Toxicology*, 2nd edn. London: Pharmaceutical Press, 2013; pp. 335–50.

² Chue P, Singer P. A review of olanzapine-associated toxicity and fatality in overdose. *J Psychiatr Neurosci* 2003; **28**: 253–61.

³ Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 9th edn. San Francisco: Chemical Toxicology Institute, 2011.

Methods used in analytical toxicology

Older and newer methods are discussed here to reflect global use. A range of chromatographic and other methods, such as radioligand immunoassays, are available for toxicological analyses. Plasma concentrations associated with serious toxicity range from µg/L in the case of drugs such as digoxin to g/L in the case of ethanol.

Specialized laboratories use a combination of solvent extraction and thin-layer chromatography (TLC) together with gas chromatography-mass spectrometry (GC-MS) or liquid chromatography tandem-mass spectrometry (LC-MS/MS) as the basis for a 'toxicology screen'. It is unwise to use TLC without corroboration of results by another method, e.g. LC-MS/MS, because the resolution power of TLC is limited and interpretation of chromatograms is subjective. A commercial kit for TLC (Toxi-lab, Marion Laboratories) is supplied with a compendium of colour plates, but even so problems can arise in the differentiation of compounds with similar mobility and colour reactions. The kit is aimed at the US market, and some common UK drugs are not included. Spectrophotometry is commonly used to measure salicylates, iron, and COHb. However, UV spectrophotometry and spectrophotofluorimetry are often used as detectors for high-performance liquid chromatography (HPLC) and in immunoassays. Spectrophotometric methods and immunoassays often suffer from interference from metabolites or other drugs. Immunoassays have the advantage of long shelf-life and simplicity, but all require confirmation with a chromatographic method if the results are to stand scrutiny. This is because immunoassays for small molecules are often not specific, e.g. some urine amphetamine immunoassays give +ve results with proguanil, isoxsuprime, labetalol, tranylcypramine, and phenylethylamine. The Syva Emit antidepressant assay cross-reacts with phenothiazines after overdose. Chromatographic methods have the advantage of selectivity and sensitivity and the ability to perform quantitative measurements, but they are more expensive. Generally, gas-liquid chromatography (GLC) or LC-MS/MS are used to measure basic drugs. Acidic or neutral moieties can also be analysed by LC-MS/MS. For screening very large numbers of compounds simultaneously or sequentially, then the Applied Biosystems Q-Trap technology is one of the most versatile—this is an LC-MS/MS where one of the quadruples of the triple quadrupole is used as an ion trap. Most laboratories that aim to be as versatile as possible have a fast GLC, in addition to the LC-MS/MS.

Modern methods of assay for heavy metals vary enormously. Inductively coupled plasma mass spectroscopy (ICPMS) is the most commonly used method in the UK. Here, ICPMS is used, instead of the flame or electro-thermal furnace for atomization of the elements and mass is the detector. Atomic absorption spectrophotometry, with either flame or electrothermal atomization is the older method. In the case of iron, reliable kits based on the formation of a coloured complex are available.

There is wide variation in the units that various laboratories use to report results. This has caused confusion and errors in treatment, and great care is needed to ensure that clinical interpretation is undertaken in full knowledge of the units used by the reporting laboratory. Particular care is also required in interpretation and application of analytical techniques in post-mortem toxicology, due to changes in concentrations of blood in storage and differential post-mortem redistribution of drug after death (Samples of medicolegal importance, p. 702).

Further reading

- Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 9th edn. San Francisco, Chemical Toxicology Institute, 2011.
- Drummer OH. Requirements for bioanalytical procedures in post-mortem toxicology. *Anal Bioanal Chem* 2007; 388: 1495–503.

Brainstem death testing and organ donation

Brain death cannot be diagnosed in the presence of drugs that mask CNS activity, e.g. baclofen. The rule of thumb, based on the pharmacological principle that most drugs need five half-lives to be effectively eliminated from the circulation, is to allow four half-lives of any drug to elapse before declaring death, or to allow at least 2–3 days for drug effects to wear off. Whether this is satisfactory for patients with organ failure, and hence impaired drug elimination, is unclear. Often in patients being assessed for organ donation, measurement of plasma concentrations of residual drugs, with expert interpretation, is required to determine whether brainstem death tests are valid or whether a drug could be interfering with the results.

Selected donor organs from those who have died from poisoning by tricyclic antidepressants, benzodiazepines, barbiturates, insulin, CO, cocaine, methanol, and paracetamol have been used in transplantation. It is important to identify which organs act as reservoirs for drugs and either not consider such organs, e.g. a liver from a paracetamol-poisoned patient, or take prophylactic precautions like acetylcysteine administration in the case of donation of a heart from a paracetamol-poisoned patient.

Further reading

- Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 9th edn. San Francisco, Chemical Toxicology Institute, 2011.
- Hanson P. Organ donation after fatal poisoning: an update with recent literature data. *Adv Exp Med Biol* 2004; 550: 207.
- Wood DM, Chan WL, Dargan P. Using drug-intoxicated deaths as potential organ donors: impression of attendees at the American College of Medical Toxicology 2014 annual scientific meeting. *J Med Toxicol* 2014; 10: 360–3.

Interpretation of arterial blood gases in poisoned patients

Interpretation of blood gas values may be found in  OHCM 10e, p. 162, pp. 188–9. Essentially four patterns emerge, which may occur together.

Respiratory acidosis

Hypoventilation results in retention of CO_2 . This can occur after an overdose with any drugs that depress the CNS, e.g. tricyclic antidepressants, opioids, and ketamine.

Respiratory alkalosis

Hyperventilation with respiratory alkalosis is classically caused by aspirin (salicylate). It can also occur in response to hypoxia, drugs, and CNS injury.

Metabolic alkalosis

Metabolic alkalosis is very uncommon in poisoning. Rarely, it may result from excess administration of alkali, e.g. deliberate alkali ingestion.

Metabolic acidosis

This is the commonest metabolic abnormality in poisoning. If acidosis is particularly severe (e.g. pH <7.2), this should raise the question of poisoning by ethanol, methanol, or ethylene glycol. Measuring the anion gap and osmolar gaps are helpful in further differentiation of the medical or toxicological cause ( Ethylene glycol, ethanol, and methanol poisoning, pp. 720–2).

Amphetamines and derivatives

For example, methylene dioxy-methamphetamine (MDMA) (ecstasy), MDEA, crystal methamphetamine ('ICE'), paramethoxy-methamphetamine (PMA).

Acute amphetamine overdose may cause sympathetic hyperstimulation, cardiovascular collapse, rhabdomyolysis, ventricular tachyarrhythmias, and death (often trauma-related). The following investigations should be considered in patients presenting to hospital with acute amphetamine(s) intoxication. In man, the half-life of methamphetamine ranges from 10 to 20h, depending on urine pH and the dose taken.

Plasma urea and electrolytes and glucose

It is critical that at least one set of U&E and creatinine are checked in every patient. Most are profoundly dehydrated and require vigorous rehydration. Some patients develop hyponatraemia, often after drinking excess water, and ADH secretion may also be responsible for this (→ OHCM 10e, p. 672). Hypoglycaemia may occur.

Dipstick test of urine for myoglobin and subsequent serum creatine kinase

Hyperthermia can develop after amphetamine exposure and may cause rhabdomyolysis. Hyperthermia may be one of the features of serotonin toxicity (accompanied by autonomic instability, ocular and peripheral clonus, and hyper-reflexia). Dipstick testing of urine is +ve for blood in rhabdomyolysis, as myoglobin is detected by the Hb assay. This is an indication that serum CK should then be measured. If found to be elevated, adequate rehydration is needed to reduce deposition of myoglobin in renal tubules and reduce the risk of ARF as a consequence. Caution in use of alkalinization as for certain drugs, e.g. amphetamines; this potentially delays drug excretion.

Full blood count

Rarely, aplastic anaemia (→ OHCM 10e, p. 364) has been reported after ecstasy (MDMA) ingestion.

Clotting studies

DIC (→ OHCM 10e, p. 353) can occur, often in the context of hyperthermia. Once liver damage ensues, INR/PT (→ OHCM 10e, p. 351) will rise.

Temperature

Hyperpyrexia can lead to rhabdomyolysis, DIC, and hepatocellular necrosis. Risks relate to the time in hours spent above 39°C. A rectal thermometer is the most accurate measure of temperature.

Blood pressure

A BP >180/120mmHg requires urgent medical care to reduce the risk of stroke.

Liver function tests

Acute liver injury can occur with a rise in AST or ALT, often of several thousands.

►► Note: do not miss a hidden paracetamol overdose—check paracetamol levels in blood, from the earliest sample you have on that patient! Checking an INR or prothrombin ratio (PTR) is essential in suspected late paracetamol poisoning and is of good prognostic value.

ECG

An ECG should be carried out in patients with tachycardia, chest pain, or reduced GCS. Patients should undergo cardiac monitoring. Cardiac arrhythmias are common and deaths, which occur soon after ingestion, may be due to these. Arrhythmias are often supraventricular, although ventricular arrhythmias also occur. Serial troponin levels are required if there has been/is chest pain.

Urine tests

Urine tests, e.g. EMIT dipstick system or by immunoassay in the laboratory, are sensitive and group-specific for amphetamines and can confirm an amphetamine has been ingested if that is in doubt, e.g. agitated patient in the emergency department. Note: amphetamine, MDMA, and MDA concentrations in blood are of no value in determining clinical management. Newer synthetic amphetamines (cathinones) may not be detected by urine dipstick tests but can be detected by chromatographic techniques in the laboratory.

Imaging

A CT brain scan should be performed for patients with altered conscious state, focal neurological signs, or severe headache.

Patients who are suspected body packers or body stuffers should undergo abdominal imaging, e.g. CT scan.

Further reading

Jones AL. Amphetamine overdose. *BMJ Best Practice* (online), Sept 2015. us.bestpractice.bmjjournals.com.
Volkow ND, Fowler JS, Wang GJ, et al. Distribution and pharmacokinetics of methamphetamine in the human body: clinical implications. *PLoS One* 2010; 5: e152369.

Anticonvulsants

For most anticonvulsants, LC-MS/MS will be the analytical method of choice. However, some (e.g. valproic acid) are difficult to assay by LC-MS/MS, and GLC may be more appropriate.

Carbamazepine toxicity

Plasma concentrations of carbamazepine and its active metabolite the 10,11-epoxide can be measured by HPLC or LC-MS/MS but do not correlate well with the degree of toxicity. They are seldom performed, unless the diagnosis is in doubt or there is concern about a therapeutic excess. The therapeutic range in plasma is between 8 and 12mg/L. Toxicity has been seen with carbamazepine concentrations above 20mg/L (85mmol/L). Coma, fits, respiratory failure, and conduction abnormalities have been seen with concentrations in excess of 40mg/L (170mmol/L). In seven fatalities due to carbamazepine overdose, femoral blood concentrations taken post-mortem averaged 45mg/L (range 35–70).⁴

An FBC should be performed. U&E and creatinine should also be checked, as hyponatraemia and SIADH (OHC 10e, p. 241) have been reported. Hypoglycaemia has also been reported. LFTs and PTR/INR should be performed to assess hepatotoxicity. An ECG should be performed in all but the most trivial carbamazepine over dosage to assess if any conduction abnormalities or interval prolongations (e.g. QRS widening, sinus tachycardia, AV block, or QTc prolongation) are present.

Lamotrigine toxicity

Overall, most patients exposed to lamotrigine in overdose experienced no clinical effects.⁵ Plasma concentrations of lamotrigine can be measured for compliance purposes (therapeutic range 1–4mg/L; upper limit may be as high as 10mg/L) but are not of value in the overdose situation. In a fatal case, the femoral blood concentration was 54mg/kg.⁴

Valproate toxicity

Plasma concentrations of sodium valproate can be measured by HPLC but do not correlate well with either the depth of coma or risk of seizures after overdose. The therapeutic plasma range is 40–100mg/L. U&E, creatinine, and glucose should be measured, as hypernatraemia, hypoglycaemia, and hypocalcaemia have been reported after overdosage. LFTs and PTR/INR should be taken to assess hepatotoxicity. Depending on the clinical need, a CT head scan and/or ECG may be required. Post-mortem blood concentration in a 34-year-old man was 720mg/L.⁴

⁴ Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 9th edn, San Francisco, Chemical Toxicology Institute, 2011.

⁵ Lofton AL, Klein-Schwartz. Evaluation of lamotrigine toxicity reported to poison centers. *Ann Pharmacother* 2004; 38: 1811–15.

Phenytoin toxicity

Most patients with acute phenytoin poisoning do not require measurement of their plasma phenytoin concentration and are treated with multi-dose activated charcoal. Blood glucose, U&E, creatinine, LFTs, and PTR/INR should be checked. An ECG should be obtained. Most cardiac complications have occurred with rapid ($>50\text{mg/min}$) IV administration. A CT head scan is needed if there is focal neurology (excluding ataxia or nystagmus) or any history of traumatic injury.

Most phenytoin toxicity is managed by repeat multi-dose activated charcoal. Rarely, an urgent phenytoin measurement may help in severe phenytoin poisoning where charcoal haemoperfusion or some other extracorporeal elimination method, such as MARS, is being considered,⁶ e.g. if the plasma phenytoin concentration is above 40mg/L and rapidly rising or is close to, or exceeding, the potential lethal level of 100mg/L. The overall clinical value of such elimination methods remains to be established. Post-mortem phenytoin concentration in femoral blood of a 4.5-year-old child who ingested 2g was 45mg/L.⁴ Phenytoin undergoes post-mortem redistribution.

Patients with suspected chronic phenytoin toxicity as a result of therapeutic dosing should have their plasma phenytoin concentration measured. The 'therapeutic range' is 10–20mg/L. Routine measurements may be useful to monitor anticonvulsant therapy or to time re-institution of chronic therapy after overdose.

⁶ Sen S, Ratnaraj N, Davies NA, et al. Treatment of phenytoin toxicity by the Molecular Adsorbents Recirculating System (MARS). *Epilepsia* 2003; 44: 265–7.

Benzodiazepines

Most patients who have taken an overdose with benzodiazepines just sleep off the drug without sequelae within 24h. However, more severe effects can occur when benzodiazepines are mixed with other drugs, especially in patients with pre-existing cardiovascular or respiratory disease. Pulse oximetry is useful for monitoring the adequacy of ventilation if significant CNS depression is present.

Generally, measuring benzodiazepine concentrations in blood or urine is not of value in the management of benzodiazepine overdose patients. Benzodiazepines can be detected in routine urine drugs screens. LC-MS/MS is the method of choice for quantitative analysis of diazepam and its metabolites. Liquid chromatography simultaneously assays diazepam and its polar metabolites, and post-mortem blood concentrations of 5 and 19mg/L have been found in fatalities.

Cannabis (marijuana)

Cannabis use may be detected in routine urine drug screening. They may be detected for up to several days after use, depending on the dose and frequency of use.

Synthetic cannabinoids may be consumed in herbal mixtures for recreational use, as an alternative to cannabis. Blood glucose, U&E, creatinine, and ECGs should be undertaken. Note that synthetic cannabinoids, e.g. JWH018, are often not detected by standard bedside urine drug screens (and thus are often the drugs of abuse selected by workers who undergo regular employment screening). However, they are detected by GC-MS, mass spectrometry, or LC-MS/MS if the sample is sent to the laboratory.

Further reading

Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 9th edn, San Francisco, Chemical Toxicology Institute, 2011.

Hermanns-Clausen M, Kneisel S, Szabo B, Auwärter V. Acute toxicity due to the confirmed consumption of synthetic cannabinoids: clinical and laboratory findings. *Addiction* 2013; **108**: 534–44.

Carbon monoxide

CO non-fire poisoning deaths have started to reduce in the UK, e.g. in 2012, compared with previous years. Incidence peaks in winter months, due to ↑ use of indoor heating and fossil fuel-powered generators and reduced external ventilation. It is highly likely that CO poisoning remains significantly underdiagnosed with patients presenting with 'flu-like' illness.

Survival rates for those resuscitated post-cardiac arrest with CO poisoning is very low, especially if there was pre-existing cardiovascular or respiratory disease. Pregnant women and their fetus are particularly susceptible to CO poisoning. Patients surviving significant CO poisoning may develop acute or delayed neurological sequelae leading to loss of consciousness, coma, and death. In the recovery phase, neurological sequelae, such as poor concentration and memory problems, may be seen. Symptoms include cognitive or personality changes, incontinence, psychosis, and Parkinsonian features such as rigidity. ~50–75% of people recover from their neurological features within 1 year.

A COHb concentration in blood *confirms recent exposure to CO* and should be measured urgently in all patients with suspected CO poisoning, including those with smoke inhalation. The space above the blood in the sample tube (headspace) should be minimized prior to blood gas analysis. Expected 'normal' values for COHb are up to 5% in non-smokers and up to 10% in smokers. However, people with pre-existing cardiovascular or respiratory disease can present with symptoms of toxicity at COHb concentrations in the low or normal range. A COHb concentration, however, *does not measure severity of poisoning* because COHb begins to dissociate from the moment of removal from the source of CO, and the rate of dissociation is also dependent on factors such as exogenous O₂ administration. Thus, any attempt to 'back-extrapolate' to find the initial highest COHb, e.g. by using nomograms, is flawed.

Management of the CO-poisoned patient is determined by the patient's clinical condition and also on circumstantial evidence such as the intensity and duration of exposure, rather than a COHb concentration per se, although a level of >40% has been used as one criterion to guide the use of hyperbaric O₂ therapy (which is of controversial clinical value). The CO-exposed patient should be administered high-flow O₂ (e.g. 12L/min through a tight-fitting non-rebreather mask) until the COHb is <5% and clinical signs of CO poisoning, such as impaired heel-toe walking and finger-nose incoordination, have resolved. If in doubt, O₂ is administered for a minimum of 6h. It is currently unknown if hyperbaric O₂ is more effective than normobaric 100% O₂ in prevention of neurological complications in patients with CO poisoning.

Arterial blood gases

Any patient with suspected poisoning by CO requires ABG analysis. O₂ saturation monitors are misleading, as they read COHb as oxyhaemoglobin (HbO), and the true O₂ saturation of the patient can only be determined by ABG analysis.

Electrocardiogram

An ECG should be performed in anyone severely poisoned (e.g. drowsiness or any neurological abnormality, chest pain, or breathlessness) or with pre-existing ischaemic cardiomyopathy. ECG changes, such as ST-segment depression, T wave abnormalities, ventricular tachycardia or fibrillation, and cardiac arrest can occur. If ischaemia/infarction is seen on the ECG or suspected clinically, then serial troponins should be requested.

Further reading

- Fisher DS, Leonardi G, Flanagan RJ. Fatal unintentional non-fire-related carbon monoxide poisoning: England and Wales, 1979–2012. *Clin Toxicol* 2014; 52: 166–70.
Smollin C, Olson K. Carbon monoxide poisoning (acute). *BMJ Clin Evid*. 2010 Oct 12; 2010–103.

Cocaine

Cocaine is snorted into the nose or injected IV.

Blood pressure monitoring

Patients with cocaine intoxication should have frequent measurements of their BP, as hypertension is a significant risk, and strokes and chest pain have been widely reported. A continuous monitoring device for repeated measurements is suitable.

ECG and cardiac markers

Cocaine-induced angina and MI are common. ECG monitoring is advised for all but the most trivial exposure to cocaine.

The predominant coronary pathology of cocaine is vasoconstriction. Thrombosis is less common, and troponin is the most sensitive indicator of myocardial damage (MI) due to cocaine. Cardiac troponins should also be performed in any patient with chest pain or ECG abnormalities. They are the most sensitive and specific markers. If elevated, then coronary angiography is usually indicated.

The ECG is of less diagnostic value. Up to 84% of patients with cocaine-related chest pain have abnormal ECGs, even in the absence of MI. In another study, 42% of patients with 'MI-like' ST elevation on their ECGs had MI excluded by cardiac markers. In a further study, total CK was elevated in 75% of patients, including 65% without MI.

Urine or blood testing

The use of cocaine can be determined by self-reports from patients or urine analysis of metabolites of cocaine (benzoylecgonine), e.g. EMIT test. This remains +ve for 24–48h after cocaine use. In assessing patients who may be a body packer, a +ve urine test may indicate pre-existing cocaine use or leaking cocaine from the packets.

GC-MS is more specific and can be carried out on blood or urine. Cocaine is unstable in blood, and samples are best taken into 1% w/v fluoride oxalate tubes if medicolegal sequelae of cocaine use are a possibility. 'Nasal insufflation of 106mg of the drug to six volunteers produced mean peak plasma concentrations of 0.22mg/L at 0.5h and 0.61mg/L for the metabolite benzoylecgonine at 3h'.⁷ Smoking 50mg in six volunteers produced mean peak plasma concentrations of 0.2mg/L at 0.08h and 0.15mg/L for benzoylecgonine at 1.5h. Patients have survived plasma concentrations of 5.2mg/L, but usually fatalities are associated with cocaine/benzoylecgonine concentrations in excess of 5mg/L, depending on the route of use. The IV route is the most dangerous. Cocaine may show significant post-mortem redistribution.

Further reading

- McCord J, Jneid H, Hollander JE, et al.; American Heart Association Acute Cardiac Care Committee of the Council on Clinical Cardiology. Management of cocaine-associated chest pain and myocardial infarction: a scientific statement from the American Heart Association Acute Care Committee of the Council on Clinical Cardiology. *Circulation* 2008; **117**: 1897–907.
- Senthilkumaran S, David SS, Jena NN, Menezes RG, Thirumalaikolundusubramanian P. Acute myocardial infarction and cocaine toxicity: One step closer. *Indian J Crit Care Med* 2014; **18**: 118.
- Shields LB, Rolf CM, Hunsaker JC 3rd. Sudden death due to acute cocaine toxicity-excited delirium in a body packer. *J Forensic Sci* 2015; **60**: 1647–51.

⁷ Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 9th edn. San Francisco, Chemical Toxicology Institute, 2011.

Cyanide

Cyanide poisoning can occur by deliberate inhalation of gas, ingestion of cyanide salts, or exposure in industrial fires.

Arterial blood gas estimation

Essential to determine the O₂ saturation and acid–base status of the patient.

Serum lactate

This is helpful in confirming suspected toxicity and can be used clinically as a surrogate for the cyanide assay. It is likely to exceed 7mmol/L in cases of significant exposure. Thus, a blood gas analyser in the emergency department can give a quick (indicative) answer to the question of whether exposure has taken place.

Electrocardiogram

All patients should have an ECG. It should be examined for evidence of ischaemic damage, e.g. ST depression, ST elevation, T wave inversion.

Cyanide assay

Blood cyanide concentrations are rarely of use in emergency management, because they cannot be measured quickly enough. However, a sample should be taken before antidote administration for assay at a later stage. Cyanide concentrations of <0.2mg/L are 'normal'; 1.0–2.5mg/L causes obtundation and coma, and >2.5mg/L is potentially fatal.

►► Note: the antidote of choice for cyanide poisoning is hydroxocobalamin, together with O₂; these are antidotes that can safely be given without certainty of cyanide ingestion. An alternative is to give sodium thiosulfate and sodium nitrite, and dicobalt edetate. However, note that dicobalt edetate should *only* be given if cyanide poisoning is certain, i.e. a proper history is available; otherwise you may kill your patient with cardiotoxicity of the antidote. Excessive administration of sodium nitrite can cause significant methaemoglobinæmia.

Further reading

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Borron SW, Baud FJ, Megarbane B, Bismuth C. Hydroxocobalamin for severe acute cyanide poisoning by ingestion or inhalation. *Am J Emerg Med* 2007; **25**: 551–8.
Hall AH, Saiers J, Baud F. Which cyanide antidote? *Crit Rev Toxicol* 2009; **39**: 541–52.

Digoxin

Patients who are already taking digoxin and those with pre-existing CVD are more susceptible to digoxin toxicity after overdosage. Digoxin toxicity can also result from progressive renal impairment or due to interactions with other drugs, as well as overdoses. Acute digoxin toxicity is an indication for oral multi-dose activated charcoal and IV hydration.

Electrocardiogram

All patients with suspected digoxin poisoning should have a 12-lead ECG, and all symptomatic patients should be attached to a cardiac monitor. Digoxin poisoning can cause virtually any type of cardiac arrhythmia due to ↑ automaticity and ↓ AV conduction. The combination of heart block with tachyarrhythmia is very common.

Plasma digoxin concentration

Absorption of digoxin often peaks at 4–6h after ingestion. Its half-life is in excess of 30h. Digitoxin is a structurally related drug that has an even longer plasma half-life (6 days). A digoxin measurement is a useful, but not absolute, guide to toxicity, as plasma digoxin concentrations correlate poorly with the severity of poisoning, particularly early in the course of acute poisoning. However, it is desirable in acute poisoning (although not essential) if anti-digoxin antibody fragments (Fab) are to be used, as it is useful in calculating the dose of fragments required (→ Digoxin, Indications for Fab fragments and doses of Fab fragments, pp. 718–19), as well as confirming exposure. Plasma digoxin concentrations cannot be interpreted after administration of digoxin Fab using normal assay procedures. Samples taken to investigate probable chronic digoxin intoxication should be taken at least 6h after dosing. They are not normally analysed urgently, unless life-threatening features of toxicity are present and use of Fab is being considered. The therapeutic range for digoxin is 0.8–2.0µg/L.

Urea and electrolytes, creatinine

It is important to ascertain if the patient has any renal impairment and plasma creatinine and urea are helpful, although of course do not exclude renal impairment completely. Hyperkalaemia is common in acute digoxin overdose and may be severe, e.g. >7mmol/L.

If possible, a magnesium level is helpful to exclude hypomagnesaemia, which contributes to risk of cardiotoxicity and is easily corrected.

Indications for digoxin antibody (Fab) fragments in acute toxicity and doses of Fab fragments

- Bradycardia or heart block associated with hypotension.
- Tachyarrhythmias associated with hypotension, especially ventricular arrhythmias

Fab fragment administration should be considered in less severe stages of poisoning in older patients and those with pre-existing CVD. At one time, the plasma K⁺ concentration was considered an indication for use of Fab fragments, but this is no longer used as an indication. Previously, use of

Fab fragments was considered for chronic toxicity, but this tends not to be used now.

The dose of Fab fragments to give for an acute ingestion of digoxin can be calculated from either the dose of digoxin ingested or the plasma digoxin concentrations. If in doubt, ten ampoules of Digibind® can be given, followed by an additional ten ampoules if clinically indicated.

Number of 40mg vials of Fab = plasma digoxin concentration
(ng/mL) × body weight × 0.0084

Or

Ingested dose (mg) × 1.2

Or

Best guess of 10–20 vials

➲ OCHM 10e, p. 842. Please note that if other types of digoxin-binding antibodies are used, then the dosage may be different to those indicated above.

Further reading

- BMJ Best Practice (last updated 2016). *Digoxin overdose*.  <http://bestpractice.bmj.com/best-practice/monograph/338.html>.
- Dasgupta A. Therapeutic drug monitoring of digoxin: impact of endogenous and exogenous digoxin-like immunoreactive substances. *Toxicol Rev* 2006; **25**: 273–81.
- Flanagan RJ, Jones AL. Fab antibody fragments: some applications in clinical toxicology. *Drug Safety* 2004; **27**: 1115–33.

Ethylene glycol, ethanol, and methanol poisoning

A history of ingestion or the presence of a metabolic acidosis raises suspicion of poisoning with these substances. Calculation of the anion gap and osmolal gaps is helpful in the assessment of such patients.

Anion gap

The normal anion gap is 12 ± 2 .

Calculating the anion gap

$$\text{Anion gap} = ([\text{Na}^+] + [\text{K}^+] - ([\text{Cl}^-] + [\text{HCO}_3^-])$$

Many toxins cause a high anion gap acidosis and these include

- Ethanol.
- Methanol. (Note: the high anion gap is due to metabolites and may take several hours to develop.)
- Ethylene glycol. (Note: the high anion gap is due to the metabolites and may take 6–24h to develop.)
- Metformin.
- Cyanide.
- Isoniazid.
- Salicylates (aspirin).

This list can be further reduced by measuring the osmolal gap.

Osmolal gap

This is the difference between the laboratory estimation of osmolality (Om) and calculated osmolality (Oc).

Calculating the osmolal gap

The osmolal gap is measured osmolality (Om) minus calculated osmolality (Oc):

$$\text{Oc} = 2([\text{Na}^+] + [\text{K}^+]) + [\text{urea}] + [\text{glucose}]$$

The osmolal gap is normally <10 .

Toxic causes of a raised osmolal gap include

- Methanol.
- Ethylene glycol.
- Diethylene glycol.
- Isopropanol.
- Ethanol.

The acronym 'MEDIE' can be a helpful mnemonic.

Ethylene glycol and methanol plasma concentrations

Often the diagnosis of ethylene glycol or methanol poisoning can be difficult, because assays for these substances are not widely available. If possible, their measurement can help manage severe intoxication. Other parameters may have to be used, i.e. anion gap, osmolal gap, and ABG analysis. A normal osmolal gap does not exclude poisoning with ethylene glycol or methanol, but if the osmolal gaps and anion gaps are both normal, and the patient is not symptomatic, then significant ingestion is unlikely to have occurred. In general, ethylene glycol or methanol measurements should not be carried out, unless metabolic acidosis is present and there is an anion gap.

Ethylene glycol and methanol concentrations in blood are useful to confirm ingestion and indicate when to stop antidotal treatment (with ethanol or 4-methylpyrazole) and/or when haemodialysis is needed ($>500\text{mg/L}$;  Indications for haemodialysis in methanol or ethylene glycol poisoning are, p. 722). However, a low concentration may just mean that most of the parent compound has been metabolized. Formate (i.e. the methanol metabolite) levels can also be checked in patients who may have taken methanol.

Microscopy of urine for oxalate crystals

In suspected ethylene glycol poisoning, microscopy can be performed to look for oxalate crystals. However, they are only present in 50% of cases and often only many hours after ingestion. Treatment of a patient should not be delayed or dependent upon looking for crystals.

Plasma ethanol concentrations

Plasma ethanol concentrations are usually not needed in patients who are drunk, unless there is doubt about the diagnosis, e.g. patients with a widening osmolal gap or the patient is so severely poisoned that haemodialysis is being considered for the ethanol poisoning. They are, however, essential to guide appropriate use of ethanol as an antidote in ethylene glycol or methanol poisoning ( Ethylene glycol, ethanol, and methanol poisoning, Indications for haemodialysis in methanol or ethylene glycol poisoning, p. 722). Rarely, a plasma ethanol measurement will be needed in child protection cases, and such sampling will need chain of custody and a specific method (GLC) by a specialist laboratory. The need for frequent monitoring of ethanol concentrations during treatment is avoided by use of the alternative antidote 4-methylpyrazole (a competitive alcohol dehydrogenase antagonist).

Antidotal therapy with ethanol

The dose of ethanol for treatment of ethylene glycol and methanol poisoning can be very difficult to predict, because ethanol metabolism is variable and unpredictable. It is therefore important to frequently recheck blood ethanol concentrations in patients receiving an ethanol infusion. The dose should be adjusted to achieve a blood ethanol concentration of 1–1.5g/L to achieve competitive inhibition of alcohol dehydrogenase.

Indications for continued ethanol therapy are

- Methanol or ethylene glycol poisoning with blood concentrations >200mg/L.
- Metabolic acidosis with pH <7.3.
- Osmolal gap >10mOsmol/kg water.
- Formate concentration >10mg/L.
- Urinary oxalate crystals.
- Severe symptoms.

Indications for haemodialysis in methanol or ethylene glycol poisoning are

- Methanol or ethylene glycol concentration >500mg/L.
- Severe metabolic acidosis (pH <7.3) unresponsive to therapy, i.e. ABGs are needed in all cases of high anion gap poisoning.
- Renal failure—hence, it is essential to check plasma U&E in all patients.
- Presence of visual problems in methanol poisoning.
- Formate concentration >500mg/L in methanol poisoning.
- Haemodialysis should be continued until the methanol/ethylene glycol concentration is well below 200mg/L.

Further reading

- Brent J. Fomepizole for ethylene glycol and methanol poisoning. *N Engl J Med* 2009; **360**: 2216–23.
McMartin K, Jacobsen D, Hovda KE. Antidotes for poisoning by alcohols that form toxic metabolites. *Br J Clin Pharmacol* 2016; **81**: 505–15.
Megarbane B, Borron SW, Baud FJ. Current recommendations for treatment of severe toxic alcohol poisonings. *Intens Care Med* 2005; **31**: 189–95.

Iron

Serum iron concentrations

In the UK, one 200mg tablet of ferrous sulfate contains 65mg of elemental iron. Iron is also found in varied amounts in many over-the-counter vitamin supplements. The elemental iron content of the preparation ingested should be checked carefully, as the important consideration is the amount of elemental iron ingested, not the weight of iron or vitamin tablets.

Serum iron concentrations should be measured urgently in all patients who may have ingested $>30\text{mg/kg}$ of elemental iron acutely, those who have ingested an unknown quantity, or those with symptoms, e.g. GI. If a sustained-release preparation of iron has been taken, a later serum iron concentration should be taken. A blood sample taken late after ingestion may underestimate the iron, as it may have already started distributing to tissues, i.e. in a late-presenting patient, a low concentration cannot be interpreted, but a high one indicates toxicity.

If the antidote desferrioxamine is given before 4h have elapsed, it interferes with the colorimetric assay for iron, and so a serum sample for iron should be taken off before it is given. If atomic absorption spectrophotometry is available for measurement of serum iron, there is no interference from desferrioxamine.

It is essential to interpret the serum iron concentration result in the context of the clinical state of the patient. If $<55\mu\text{mol/L}$ ($<300\text{mg/dL}$), mild toxicity is expected. If above $90\mu\text{mol/L}$ (500mg/dL), severe toxicity is expected and treatment with desferrioxamine is necessary. Do not wait for an iron concentration if altered conscious state, shock, or severe acidosis ($\text{pH} <7.1$) is present. Antidotal treatment is also indicated for patients with iron concentrations of $>55\mu\text{mol/L}$ if there is additional clinical evidence of toxicity, e.g. GI symptoms, leucocytosis, or hyperglycaemia. Antidotal therapy with desferrioxamine is indicated without waiting for the serum iron concentration in patients with severe features (e.g. fitting, unconscious, or hypotensive). Desferrioxamine is usually continued until the urine has returned to a normal colour, symptoms have abated, and all radio-opacities of iron tablets on AXR have disappeared. Urine free iron estimation is the best test of when to stop chelation therapy with desferrioxamine but is not widely available.

Working out if the patient needs a serum iron level checked

If a patient has ingested $<30\text{mg/kg}$ body weight of elemental iron (a 200mg ferrous sulfate tablet = 65mg of elemental iron), then no serum iron level is required. If in doubt, a plain AXR will usually indicate if lots of tablets are present. A serum concentration of $<55\mu\text{mol/L}$ ($<300\text{mg/dL}$) also indicates low risk (☞ Iron, Serum iron concentrations, p. 724).

Abdominal X-ray

This is required in patients who have ingested in excess of 30mg of elemental iron/kg body weight. The AXR determines the need for gut decontamination either by gastric lavage or whole bowel irrigation with polyethylene glycol. Undissolved tablets appear radio-opaque, but they disappear once dissolved, so the absence of radio-opacities does not exclude the possibility of toxicity.

Full blood count

This is needed in all cases of iron poisoning. Leucocytosis ($>15 \times 10^9/\text{L}$) is common with significant toxicity. Cross-match is a wise precaution in potentially serious poisoning.

Urea and electrolytes and creatinine, baseline liver function tests, and clotting

This is needed in all cases.

Blood glucose

Hyperglycaemia is common in serious poisoning.

Arterial blood gases

These should be checked in symptomatic or severely poisoned patients. Metabolic acidosis can occur.

Total iron binding capacity

This has no role in the assessment of acute iron poisoning.

What to do if estimation of serum iron concentration is unavailable

If serum iron assay is not available, the presence of nausea, vomiting, leucocytosis ($>15 \times 10^9/\text{L}$), and hyperglycaemia ($>8.3\text{mmol/L}$) suggests significant ingestion and the need for treatment with desferrioxamine.

⇒ OHCM 10e, p. 842.

Further reading

Chang TP, Rangan C. Iron poisoning: a literature-based review of epidemiology, diagnosis, and management. *Pediatr Emerg Care* 2011; **10**: 978–85.

Royal Children's Hospital Melbourne. *Iron poisoning*. Clinical practice guidelines. http://www.rch.org.au/clinicalguide/guideline_index/Iron_poisoning/.

Lead exposure and poisoning

Blood lead concentrations

Blood lead concentrations are used to confirm exposure and decide on whether environmental exposure reduction measures or (rarely) iron chelation therapy is required. Samples are not 'urgent' (except in the case of suspected acute lead encephalopathy) and must be taken into an EDTA tube.

A blood lead level of $5\mu\text{g}/\text{dL}$ or more requires further testing and monitoring, and the source of lead to be found and removed. A lead level of $>45\mu\text{g}/\text{dL}$ in a child usually indicates the need for chelation treatment. Occupational lead levels and appropriate responses for adults are enshrined in Worker/Occupational Health and Safety legislation.

There are two agents used for chelation therapy in lead poisoning—IV EDTA (disodium calcium edetate) and oral DMSA (2,3-dimercaptosuccinic acid). Before use, chelation therapy should be discussed with a toxicologist. In general, patients with a blood lead concentration of $>45\mu\text{g}/\text{dL}$ should be treated with chelation therapy and removal from further exposure. Children with encephalopathy or a blood lead concentration of $>75\mu\text{g}/\text{dL}$ require admission to hospital for urgent chelation therapy.

Abdominal X-ray

A plain AXR should be performed in all children, particularly if there is a history of pica, to exclude ingested paint or lead foreign bodies such as curtain pulls or fishing sinkers. Long bone X-rays in children may show lead lines.

Zinc protoporphyrin estimations

Zinc protoporphyrin (ZPP) estimations can be helpful in individuals with moderate ($>20\mu\text{g}/\text{dL}$) to high ($>40\mu\text{g}/\text{dL}$) blood lead concentrations in whom one is trying to determine the chronicity of exposure. There is a poor correlation between ZPP and blood lead at lower blood lead concentrations. There are other conditions (e.g. iron deficiency) that can ↑ ZPP, and there is significant inter-individual variation. ZPP has been proposed as a surrogate marker for blood lead, but blood lead is the best marker and should not be replaced by ZPP.

Other essential investigations

Patients should also have FBC and a blood film (for basophilic stippling), U&E, LFTs, and serum Ca^{2+} measured. All children should have their serum iron measured, as iron deficiency is an important diagnosis and, if corrected, can reduce ongoing lead absorption from the gut.

Further reading

- Boreland F, Lesjak MD, Lyle DM. Managing environmental lead in Broken Hill: a public health success. *NSW Public Health Bull* 2008; **19**: 174–9.
- Gulson B, Korsch M, Matisons M, Douglas C, Gillam L, McLaughlin V. Windblown lead carbonate as the main source of lead in the blood of children from a seaside community: an example of local birds as "canaries in the mine". *Environ Health Perspect* 2009; **117**: 148–54.

Lithium

Blood lithium concentration

Lithium is available as sustained-release and non-sustained-release tablets and liquid. After ingestion of liquid preparations, plasma lithium concentrations peak at 30min. With sustained-release preparations, peak concentrations occur at 4–5h. The plasma half-life of lithium is often in excess of 24h. Interpretation of plasma lithium concentrations depends on the clinical circumstances of exposure (→ Acute overdose in lithium-naïve patient, p. 728, → Chronic excess of lithium, p. 728, → Acute-on-chronic lithium poisoning, p. 728). Do not take blood for lithium levels into a lithium heparin tube!

Acute overdose in lithium-naïve patient

A single overdose in a lithium-naïve patient is of low risk. However, onset of toxicity may be delayed for as much as 24h. Plasma samples for lithium assay should be taken at 6h post-ingestion and measured urgently. The patient should have IV fluids to facilitate lithium elimination. Consider haemodialysis if plasma lithium concentration is >7.5mmol/L.

Chronic excess of lithium

Lithium toxicity can occur if the patient has been taking too high a dose or is dehydrated, or if an interaction with thiazide diuretics, NSAIDs, ACE inhibitors, or tetracyclines has occurred. Risk of toxicity is further enhanced by the presence of hypertension, diabetes, cardiac failure, renal failure, or schizophrenia. Blood for plasma lithium assay should be taken at presentation. Often good IV hydration suffices to clear the lithium; rarely haemodialysis is needed. Consider haemodialysis if the plasma lithium exceeds 2.5mmol/L.

Acute-on-chronic lithium poisoning

A patient taking lithium chronically who then takes an acute overdose is at risk of serious toxicity, because tissue binding of lithium is already high. The plasma lithium levels should be measured urgently at 6h post-ingestion. Lithium measurements should be repeated 6- to 12-hourly in symptomatic patients until clinical improvement occurs. Consider haemodialysis if plasma concentrations exceed 4mmol/L.

Indications for haemodialysis or arteriovenous haemodiafiltration

Lithium is effectively removed by haemodialysis (preferred) or arteriovenous haemodiafiltration. It is indicated in all patients with severe lithium poisoning, i.e. coma, convulsions, respiratory failure, or ARF. Plasma lithium concentrations can also guide the need for haemodialysis/haemodiafiltration. Each hour of dialysis will reduce the plasma lithium by 1mmol/L, but plasma lithium often rebounds after haemodialysis has stopped, so the assay should be repeated at the end of dialysis and again 6–12h later.

Urea and electrolytes, serum creatinine

Hyponatraemia is common in lithium toxicity. It is also important to check the serum K⁺ concentration and urea, as lithium is renally excreted and renal failure delays its elimination.

ECG

Lithium poisoning may result in arrhythmias, and complete heart block has been reported. An ECG (and sometimes monitoring) is required. Chronic lithium toxicity frequently has non-specific and diffuse depressed ST-segments and T wave inversion, which are seldom of sinister consequence.

Further reading

- Bellomo R, Kearly Y, Parkin G, et al. Treatment of life-threatening lithium toxicity with continuous arterio-venous hemodiafiltration. *Crit Care Med* 1991; **19**: 836–7.
- Eyer F, Pfab R, Felgenhauer N, et al. Lithium poisoning pharmacokinetics and clearance during different therapeutic measures. *J Clin Psychopharmacol* 2006; **3**: 325–30.

Methaemoglobinemia

Oxidizing agents convert Hb to methaemoglobin (MetHb), and this renders it incapable of carrying O₂. Common agents causing methaemoglobinemia include: dapsone, sulfonamides, chlorates, nitrites, nitrates, and local anaesthetics including lidocaine. The onset and duration of symptoms will depend on the agent. Nitrites cause breathlessness and flushing within minutes of exposure, but dapsone may cause methaemoglobinemia several hours after ingestion and the methaemoglobinemia may then persist for days.

Essential investigations

Patients with suspected methaemoglobinemia should have the following

- ABGs.
- FBC (especially if dapsone has been taken → haemolytic anaemia).
- Blood MetHb concentration.

MetHb can produce a normal PO₂ in the presence of reduced O₂ saturation. Pulse oximetry measures both MetHb and oxygenated Hb so can give false results.

Methaemoglobin estimation in blood

Measurement of blood MetHb is required to confirm the diagnosis and assess the severity of poisoning. The measurement must be done urgently when administration of the antidote methylthioninium chloride (methylene blue) is contemplated. Samples for MetHb estimation need to be analysed as soon as possible after collection, as if left to stand around, the MetHb will be falsely low owing to a reduction by endogenous MetHb reductase. The severity of symptoms correlates roughly with the measured MetHb concentrations. Anaemia and cardiac or pulmonary disease will lead to more severe symptoms at a lower MetHb level (see Table 11.2).

If the patient has severe clinical features of toxicity or if the blood MetHb concentration is >30%, the patient should be given methylene blue. Methylene blue can be given at lower blood MetHb concentrations in those who are symptomatic.

Table 11.2 Clinical effects

MetHb concentration (%)	Clinical effects
0–15	None
15–30	Mild: cyanosis, tiredness, headache, nausea
30–50	Moderate: marked cyanosis, tachycardia, dyspnoea
50–70	Severe: coma, fits, respiratory depression, metabolic acidosis, arrhythmias
>70%	Potentially fatal

Opioids

Classic features of opioid poisoning

- Depressed respiratory rate and volume.
- Pinpoint pupils.
- Coma
- Signs of parenteral drug use, e.g. needle marks.

Toxicity can be prolonged for 24–48h, particularly after ingestion of methadone, which has a long half-life. The lifesaving measure is prompt administration of adequate doses of naloxone, before waiting for results of any investigations. This often needs to be repeated or an infusion started, as the half-life of the antidote is much shorter than opioid drugs.

Adequacy of ventilation

O_2 saturation monitoring and/or ABG analysis demonstrates the adequacy of ventilation in those whose respiration is depressed, together with accurately measuring the respiratory rate.

Urine drug screening

Qualitative screening of the urine (group-specific immunoassay) confirms recent use. This may, however, not detect fentanyl derivatives, tramadol, and other synthetic opioids.

Measuring opioids in blood

Quantitative analysis is usually undertaken by LC/Q-TOF-MS or GC-MS or MS. This is often required for medicolegal purposes, particularly where a fatality or a childcare issue is involved.

'Plasma morphine free and total morphine concentrations were an average of 88 and 277 $\mu\text{g}/\text{L}$ in 54 people treated for heroin overdose'.⁸ Interestingly, the degree of poisoning correlated better with total morphine concentrations than free concentrations.

Post-mortem morphine levels in heroin overdose deaths vary, depending on prior narcotic history, but in general exceed 0.3mg/L.⁸

'Blood tramadol concentrations in 105 people arrested for impaired driving performance averaged 850 $\mu\text{g}/\text{L}$ '.⁸ Post-mortem concentrations in five tramadol deaths averaged 6.1mg/L. Heroin metabolites and tramadol do not undergo significant post-mortem redistribution.

Paracetamol screening

Opioid tablets are frequently combined with paracetamol. All unconscious patients should therefore have plasma paracetamol level measured.

Further reading

Jones AL. Management of opioid poisoning. In: A Webb, D Angus, S Finfer, L Gattinoni, M Singer (eds.). *Oxford Textbook of Critical Care*, 2nd edn. 2016; pp. 1522–5.

⁸ Baselt RC, Cravey RH. *Disposition of Toxic Drugs and Chemicals in Man*, 9th edn. San Francisco: Chemical Toxicology Institute, 2011.

Paracetamol (acetaminophen) poisoning

Overview

Paracetamol is the commonest drug taken in overdose in the UK. Paracetamol can be measured by a variety of assay methods, but HPLC or LC-MS is less susceptible to interference than some enzyme-based assays.

Measurement of plasma paracetamol concentration is essential for assessing the need for antidotal treatment (NAC) in most cases of paracetamol poisoning. The nomogram for paracetamol concentrations and protocols for when and how to give NAC differs between countries, and it is important to consult your own national guidelines. If NAC is given within 12h of the overdose, it provides complete protection against liver injury and renal failure. Beyond 12h after ingestion, the protection is less complete and assessment of liver damage is required. Paracetamol poisoning can be deceptive, as there is a latent phase of many hours where the patient remains well before liver damage develops. The co-ingestion of opioids may delay gastric emptying and peak plasma paracetamol concentrations.

INR/PT

The most sensitive marker of prognosis in paracetamol poisoning is the PT or INR. This often starts to ↑ within 24–36h of the overdose and peaks at 48–72h. Once the INR/PT starts to improve, this is a sign that hepatotoxicity is starting to improve and the patient will not go on to develop acute liver failure. Approximately half of patients with a PT of 36s at 36h post-ingestion will develop acute liver failure.

Plasma alanine and aspartate aminotransferases

These may begin to rise as early as 12h post-ingestion but usually peak at 72–96h. AST or ALT values in excess of 10,000IU/L are not unusual, and a plasma ALT of >5000IU/L is very suggestive of paracetamol poisoning (see Fig. 11.1). Serum bilirubin may peak after the aminotransferase, and this should not lead to concern for patients in whom the INR or PT have begun to fall.

Do not correct abnormalities in PT or INR with FFP or cryoprecipitate, unless life-threatening bleeding is taking place; otherwise the most sensitive marker of how the patient is progressing will be lost.

Other blood test abnormalities in paracetamol poisoning

Hypoglycaemia and metabolic acidosis are common. Early metabolic acidosis is often associated with very high plasma paracetamol concentrations, e.g. >400mg/L. Later, development of acidosis indicates incipient acute liver failure and the need to urgently check ABGs, LFTs, and INR/PT.

Pancreatitis with ↑ serum amylase/lipase has been reported. Several cases of thrombocytopenia have been reported.

U&E and creatinine should be checked. Renal failure can occur in the context of hepatic failure, but also in its absence (in 1 in 100 patients). It is treated with NAC and supportive measures, e.g. haemodialysis, if needed. Full recovery with supportive care is common.

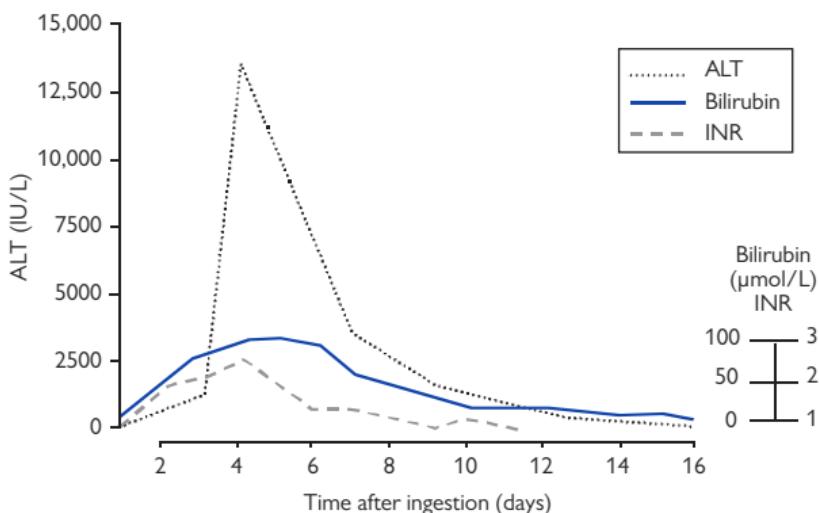


Fig. 11.1 Time course of liver function tests in paracetamol poisoning.

Investigating the patient who has taken a paracetamol overdose <4h ago

Ingestion of >75mg/kg of paracetamol or a paracetamol-containing product should be recognized as a potential hepatotoxic dose for most people. If ingestion of this amount or more has occurred within the last 1h, activated charcoal should be given orally (50g for an adult). A plasma paracetamol level should then be checked at 4h from the time of ingestion, to determine the need for NAC treatment from the nomogram (see Fig. 11.2).

Very rarely, e.g. after ingestion of 4 × 500mg tablets by an adult, a confirmatory plasma paracetamol level is not needed, but in general it is safer to be certain by checking a blood concentration.

Investigating the patient who has taken a paracetamol overdose between 4 and 8h ago

A plasma paracetamol level should be checked as soon as possible. If a single acute ingestion has taken place, then the result is plotted on the relevant national nomogram against the time since ingestion (e.g. see Fig. 11.2). This determines the need for NAC antidote treatment (i.e. if the level is above or very close to the line).

If the overdose is staggered or repeated, supratherapeutic, then specific toxicology, advice is needed. If in doubt, treat the patient with NAC. Some countries have modified this nomogram to treat patients at lower paracetamol levels.

Investigating the patient who has taken a paracetamol overdose between 8 and 24h ago

Start treatment with NAC straightaway. Take blood for a paracetamol level, INR/PT, creatinine, and plasma venous HCO_3^- (if plasma venous HCO_3^- is abnormal, check ABGs). Check results and refer to the graph to determine whether treatment with NAC needs to be continued (i.e. is the plasma level above the treatment line?) or can be stopped (below the line). ►► Beyond

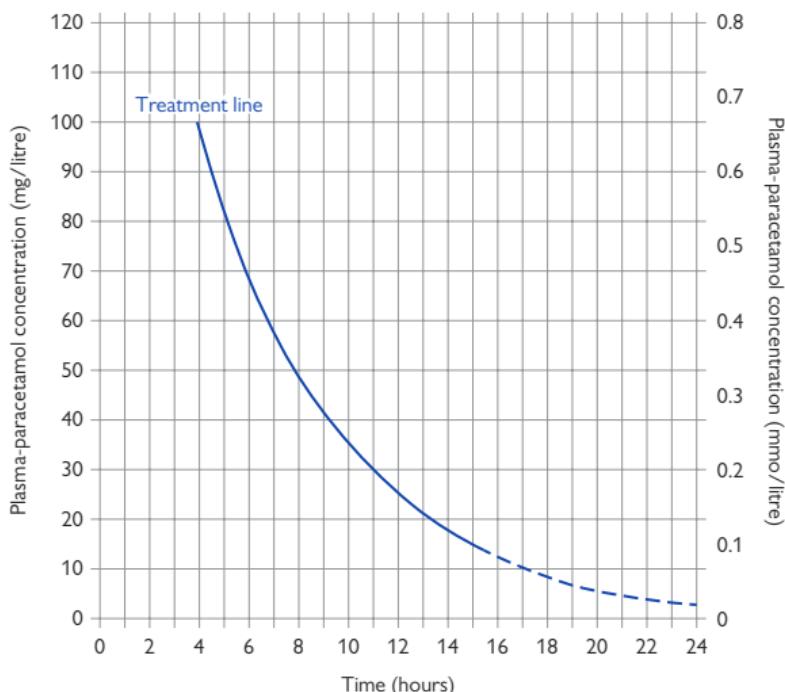


Fig. 11.2 An acetylcysteine treatment graph.

Note: The most up-to-date national nomogram should be accessed for your country.

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16h after ingestion, the sensitivity of the assay for paracetamol may be too low to detect a treatable level—check and, if in doubt, treat the patient with NAC! On completion of NAC, check blood INR/PT, creatinine, and plasma venous HCO_3^- (if abnormal, check ABGs). If the patient is asymptomatic and the INR or creatinine is normal or falling, discontinue NAC. If the patient has symptoms (abdominal pain or vomiting) or the INR or creatinine is rising, continue maintenance NAC (50mg/kg in 500mL of glucose every 4h) until the INR improves. Contact a poisons centre/liver unit.

Investigating the patient who has taken a paracetamol overdose >24h ago or the timing is not able to be established

Start treatment with the antidote NAC straightaway, unless a trivial amount has been taken.

Take blood for baseline INR/PT, creatinine, venous HCO_3^- (if abnormal, check ABGs), and paracetamol.

If the patient is asymptomatic and the laboratory tests normal (INR <1.3, paracetamol concentration <5 mg/L, and ALT <2 times the upper limit of normal), discharge the patient and advise to return if vomiting/abdominal pain develops. If the blood results are abnormal, continue NAC, and phone a liver unit/poisons centre for advice.

Investigating the patient who has taken a staggered overdose

The Commission on Human Medicines currently recommends that a Xmg/kg/24h dose ingested calculation is not used to guide therapy. Current advice is that all staggered overdoses should be treated with NAC and discussed with a toxicologist.

Blood is taken for paracetamol level, U&E, creatinine, HCO_3^- , ALT, and INR. Hepatotoxicity is not likely if the patients is asymptomatic, the plasma paracetamol concentration is <5 mg/L, INR is <1.3, and ALT is <2 times the upper limit of normal. If all of the above are found, then NAC may be stopped and the patient discharged, with advice to return if they experience vomiting or abdominal pain.

Investigating the patient who has a repeated supratherapeutic ingestion

All patients with a supratherapeutic overdose should be considered for NAC treatment and discussed with a toxicologist. Currently, patients with symptoms or signs of hepatotoxicity or those who have definitely ingested 75mg or less of paracetamol require no treatment.

Further reading

- BMJ Best Practice. *Paracetamol overdose*.  <http://bestpractice.bmj.com/best-practice/monograph/337.html>.
- TOXBASE.  <https://www.toxbase.org/>.
- UK National Poisons Information Service.  <http://www.npis.org/>.

Salicylate poisoning

Features of severe poisoning

Ingestion of >150, 250, and 500mg/kg body weight of aspirin, respectively, produces mild, moderate, and severe poisoning, respectively. Aspirin poisoning is becoming increasingly rare in developed countries. Signs of serious salicylate* poisoning include metabolic acidosis, renal failure, and CNS effects such as agitation, confusion, coma, and convulsions. Death may occur as a result of CNS depression and cardiovascular collapse. The development of metabolic acidosis is a bad prognostic sign, as it also indicates ↑ CSF transfer of salicylate.

Note: (*) aspirin, oil of wintergreen.

Plasma salicylate concentration

Plasma salicylate should be measured urgently in all, but the most trivial overdose, i.e. all those thought to have ingested >150mg/kg body weight of aspirin or any amount of oil of wintergreen. It should be performed at 4h post-ingestion, because delayed absorption of the drug renders such levels uninterpretable before this time. As salicylates may form concretions in the stomach, which delay absorption, it is recommended that a salicylate level is rechecked 3–4h after the first sample, to catch the peak salicylate concentration. There is no evidence for indiscriminate requesting of salicylate concentrations in every unconscious patient (unlike paracetamol) or in conscious patients who deny taking aspirin and who have no features suggesting salicylate toxicity. The plasma salicylate concentration is not an absolute guide to toxicity, as paracetamol levels are in paracetamol poisoning, but should be interpreted together with clinical features and acid–base status of the patient.

Urinary alkalinization (➡ OCHM 10e, p. 844) is indicated for patients with salicylate concentrations of 600–800mg/L in adults and 450–700mg/L in children and the elderly. Metabolic alkalosis is not a contraindication to bicarbonate therapy, as patients may have a high base deficit in spite of an elevated serum pH.

Haemodialysis is very effective at salicylate removal and correction of acid–base and electrolyte abnormalities. It should be considered if the plasma salicylate levels are >700mg/L in children and >800mg/L in adults. Other indications for haemodialysis include resistant metabolic acidosis, severe CNS effects, such as coma, convulsions, pulmonary oedema, and ARF.

Arterial blood gases

Acid–base problems are common in salicylate poisoning. Respiratory centre stimulation causes respiratory alkalosis. Uncoupled oxidative phosphorylation and interruption of glucose and fatty acid metabolism by salicylates often cause concurrent metabolic acidosis. Serial ABGs are needed in severe salicylate poisoning.

Further reading

Ghosh D, Williams KM, Graham CG, et al. Multiple episodes of aspirin overdose in an individual patient: a case report. *J Med Case Rep* 2014; 19: 374.

Theophylline

Acute theophylline poisoning can carry a high mortality, and its management is best guided by the Shannon severity grading scheme, bearing in mind that delayed effects tend to occur after sustained-release formulations have been ingested. The adult therapeutic range is 10–20mg/L. Serious toxicity occurs at >100mg/L (770mmol/L).

Urea and electrolytes, creatinine, glucose

It is vital to check the plasma K⁺ concentration frequently, as hypokalaemia is a life-threatening complication and the serum K⁺ concentration is a useful guide to severity. If >2.5mmol/L, the patient is less severely poisoned (grade 1) than if it falls to <2.5mmol/L (grade 2). Check blood glucose since hyperglycaemia is a common complication.

Arterial blood gases

In potentially serious poisoning (e.g. ingestion of >20mg/kg body weight), abg analysis is helpful in optimizing the acid–base status of the patient. An initial phase of hyperventilation with respiratory alkalosis can be followed by a further stage of metabolic acidosis.

ECG

In potentially serious poisoning (e.g. ingestion of >20mg/kg body weight), an ECG is required. Cardiac monitoring is helpful for early identification of arrhythmias in such patients.

Plasma theophylline concentrations

Measuring plasma theophylline concentrations confirms theophylline ingestion if this is in doubt, and it is usually undertaken by HPLC or LC-MS/MS. However, for the vast majority of poisoned patients, obtaining a plasma theophylline concentration does not guide their management. Therapeutic levels rarely exceed 20mg/L (155mmol/L). Theophylline peak concentration in plasma may occur at 1–3h after ingestion of a standard-release formulation. However, overdose is often with sustained-release products, and delayed absorption can result in delayed peak plasma concentration and toxicity, often 12–24h later.

Plasma concentrations are also helpful in deciding when to employ charcoal haemoperfusion in seriously poisoned patients (particularly if plasma concentrations are >100mg/L (770mmol/L)). Charcoal haemoperfusion can be considered at lower concentrations, e.g. 80mg/L, in the elderly or those with pre-existing IHD, and those with persistent seizures or hypotension not responding to IV fluids. Charcoal haemoperfusion can also be decided on the basis of grade 3 or 4 severity grading alone. If charcoal haemoperfusion is not possible, then haemodialysis with multi-dose activated charcoal is probably an alternative.

Urine testing for myoglobinuria and measuring serum creatine kinase

Theophylline poisoning can be accompanied by rhabdomyolysis. Hence, the urine should be dipstick-tested, and if found +ve for blood, a serum CK should be obtained. This will then indicate that renal function should be closely monitored and whether the urine should undergo alkalinization.

Further reading

Shannon M. Predictors of major toxicity after theophylline overdose. *Ann Intern Med* 1993; 119: 1161–7.

Tricyclic antidepressants

The main risks of overdose with these drugs are the cardiovascular system (CVS) and CNS toxicity.

Electrocardiogram

An ECG should be performed in all but the most trivial cases of overdose.

ECG abnormalities are common in moderate to severe poisoning and include

- *QRS prolongation:* >110ms in adults predicts the risk of ventricular cardiac arrhythmias (and the need for IV sodium bicarbonate), and a QRS >160ms predicts the risk of fits. In children, a QRS >110ms is predictive of the risk of arrhythmias, but not fits.
- Note: ECG criteria are not the only factors assessing the risk of arrhythmias, fits, and acidosis—electrolyte disturbances contribute. Supraventricular, and potentially fatal ventricular, arrhythmias can occur.

Cardiac monitoring

This is essential if ingestion of >10mg/kg body weight has taken place. It is seldom necessary beyond 24h after ingestion.

Arterial blood gas analyses

These should be done on all patients with marked symptoms and signs, particularly those with a reduced GCS score. It should also be performed on those with widened QRS or seizures, not least because such patients are receiving IV sodium bicarbonate therapy and a pH of 7.5 should not be exceeded.

Plasma concentrations

This is of no value, as plasma concentrations of tricyclic antidepressants correlate poorly with clinical features of toxicity.

Warfarin and superwarfarins

INR/PTR

This is a key test in possible warfarin/superwarfarin overdose. In the case of warfarin, the INR often begins to rise from day 2 after the overdose and settles within a week, but with superwarfarins, the time course of clotting abnormality may stretch to weeks. Thus, repeated INR/PTR estimations may guide progress and the need for vitamin K1 antidote in warfarin toxicity. Individual factor assays have been done in some cases but are not routinely necessary.

Liver function tests

Assessment of liver function is helpful in warfarin overdose. A congested, dysfunctional liver would be expected to handle the consequences of a warfarin overdose less well.

Table of conversion factors between mass and molar units

(See Table 11.3.)

Table 11.3 Conversion factors between mass and molecular units

Drug	Mass units	Molar (SI) units	Conversion factor
Carbamazepine	mg/L	µmol/L	4.23
Digoxin	µg/L or ng/mL	nmol/L	1.28
Ethanol	g/L	mmol/L	1.28
Iron	mg/L	mmol/L	0.179
Lead	mg/L	mmol/L	0.0048
Paracetamol	mg/L	mmol/L	0.0066
Phenytoin	mg/L	mmol/L	3.96
Salicylate (aspirin)	mg/L	mmol/L	0.0072
Theophylline	mg/L	mmol/L	7.7

Rheumatology

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Rheumatological investigations

Investigations in rheumatology are important not only in diagnosis, but also in assessing disease activity and monitoring treatment. They are complementary to a careful history and physical examination and should only be requested in the correct clinical context where results are likely to affect management. In isolation, ‘abnormal’ results can lead to unwarranted anxiety, investigations, and treatment.

Inflammatory markers

Inflammatory rheumatic disease is usually associated with an acute phase response. The ESR and CRP are the most commonly used to screen for inflammation. However, they are non-specific, also ↑ in infection and malignancy. Obesity is also recognized as a cause for high CRP. Conversely, a normal ESR or CRP does not exclude rheumatic disease, e.g. spondyloarthropathies.

Erythrocyte sedimentation rate

ESR is measured by observing how fast RBC fall through a column of blood in 1h. It is dependent upon many variables, including serum Igs, fibrinogen, and Hct. Therefore, a raised ESR may not reflect inflammation, but other factors, e.g. hypergammaglobulinaemia. Normal ESR levels also ↑ with age. In some centres, plasma viscosity is preferred as a screening for acute phase response, rather than the ESR, as it is independent of age, sex, and Hb (→ Erythrocyte sedimentation rate, pp. 252–3).

C-reactive protein

CRP production is driven by IL-1 and IL-6 on hepatocytes. It generally provides a more accurate reflection of inflammatory or infective processes than ESR, as it changes more rapidly and is independent of the variables that complicate ESR interpretation.

ESR and CRP in diagnosis and monitoring

Although ESR or CRP are not specific, the degree of elevation and relationship of ESR to CRP may suggest a diagnosis. For example CRP and ESR may be very high in infection and gout but are usually only moderately raised in RhA (see Table 12.1). Isolated elevation of CRP may also reflect auto-inflammatory disorders, e.g. familial Mediterranean fever and amyloidosis or infection.

In cases where ESR/CRP are ↑ in active disease, they are extremely useful in monitoring disease activity and response to treatment.

Other acute phase markers

In general, it is not helpful to measure other acute phase reactants, e.g. caeruloplasmin, α 1-antitrypsin, fibrinogen, and Hp. Two exceptions are serum ferritin and complement, which can be useful in diagnosis. Other common biochemical and haematological tests discussed below also reflect levels of inflammation, e.g. albumin and Hb fall, whilst ALP and γ GT rise.

In a clinical scenario where CRP is very high and it is difficult to distinguish between systemic infection or inflammation (e.g. vasculitis), measuring PCT might be considered. This is usually used in the ITU/acute medicine setting. A high level suggests a high risk of progression to systemic sepsis, whilst a low level may support an inflammatory cause for the raised CRP.

Table 12.1 ESR and CRP in diagnosis

Diagnosis	ESR	CRP
Infection	↑↑ or ↑↑↑	↑↑ or ↑↑↑
Malignancy	Normal to ↑↑	Normal to ↑
RhA	↑↑	↑↑
SLE*	↑	Normal to ↑
SLE with serositis	↑	Normal to ↑↑
Gout/CPPD**	↑↑ or ↑↑↑	↑↑ or ↑↑↑
Sjögren's syndrome	↑ or ↑↑	↑
Vasculitis	↑↑ or ↑↑↑	↑↑ or ↑↑↑
Osteoarthritis	Normal	Normal
Psoriatic arthritis/spondyloarthropathy	Normal to ↑↑	Normal to ↑↑

* Systemic lupus erythematosus.

** Calcium pyrophosphate disease.

Serum ferritin

Ferritin may be used as a surrogate marker for body iron stores but is also an acute phase reactant. In the presence of active inflammation or infection, a normal or high serum ferritin may be found, even if the patient has true iron deficiency (➔ Assessment of iron status, pp. 244–7). Serum ferritin levels are often very high in active adult Still's disease and fall with treatment. Elevated ferritin associated with a transferrin saturation of over 45% is also seen in haemochromatosis arthropathy.

Complement

Complement components C3 and C4 usually rise in the presence of active inflammation or infection. However, in SLE, they are commonly low. In some, but not all, SLE patients, they are abnormal when disease is active and normalize on treatment, making them useful in monitoring disease activity, especially lupus nephritis. Low levels of C1 can be associated with the development of SLE.

Haematology

Haemoglobin

There are three common scenarios for anaemia (\downarrow Hb) in patients with rheumatic symptoms.

- Related to the rheumatic disease—most commonly ‘anaemia of chronic disease’. Rarely AIHA (associated with +ve Coombs’ test, high reticulocytes, high bilirubin, low Hp.)
- As a manifestation of an alternative systemic disorder that presents with arthralgia (e.g. coeliac disease/leukaemia).
- Related to drug side effects, e.g. GI bleeding due to NSAIDs or pancytopenia caused by immunosuppressive agents (e.g. methotrexate).

The degree, speed of onset, and type of anaemia should be assessed, taking into account the clinical picture. Trends in Hb are useful in monitoring disease activity and treatment.

Microcytic hypochromic anaemia

The major differential diagnoses are:

- Chronic iron deficiency anaemia.
- Thalassaemia trait.
- Anaemia of chronic disease (typically associated with normal MCV, but if *chronic*, the MCV may reduce).

Iron deficiency related to acute or chronic blood loss (e.g. GI bleeding), or nutritional or bowel disease, such as Crohn’s disease or coeliac disease, should be considered.

Macrocytic anaemia

Vitamin B₁₂ or folate deficiency related to nutritional deficiency, e.g. scleroderma causing jejunal diverticulosis, Whipple’s disease causing malabsorption, or due to drug therapy such as methotrexate, sulfasalazine, and azathioprine. Excessive alcohol consumption may also cause macrocytosis without significant anaemia.

Normocytic normochromic anaemia

Usually reflects chronic disease, and the degree of anaemia may vary with disease severity, e.g. RhA, crystal arthritis, vasculitis.

Dimorphic anaemia (large and small red cells)

This picture is seen in mixed deficiency, e.g. iron deficiency plus vitamin B₁₂ or folate deficiency, post-transfusion, or during iron replacement therapy in a patient with iron deficiency. It is a feature of malabsorption associated with coeliac-related arthritis, scleroderma of the gut, and jejunal bypass arthritis.

White cell count

White cell count may be helpful in diagnosis and monitoring disease activity and is essential for monitoring immunosuppressive therapies. Diagnoses to consider where levels are \uparrow/\downarrow are listed. BM biopsy may need to be considered to distinguish from 1° haematological disorders.

Increased

- **Neutrophilia:** septic arthritis, crystal arthropathy (gout and pseudogout), systemic corticosteroid administration, myeloproliferative disorders.
- **Lymphocytosis:** consider lymphocytic leukaemia with joint symptoms and viral infection.
- **Eosinophilia:** eosinophilic granulomatosis with polyangiitis (EGPA; formerly known as Churg–Strauss syndrome) and other vasculitides, hypereosinophilic syndromes, eosinophilic fasciitis, and also Addison's disease (can complicate rheumatological diseases). Newly raised eosinophils should heighten awareness of potential adverse reactions to disease-modifying drugs. Asthma and allergy.

Reduced

- **Neutropenia:** consider autoimmune neutropenia, e.g. associated with SLE and Felty's syndrome in RhA. May be drug-induced (very common with tocilizumab and seen as adverse response to sulfasalazine, methotrexate, azathioprine, cyclophosphamide, ciclosporin, mycophenolate, leflunomide, and other cytotoxics). Normal range for neutrophils also vary between races, e.g. neutrophils may be lower in Afro-Caribbean populations.
- **Lymphopenia:** common in SLE.

Platelet count

- **Thrombocytosis:** reflects active inflammation, e.g. RhA, but should be distinguished from 1° polycythaemia vera (JAK2 kinase and BM biopsy if appropriate).
- **Thrombocytopenia:** autoimmune thrombocytopenia, may also be related to connective tissue disease, including SLE, RhA (Felty's syndrome), and antiphospholipid syndrome (APS).
- Can also reflect drug toxicity, including those listed under neutropenia above.

Pancytopenia may also occur

- As part of autoimmune disease, e.g. SLE/Felty's syndrome.
- As a side effect to immunosuppressive drugs previously mentioned.
- As a severe complication of disease. Macrophage activation syndrome is a life-threatening disorder that can manifest with pancytopenia in systemic juvenile arthritis, adult Still's disease, lupus, and Kawasaki disease. Pancytopenia is associated with high fever, hepatosplenomegaly, high ferritin, and hypertriglyceridaemia and is associated with unexpectedly low ESR.

Biochemistry tests

Although biochemical tests do play a role in the diagnosis of rheumatic disease (e.g. metabolic bone disease), their major use is in the assessment of systemic involvement of disease, e.g. renal or hepatic. In addition, they are essential for monitoring treatment.

Renal function

Renal function is measured in the diagnosis and monitoring of systemic disease. eGFR is widely used as a surrogate marker for the GFR. A wide range of diseases may involve the kidney, including vasculitis, SLE, Sjögren's syndrome, and gout.

The dose of many drugs should be adjusted according to renal function, including immunosuppressive agents, e.g. cyclophosphamide and methotrexate. Some drugs are contraindicated with low eGFRs, e.g. IV zoledronate and other bisphosphonates for osteoporosis. Drug treatment should also be considered as a cause for decline in renal function in rheumatology patients, e.g. NSAIDs, sulfasalazine.

Uric acid

Ninety per cent of cases of gout will have a raised serum urate. However, it may also be elevated in normal health and be ↑ by diuretic treatment. Definitive diagnosis of gout can only be made by identification of urate crystals by polarizing light microscopy of joint fluid or in biopsy.

Gamma globulins

γ-globulins are often elevated in active inflammatory disease. They are usually globally ↑ in Sjögren's syndrome but may also be raised in RA, sarcoidosis, SLE, and other connective disease.

Liver function tests

These are measured in the monitoring of many disease-modifying agents, e.g. methotrexate and sulfasalazine. Elevated γGT and ALP can also reflect active inflammation. They may be relevant in considering underlying diagnoses of arthralgia, e.g. haemochromatosis.

Bone function

Serum calcium

Hypercalcaemia should raise suspicion of malignancy, metabolic bone disease, and sarcoidosis. In metabolic bone disease/hyperparathyroidism, Ca^{2+} is not always elevated and a 'high normal' result may be significant in the correct clinical context. Hypocalcaemia may indicate hypoparathyroidism (⇒ Hypercalcaemia, pp. 190–2; ⇒ Hypercalcaemia/osteomalacia, pp. 188–9).

Alkaline phosphatase

ALP may be elevated due to bone, liver, or GI disease. If γ GT is concomitantly raised, one would suspect it originates from the liver. In isolation, it is more likely to be related to bone and further investigation, e.g. PTH, vitamin D, urinary Ca^{2+} , should be considered. ALP is high in Paget's disease, hyperparathyroidism, fractures, and bony metastases. Low ALP may be the only biochemical clue to hypophosphatasia.

Phosphate

Hypophosphataemia occurs in hyperparathyroidism and hereditary and acquired hypophosphatasia.

Serum parathyroid

Where bone biochemistry is abnormal, PTH should be measured. It is elevated in hyperparathyroidism and vitamin D deficiency. In cases of hypocalcaemia, low PTH suggests 1° parathyroid disease, whereas normal or raised PTH suggests PTH resistance (→ Hypercalcaemia, pp. 188–9; ← Hypercalcaemia/osteomalacia, pp. 190–2).

Urinary calcium

Urinary Ca^{2+} is useful in the investigation of hypercalcaemia and elevated PTH. In hyperparathyroidism, urinary excretion is ↑. If it is low, familial hypocalciuric hypercalcaemia should be considered. Hypercalciuria may also be associated with renal calculi.

Bone markers

Bone turnover markers are available but have limited use due to the requirement for consistent sample collection, especially for bone resorption markers. Serum and urinary C-terminal and N-terminal telopeptide type 1 collagen (CTX and NTX) are the most commonly used markers of bone resorption, and procollagen type 1 N-terminal propeptide (P1NP) of bone formation.

Autoantibodies

Autoantibody tests are an integral part of rheumatological investigation. Detection of antibodies against specific antigens is useful for both diagnostic and prognostic purposes and less often monitoring disease activity. However, these tests have their limitations. Most autoantibodies are not specifically associated with a single diagnosis but can occur in a range of diseases and in healthy individuals. ELISA is often used for screening, as it is more automated and cheaper than other methods and provide some quantitative information. Other methods, e.g. indirect immunofluorescence, are used for screening or to detect specific antibodies, depending on the tissue substrate used.

Rheumatoid factor

RF is an antibody to the Fc portion of IgG, forming immune complexes, first described in the 1940s. Although included in the American College of Rheumatology criteria for rheumatology, RF is not specific to RhA. It can be found in 4–16% of healthy individuals (prevalence ↑ with age). It may also be +ve in other autoimmune diseases, malignancy, and chronic infection. The rheumatoid arthritis particle agglutination (RAPA) test may still be used to confirm a diagnosis of rheumatoid disease in the presence of an appropriate clinical picture of symmetrical inflammatory polyarthritis. A high titre, e.g. 1:320, is of more relevance than a borderline result, e.g. 1:20. It is less helpful in early diagnosis, compared to established disease, with RF being +ve in around one-third of rheumatoid patients at 3 months post-diagnosis and two-thirds at 6 months. Other methods e.g. ELISA, are more commonly used in modern practice but risk false +ve results.

Anti-cyclic citrullinated peptide antibodies

Anti-cyclic citrullinated peptide antibodies (ACPA) may be considered more useful in clinical practice in the diagnosis of RhA. In some centres, RF is only checked if ACPA are –ve. First described in 1998, they are high-affinity IgG class antibodies that react with the amino acid citrulline and are measured by ELISA. They are found in the sera of about 75% of rheumatoid patients and are about 96% specific for RhA. They appear earlier than RF, are unaffected by treatment, and predict the development of erosions. However, they are not pathognomonic.

Antinuclear factor

ANA, commonly detected by ELISA, is often thought of as a screening test for SLE or other connective tissue disease. Indeed, it is +ve in 90–99% of cases of lupus, in over 90% of cases of scleroderma, and in many cases of Sjögren's syndrome, mixed connective tissue disease, and myositis. ANA, however, can be +ve in 3–5% of healthy individuals and many diseases, including hepatic disease, e.g. PBC, pulmonary disease, e.g. 1° pulmonary hypertension, chronic infections, malignancy, or may be drug-induced. It should therefore be interpreted only in the context of clinical symptoms.

Indirect immunofluorescence is another method of detecting ANA, and results are presented as patterns of fluorescence, e.g. homogenous, speckled, nucleolar. Although not specific, some clinicians may use these in determining the relevance of the test, e.g. a strong +ve homogenous ANA is more likely to be associated with SLE than a weak speckled pattern.

Anti-DNA antibody

In contrast to ANA antibodies, dsDNA is less sensitive (50–80% of cases), but highly specific for SLE. They are seen rarely in healthy individuals and in disorders such as chronic active hepatitis and Sjögren's syndrome. They are also useful in monitoring disease activity in some, but not all, individuals with SLE. A rising titre of dsDNA may precede a flare in disease.

Extractable nuclear antigen antibodies

Where ANA is +ve, ENA antibodies should be measured as their presence can suggest a risk of certain clinical scenarios.

- *Anti-Ro and anti-La (SSB)*: occur in Sjögren's syndrome and SLE, but also other autoimmune diseases. They have been associated with fetal heart block when they are present in the mother.
- *Anti-Sm*: lupus nephritis.
- *Anti-Scl-70 (anti-topoisomerase 1) and anti-centromere antibodies (anti-CENP A/B/C)*: these antibodies are associated with scleroderma. Anti-centromere antibodies are particularly associated with limited cutaneous scleroderma, and anti-Scl-70 with diffuse systemic sclerosis. They may be a marker of pulmonary hypertension. Anti-Scl-70 is associated with pulmonary fibrosis.
- A 'myositis panel' can be requested where polymyositis or dermatomyositis are suspected, including Jo-1, Mi-2, PM-Scl, SRP, Ku and antisynthetase antibodies (PL-7, PL-12, EJ, OJ).
- Presence of Mi-2 is more commonly associated with dermatomyositis and associates with better prognosis.
- PM-Scl suggests polymyositis/scleroderma overlap, SRP associates with chronic progressive disease.
- Antisynthetase antibodies suggest risk of interstitial lung disease.
- *Anti-RNP antibodies*: have been associated with 'mixed connective tissue disease' where features of several clinical syndromes can occur in combination.
- *Anti-histone antibodies*: have been associated with drug-induced lupus.

Antiphospholipid antibodies

1° APS (thrombosis, thrombocytopenia, and fetal loss) and other connective tissue diseases are associated with antiphospholipid antibodies. They should only be requested in the correct clinical context, as they can also be detected in normal health and many other clinical contexts, e.g. infection. IgG and IgM anticardiolipin antibodies (ACL) and β2-glycoprotein antibodies are most commonly measured via ELISA. A single +ve result should be interpreted with caution, and the test should be repeated after 6 weeks to determine relevance. A high titre and IgG-type antibody may be of greater clinical relevance than a low titre and IgM.

Lupus anticoagulant tests are also undertaken and are +ve when an ↑ in clotting time is not reversible when control plasma is added *in vitro*.

Antineutrophil cytoplasmic antibody

First described in 1982, classical ANCA (cANCA) gives a characteristic bright central granular cytoplasmic staining of ethanol-fixed human neutrophils by immunofluorescence (IIF). pANCA was later described to give a perinuclear IIF pattern. ELISA is used to identify antigen-specific ANCA, and PR3 and MPO are now usually tested routinely.

ANCA is often thought to be a screening test for vasculitis. Indeed, the presence of cANCA and PR3 in combination has been reported to be 99% specific (and ~55% sensitive) for granulomatous polyangiitis (formerly known as Wegener's granulomatosis). Similarly (~90% specific), the presence of pANCA and MPO together is associated with microscopic polyangiitis. However, like other autoantibodies, ANCA (especially pANCA) may be +ve in many other situations, including infection, TB, malignancy, and IBD. Therefore, the full clinical picture must be used in making treatment decisions, and reliance should not be put on ANCA alone. In particular, the absence of a +ve PR3 or MPO or a pANCA with weak +ve MPO should raise suspicion to carefully exclude an alternative explanation to vasculitis.

HLA-B27

HLA-B27 has been strongly associated with ankylosing spondylitis (present in 90% of patients) and is also linked to other diseases, including reactive arthritis, IBD, and uveitis. However, as it occurs in about 8% of the population, it is not helpful as a screening test in such a common symptom as back pain (approximately two-thirds of HLA-B27 +ve individuals with back pain would not have ankylosing spondylitis).

Reactive and infection-related arthritis

Where reactive arthritis is suspected, appropriate serology and swabs should be sent. These may include ASOT and anti-DNAse for *Streptococcus*, serology for *Campylobacter*, *Salmonella*, *Yersinia*, and EBV, stool samples for culture, and urethral swab for *Chlamydia*. Investigations chosen and results depend upon the clinical presentation. Where an infection-related arthritis is suspected, specific investigations should be requested, e.g. *Borrelia* for Lyme disease, leptospirosis for Weil's disease, etc.

Urinalysis

Where systemic disease is a possibility, e.g. vasculitis and SLE, urinalysis should be tested both in diagnosis and routine monitoring of disease. The presence of RBCs and casts are early indicators of renal disease. Proteinuria in long-standing inflammatory disease should raise the suspicion of amyloidosis.

Urinalysis is also used in monitoring of drugs, including gold, ciclosporin, and penicillamine.

Arthrocentesis

This refers to the aspiration of fluid from a joint. Examination of the fluid can be helpful in diagnosis and is essential in septic arthritis, crystal arthropathy, and haemarthrosis. Where infection is excluded, the joint may be therapeutically injected with a steroid (e.g. methylprednisolone or triamcinolone).

Synovial fluid examination

Physical characteristics

Observation of the colour and consistency of the synovial fluid is helpful in diagnosis. In general, inflammatory arthritis, e.g. sepsis/RhA/gout, is associated with a turbid fluid with low viscosity, whilst osteoarthritis or normal joint fluid is clear with higher viscosity. Crystal arthropathies are often associated with bloodstained fluid.

Microscopy

White cell count and the percentage of polymorphonuclear cells (neutrophils) should be measured in synovial fluid. They are higher in inflammatory arthritis, e.g. RhA ($5000\text{--}75,000/\text{mm}^3$, $>50\%$), and septic arthritis ($>50,000/\text{mm}^3$, $>75\%$) than normal ($<200/\text{mm}^3$, $<25\%$) or osteoarthritis ($200\text{--}10,000/\text{mm}^3$, $<50\%$). A Gram stain and culture should be performed to detect organisms in suspected septic arthritis. Culture for less common organisms, such as TB, may also need to be considered.

Polarized light microscopy should be used to detect the presence of crystals. Urate is associated with characteristic negatively birefringent crystals, whilst calcium pyrophosphate crystals are positively birefringent. Other crystals may also be observed, e.g. hydroxyapatite in Milwaukee shoulder.

Arthroscopy

Arthroscopy and synovial biopsy may occasionally be required for the diagnosis of chronic indolent infections, such as TB, or unusual slow-growing organisms such as *Coxiella* (fever polyarthritis). Arthroscopy is essentially a diagnostic procedure used by orthopaedic surgeons where direct visualization of the affected joint is required.¹

¹ American Academy of Orthopaedic Surgeons.  <http://www.orthoinfo.aaos.org/>.

Neurophysiology

These are dynamic electrical nerve and muscle tests that are performed in the context of appropriate clinical history and examination. They may be considered in rheumatology, including:

- Assessment of muscle disorders, e.g. to distinguish inflammatory muscle disease (polymyositis/dermatomyositis) and other 1° muscle disease, e.g. muscular dystrophy.
- Where mononeuritis multiplex/peripheral neuropathy is suspected as part of a rheumatological diagnosis, e.g. vasculitis, RhA, amyloidosis.
- Nerve entrapment syndromes, e.g. carpal tunnel syndrome, tarsal tunnel, or ulnar nerve compression where the clinical diagnosis is uncertain.
- Spinal cord/nerve root compression.

Diagnostic imaging

Imaging techniques are essential tools in the management of rheumatological conditions and are useful if performed under appropriate indications. It is not a substitute for clinical examination, and findings should be interpreted in the context of clinical abnormalities. It is important to follow the guidelines for radiological investigations published by the Royal College of Radiologists.

Inflammatory arthritis

It is important to diagnose inflammatory arthritis as early as possible, as timely treatment with disease-modifying anti-rheumatic drugs improves outcomes in terms of function, joint damage, quality of life, and costs to health service usage. Although conventional X-rays can be useful in diagnosis, this is generally only when disease is established and structural damage to the joints has already occurred. The challenge to identify early arthritis has led to widespread use of alternative imaging techniques, including musculoskeletal US and MRI.

Plain radiography

Arthropathy

Hand and feet radiographs are useful in diagnosis. Soft tissue changes can show swelling, effusions, and specific abnormalities, such as calcification, which can be helpful even in early disease. Bony changes occur usually in more established arthritis. The pattern and type of radiological damage can suggest the diagnosis (see Table 12.2), and serial films can be used to assess disease progression. Other findings may suggest specific diseases, e.g. calcinosis in limited cutaneous scleroderma and chondrocalcinosis in CPPD.

Radiographs of the lumbar spine and sacroiliac joints may be utilized in distinguishing mechanical and inflammatory (e.g. ankylosing spondylitis/psoriatic arthritis) back pain. Changes seen in inflammatory back disease include sacroiliitis (sclerosis and joint space loss), squaring of the vertebrae, bony proliferation along vertebrae (syndesmophytes), and spondylodiscitis. However, changes do not usually occur early in disease, and it can be difficult to distinguish changes from degenerative disease (osteophytes, loss of disc height). Evidence of bony change elsewhere, e.g. enthesitis, is helpful in diagnosis. Isotope bone scan or MRI may be appropriate further investigations.

Other joints should be imaged according to an individual's clinical needs. For example, a knee or hip would be required if joint replacement surgery may be appropriate, or a shoulder if calcific tendinitis is considered. Plain radiography is also useful in assessing complications of rheumatic disease, e.g. rheumatoid lung disease. Limitations of plain films are that only limited information is provided about soft tissues and bone texture and early joint damage is missed.

Bone lesions

Osteopenia should be further investigated with dual-energy X-ray absorptiometry (DXA) scanning (Nuclear medicine imaging, p. 763) and appropriate blood tests, e.g. PTH, Igs, and vitamin D. The underlying diagnosis

Table 12.2 X-ray appearance in arthritis of the hands

Diagnosis	Pattern	Joints involved	Radiographic features
Osteoarthritis	Asymmetrical	DIPs, PIPs	Joint space loss Peri-articular sclerosis Bone cysts Osteophytes
RhA	Symmetrical	MCPs, PIPs	Joint space loss Peri-articular osteopenia Erosions Psoriatic
Psoriatic arthritis	Asymmetrical/ symmetrical	DIPs, PIPs (MCPs)	Periostitis Resorption of terminal phalanx Bony proliferation Ivory digit Peripheral erosions Pencil-in-cup deformity Soft tissue dactylitis
Gout	Asymmetrical	DIPs, PIPs (MCPs)	Juxta-articular erosions— can be 'punched out' Soft tissue tophi
CPPD	Asymmetrical	DIPs, PIPs, MCPs	Erosions Chondrocalcinosis

DIP, distal interphalangeal; MCP, metacarpophalangeal; PIP, proximal interphalangeal.

can be suggested by typical bony appearances, e.g. Looser's zones are lucent lines visualized at 90° to the bony cortex in osteomalacia (vitamin D deficiency). Periosteal reactions and, in more advanced disease, bone resorption, can be seen in hyperparathyroidism and renal osteodystrophy. Hypertrophic osteoarthropathy is another cause of periostitis, which can signify underlying disease, e.g. bronchial carcinoma.

Sclerotic bone lesions (\uparrow bone density) can also be seen in renal osteodystrophy.

Paget's disease is characterized by focal excessive bone resorption (osteolytic phase), followed by excessive bone formation (osteoblastic phase). Radiological features are of radiolucent areas associated with bone widening, then subsequent coarsening of trabeculae and areas of \uparrow radiodensity/sclerosis. In advanced disease, there may be bowing of the bone, pathological fracture, and \uparrow risk of osteosarcoma.

Other causes of sclerotic bone lesions include neoplasms (1° and metastases), osteomyelitis, fluoride toxicity, and haemangiomas. Myeloma and other neoplasms should be considered in osteolytic lesions.

Figures 12.1–12.4 show the use of plain radiology in a variety of rheumatological disorders.



Fig. 12.1 Hands in rheumatoid disease.



Fig. 12.2 Hands showing calcinosis, especially prominent distally.



Fig. 12.3 Plain CXR showing pulmonary fibrosis in rheumatoid disease.



Fig. 12.4 X-ray of pelvis showing avascular necrosis of the femoral heads.

Ultrasound

USS is ideally suited to rheumatology. It is a non-invasive, 'X-ray-free' technique that can be used dynamically in a clinic setting as an extension to clinical examination. Grey-scale US can be used in inflammatory arthritis to detect synovial thickening, joint effusions, and bony erosion in RA, enthesal changes in spondyloarthropathy, and crystal deposition in calcium pyrophosphate.

Ultrasonography

US has an important role in the diagnosis of early arthritis, because erosions can be identified before they are visible on radiographs and subclinical synovitis can be detected. It can also be helpful in monitoring disease progression/treatment response. Power Doppler can be used to assess joint activity in addition to joint damage.

US is also ideal for imaging tendon pathology, including tendinitis, tenosynovitis, calcific tendinitis, and tears. It has been reported to be highly sensitive and specific for rotator cuff tears, and the dynamic nature of examination means that a tendon/joint can be examined, whilst in motion. Bursae, fluid-filled cysts, and ganglia are also readily identified.

More advanced US assessments include soft tissue and muscle disorders, assessment of masses, and nerve entrapment. Imaging of temporal arteries in GCA is also reported to show diagnostic changes in experienced hands, with a hypoechoic halo being demonstrated due to artery oedema and stenosis. This may potentially be used to guide biopsy. US has also been used to assess blood flow in vasculitis, e.g. brachial and axillary arteries.

Limitations of US include difficulty in imaging less accessible areas, e.g. carpal bones, and no information beyond the bony cortex.

Figures 12.5–12.8 show the use of US in a variety of rheumatological disorders.

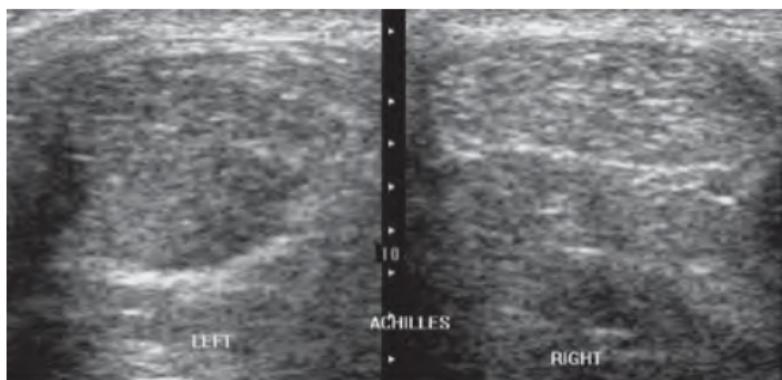


Fig. 12.5 Achilles tendinosis.

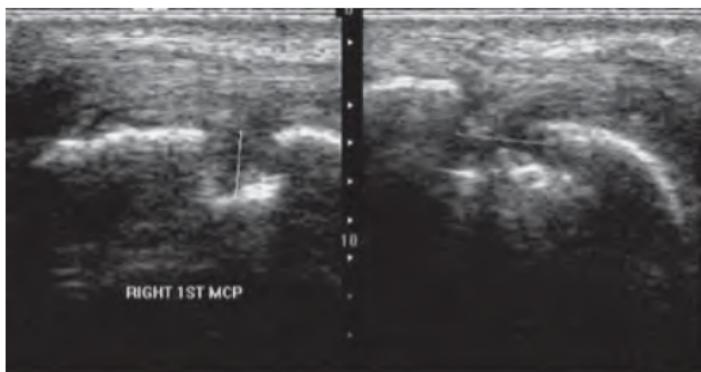


Fig. 12.6 Erosion of MCP joint.

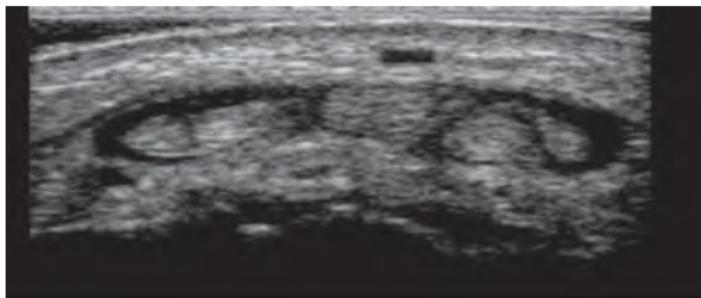


Fig. 12.7 Extensor tenosynovitis of the wrist.



Fig. 12.8 Flexor tenosynovitis of the middle finger.

Magnetic resonance imaging

In contrast to plain films and US, MRI has the strength that all components of a joint can be visualized at once. Not only can the bony cortex be appreciated, but also the composition of the bone. Use of MRI is limited by accessibility, expense, duration of scans, and presence of metal implants, e.g. pacemakers preclude its use. MRI has a role in the diagnosis of inflammatory arthritis, especially in early disease, to optimize treatment and outcomes, but also in distinguishing alternative, often significant, bone and joint disease, including neoplasm.

Inflammatory arthritis

In RhA, MRI detects erosions earlier than X-ray and can identify 'pre-erosion'—areas of bony oedema, which proceed to erosions in time. This suggests that patients at risk of erosive arthritis could be identified prior to permanent structural damage and treatment tailored accordingly. However, not all such areas do progress to erosions and similar features have been seen in bone disease (➡ *Bony lesions*, p. 761), healthy joints, bone cysts, and ganglia.

Spondyloarthropathy can be difficult to diagnose, traditionally requiring X-ray evidence of sacroiliitis. MRI can detect both active and chronic lesions of spondyloarthritis in the spine, sacroiliac joints, and entheses. Active inflammation/BM oedema is detected on short tau inversion recovery (STIR) and fat-suppressed sequences, whereas chronic lesions are seen best on T1-weighted (T1W) images. MRI is now included in the ASAS (Assessment of SpondyloArthritis International Society) international classification of axial spondyloarthritis.

Muscle disease

MRI is useful in identifying abnormal areas of muscle. In the context of myositis, an MRI scan can be used to guide a muscle biopsy to ensure sampling of abnormal tissue.

Soft tissue and back disorders

MRI is able to visualize tendon and cartilage lesions and bone tumours. MRI is particularly useful in the assessment of the back, especially in identifying infective lesions, e.g. discitis/abscess, in addition to identifying inflammatory lesions, sacroiliitis, spinal stenosis, myelopathy, disc prolapse disease, and nerve compression. It is used in the assessment of orthopaedic lesions, such as meniscal tears in the knee, especially prior to surgery.

Bony lesions

MRI is an excellent modality for investigating localized musculoskeletal symptoms, identifying significant lesions.

Bone oedema is also seen in a variety of bone lesions including avascular necrosis and transient osteoporosis of the hip, trauma, e.g. stress fractures, infection including osteomyelitis, metabolic bone disease, e.g. Paget's disease, chronic pain syndrome ('reflex sympathetic dystrophy'), and neoplasm.

Vasculitis

MRA is used in the investigation of vasculitis, including large-vessel vasculitides, e.g. GCA and Takayasu's arteritis, and smaller-vessel disease, e.g. Buerger's disease.

Computed tomography scans

CT scans are rarely used in imaging of the musculoskeletal system, especially as associated radiation dose is high. CT/HRCT does, however, have a role in the confirmation of diagnosis in bony lesions, e.g. infiltrative lesions, haemangiomas, lipomas, osteoid osteomas, and other bony lesions. Sacroiliac inflammation, erosions, and calcification are also well visualized. HRCT has been used in the assessment of osteoporosis but is largely superseded by DXA scanning (➡ Nuclear medicine imaging below).

The major role for CT is in identifying complications of rheumatic disease, e.g. pulmonary fibrosis associated with RhA.

Nuclear medicine imaging

Bone scan

Radionuclide imaging with 99m technetium or 67 gallium is relatively easy to perform and gives information about the whole body. In rheumatology, it is used to assess the pattern of joint involvement in arthritis and to detect other causes of bony pain, including Paget's disease, metastases, stress fractures, and other specific diagnoses, e.g. chronic regional pain syndrome. Although bone scans are sensitive to abnormalities, they have poor specificity so should be used in clinical context and may be a guide to further additional investigation, e.g. MRI.

Positron emission tomography

(See Fig. 12.9.)

18 -FDG PET was originally used for tumour imaging but has gained wide acceptance in its use in the imaging of medium- to large-vessel vasculitis. It is

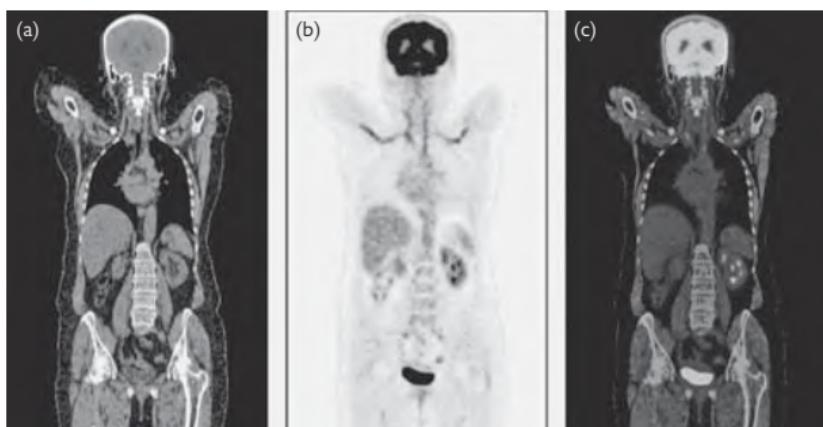


Fig. 12.9 Coronal images of CT (a), PET (b), FDG-PET/CT (c) of a 90-year-old woman with bilateral axillary artery thrombosis, arthralgia, and fatigue. These demonstrate increased FDG uptake in the carotid, subclavian and axillary vessels, consistent with large vessel vasculitis.

Reproduced from Watts, Richard, et al., *Oxford Textbook of Rheumatology*, 4th edition (2013), with permission from Oxford University Press.

of particular benefit in the diagnosis and assessment of the extent of vessel involvement in GCA, Takayasu's arteritis, and aortitis. It can also be used in the monitoring of disease activity. Other potential uses for PET-CT include inflammatory myositis and investigation for underlying malignancy associated with rheumatic disease.

Dual-energy X-ray absorptiometry scan

Osteoporosis may not be recognized clinically, unless a fracture occurs. DXA is a non-invasive, low-radiation assessment of bone density. Potential fracture sites (lumbar spine, hip, neck of femur, and wrist) are imaged, and the result is compared with that expected in a standard population to give a T-score and an age-adjusted population to give a z-score. For every standard deviation below the mean of the standard population, the fracture risk is ↑ by 2–3 times. Vertebral fracture analysis may also be offered to identify fractures at the time of the scan. DXA is offered to patients at risk of developing osteoporosis (see Table 12.3). Online calculators have been developed to identify those most at risk of developing fragility fractures and to determine who needs a DXA scan and/or treatment. The Q fracture method is most suited to the elderly, and the FRAX/NOGG (National Osteoporosis Guideline Group) calculator is intended for 40- to 90-year-olds and can be used with the bone mineral density of the femoral neck, following DXA, to improve prediction of the 10-year fracture risk. Ideally, repeat scans should be carried out on the same DXA machine, as results cannot be reliably compared between different machines.

Table 12.3 Risk factors in osteoporosis

Osteoporosis	Diseases associated with osteoporosis/bone fragility
1°	
Menopause <45(+ <5-year HRT)	RhA
2° amenorrhoea	Ankylosing spondylitis
Radiological osteopenia	Lupus
History of maternal hip fracture	Hyperparathyroidism
Previous fragility fracture*	Hyperthyroidism
Heavy long-term smoking	♂ hypogonadism
Low BMI (kg/m^2) <19	Chronic liver disease
Prolonged immobilization, e.g. MS	Chronic IBD
Excessive alcohol consumption	Malabsorption
	Chronic renal failure
2°	
Oral steroids	Hypopituitarism
Aromatase inhibitors	Haemochromatosis
Androgen deprivation treatment for prostate cancer	Organ transplant patients
Excessive thyroxine/anticonvulsants	Anorexia/bulimia

* Fracture sustained with no trauma or fall from standing height.

Radiology

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Radiology and the role of imaging

Effective use of the radiology department relies on good communication between radiologists and their clinical colleagues. The overall aim must be to target investigations efficiently in order to provide answers to clinical dilemmas at minimal cost and radiation dose (see Table 13.1). The investigation of neurological problems has been transformed by the advent of CT and MRI. Local availability varies and CT, in particular, can add considerably to the radiation burden. Conversely, if a CT is likely to provide the best answer and minimize overall costs by resulting in an early discharge, then it should be the investigation of choice. It is helpful to consider plain films, contrast studies, US, and then CT/MRI as a hierarchy where plain films are requested as an initial investigation. This hierarchy may be circumvented if a more expensive investigation is likely to produce the definitive result.

The following are important points to consider:

- *Will the investigation alter patient management?* That is, is the expected outcome clinically relevant? *Do you need it?*
- *Investigating too often or repeating investigations before there has been an adequate lapse of time to allow resolution or to allow treatment to take effect.* Similarly, investigations performed too early may be non-contributory. *Do I need it now?* Especially relevant when investigations may have been performed elsewhere. Make every effort possible to obtain prior studies. Transfer of digital data through electronic links will assist in this process. *Has it been done already?*
- *Would an investigation that does not use ionizing radiation be more appropriate, e.g. USS/MRI?*
- *Failure to provide accurate clinical information and questions that you are hoping will be answered by the investigation may result in an unsatisfactory outcome or an inappropriate focus in the report. Have I explained the problem?*
- *Would another technique be more appropriate?* The advances in radiology mean that discussion with a radiologist may be helpful in determining the best possible test. *Is this the best investigation?*
- *Over-investigating.* Are you taking comfort in too many tests or providing reassurance to the patient in this way?

Table 13.1 Typical effective doses from diagnostic medical exposures

Procedure	Typical effective dose (mSv)	Equivalent number of CXRs	Equivalent period background radiation
Chest (P-A)	0.015	1	2.5 days
Lumbar spine	0.6	40	3 months
Abdomen	0.4	30	2 months
IVU	2.1	140	11.5 months
Mammography (two views)	0.5	35	3 months
Barium enema	2.2	150	1 year
CT head	1.4	90	7.5 months
CT chest	6.6	440	3 years
CT abdomen	5.6	370	2.5 years
CT colonography	10	670	4.5 years
Bone scan (^{99m} Tc)	3	200	1.4 years
PET scan (body) (F18-FDG)	18	1200	8.1 years

UK average background radiation = 2.2mSv/year.¹

Table adapted from Royal College of Radiologists (2012) Guidelines for Doctors. Making the best use of clinical radiology services. Doses for conventional X-ray examinations are based on data compiled by the Health Protection agency (HPA) from a survey of UK hospitals in 2008. The doses for CT examinations and radionuclide studies are compiled from surveys conducted by the HPA and the British Nuclear Medicine Society.

¹ Royal College of Radiologists. *Making the best use of clinical radiology services*, Version 7.0.2. London: Royal College of Radiologists, 2012.

Plain X-rays

Wilhelm Roentgen discovered X-rays in 1895. X-rays form part of the electromagnetic spectrum, with microwaves and radio waves lying at the low-energy end, visible light in the middle, and X-rays at the high-energy end. They are energetic enough to ionize atoms and break molecular bonds as they penetrate tissues and are therefore called ionizing radiation. Diagnostic X-rays are produced when high-energy electrons strike a high atomic number material. This interaction is produced within an X-ray tube. A high voltage is passed across two tungsten terminals. One terminal (cathode) is heated until it liberates free electrons. When a high voltage is applied across the terminals, the electrons accelerate towards the anode at high speed. On hitting the anode target, X-rays are produced.

The X-ray picture is a result of the interaction of the ionizing radiation with tissues as it passes through the body. Tissues of different densities are displayed as distinct areas, depending on the amount of radiation absorbed. There are four basic densities in conventional radiography: gas (air), fat, soft tissue and fluid, and calcified structures. Air absorbs the least amount of X-rays and therefore appears black on the radiograph, whereas calcified structures and bone absorb the most, resulting in a white density. Soft tissues and fluid have a similar absorptive capacity and therefore appear grey on a radiograph.

Digital radiology

X-ray film is exposed by light photons emitted by intensifying screens sensitive to radiation transmitted through the patient. Storage phosphor technology uses photostimulatable phosphor screens to convert X-ray energy directly into digital signals. The ↑ dynamic range and image contrast of digital radiography, compared with conventional film screen combinations, and the facility to manipulate signal intensity after image capture reduce the number of repeat exposures. This ↑ efficiency and minimizes patient radiation dose. Digital images can be made available on a local network for reporting by a radiologist or for review on a ward-based computer. Picture archiving and communication systems (PACS) are efficient at image production and manipulation and in the storage, retrieval, and transmission of data. PACS facilitates remote radiology reporting and alleviates workflow pressures. The vast majority of imaging studies conducted in the UK are stored, manipulated, and shared via a picture archiving and communication system. PACS has been rolled out to all healthcare organizations over the last decade or more as local, regional, or national implementations with different degrees of connectivity to radiology information systems (RIS), either via a local RIS or shared with other organizations (domain-based). Global challenges to PACS systems include management of ↑ volumes of imaging data and the ability to data-share across a spectrum of healthcare communities. Advantages include inventive ways of providing diagnostic imaging coverage, for instance in remote or scarcely populated communities.

Chest X-ray: useful landmarks

In order to interpret a plain P-A or lateral CXR, some knowledge of chest anatomy and the major landmarks on the film is required. We have highlighted the major bony and soft tissue structures visible on the plain film in order to make it easier to spot abnormalities. Patient positioning for a P-A CXR (see Figs 13.1–13.3) and lateral CXR (see Figs 13.4–13.6) is illustrated below.



Fig. 13.1 Patient position for P-A CXR.



Fig. 13.2 P-A CXR.

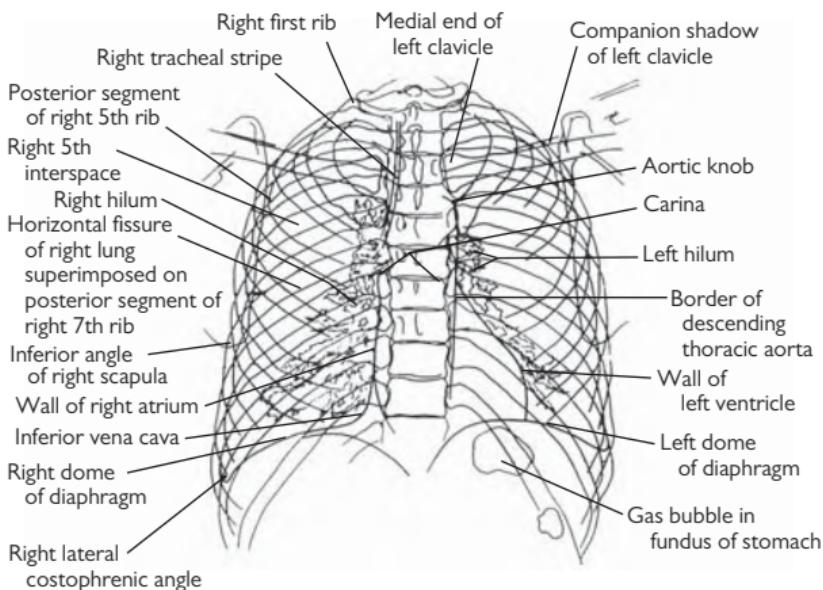


Fig. 13.3 P-A CXR landmarks.

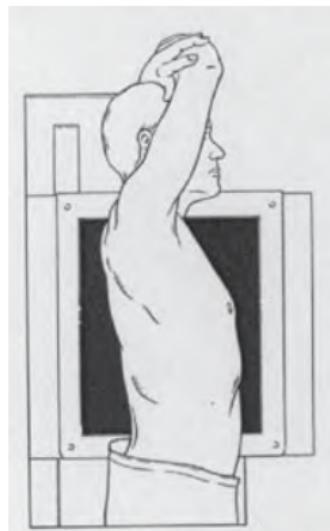


Fig. 13.4 Patient position for lateral CXR.



Fig. 13.5 Lateral CXR.

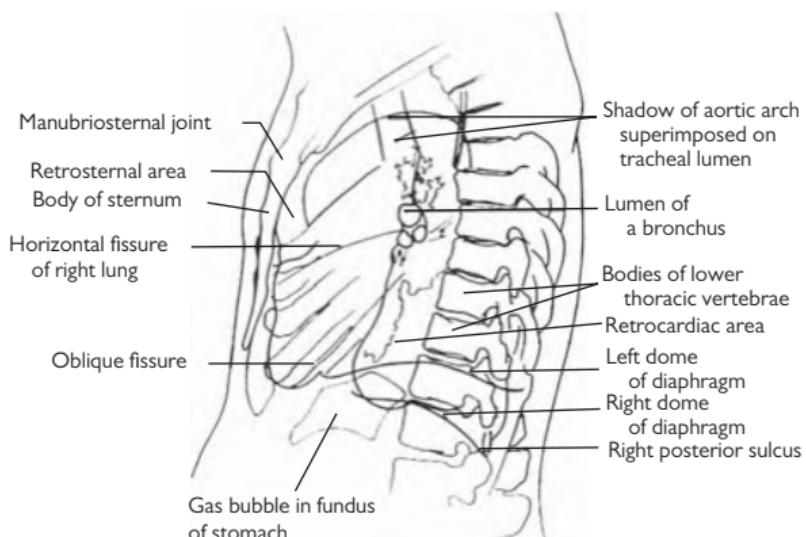


Fig. 13.6 Lateral CXR landmarks.

Chest radiograph

The chest film is the most widely requested, yet most easy to misinterpret, investigation. Using a logical approach will avoid most pitfalls. This should be the initial imaging modality in all patients with suspected thoracic pathology.

Points to consider

- Always obtain prior imaging, if available; temporal changes assist greatly in image interpretation and differential diagnosis.
- Standard projections of the chest are P-A (posteroanterior) vs AP (anteroposterior). See Projection below.
- Additional views to aid problem-solving include lordotic, oblique, and decubitus projections (see below).
- Initially assess the technical quality.

Projection

P-A vs AP will determine whether assessment of the cardiac size is reliable.

Potential other views include:

- Lateral: improves visualization of the retrocardiac space and thoracic spine; earlier and more sensitive detection of effusions.
- Lateral decubitus: assesses for pleural effusion or pneumothorax in immobile patients (portable US also has a utility in this setting).
- Lordotic: angled beam allows better view of the apices which are typically obscured by the clavicles and anterior ribs.

Posture

Erect films enable a more accurate assessment of the mediastinum, since the lungs are more expanded, and allow detection of air–fluid levels, pleural thickening, and comment on the size of the pulmonary vasculature.

Rotation

Look for the relationship of the medial ends of the clavicles to the spinous process at the same level; a common cause of unilateral transradiancy is rotation.

Degree of inspiration

Ideally, six ribs should be seen anteriorly and ten ribs posteriorly. If more, this suggests hyperinflation (does the patient have asthma or COPD?). If less (e.g. poor inspiratory effort, obesity, or restrictive chest disorders), there will be apparent cardiomegaly, ↑ basal shadowing, and less commonly tracheal deviation.

Quality of image can be assessed by the degree of penetration. The thoracic disc spaces should be just visible through the heart. Absence of respiratory or motion artefact.

The heart and mediastinum

Sequentially consider the heart, mediastinum, lungs, diaphragms, soft tissues (breast shadows), and bones. Remember to assess your review areas—the lung apices, behind the heart, under the diaphragm, and the costophrenic angles.

Diaphragm

This should lie between the fifth and seventh ribs. If flattened, consider hyperinflation. In 90% of cases, the right is higher than the left by 3–4cm. Effacement of the interface between the lung and diaphragm suggests pleural or pulmonary pathology. Loss of smooth contour suggests localized herniation (eventration). Peaks laterally may be due to subpulmonary effusion.

Root of neck and trachea

The upper trachea is central with a slight displacement to the right inferiorly due to the oesophagus. Thickening of the paratracheal line (>5mm) may imply nodal enlargement.

CXR in the intensive care unit patient

Look at the following parameters:

1. Patient demographics.
2. Date and time of study should be included in the report.
3. Has there been surgery?
4. If there are lines, catheters, drains, endotracheal tube (ETT), etc., comment on the position. If new lines, comment on changes in position or if any removed.
5. Cardiac and mediastinal size and shape.
6. Presence of pneumothorax.
7. Lung disease. Pattern of disease as well as its evolution over time.
8. Interval changes from one film to the next as well as over time.

Mediastinum

The mediastinum should be central. The heart is normally <50% of the thoracic width. Mediastinal enlargement or widening is a non-specific finding. The silhouette sign may help, but a lateral film is helpful for localization. Table 13.2 gives a list of distinguishing features that enable distinction of mediastinal masses from intra-pulmonary masses.

Normal variants mimicking a wide mediastinum are:

- AP projection (not P-A).
- Mediastinal fat (steroids, obesity).
- Vascular tortuosity—elderly patients.
- Low inspiratory supine position.

Table 13.2 Differentiating mediastinal from pulmonary masses

Mediastinal mass	Pulmonary mass
Epicentre lies in mediastinum	Epicentre in lung
Obtuse angles with lung	Acute angles
No air bronchograms	Air bronchograms possible
Smooth and sharp margins	Irregular margins
Moves on swallowing	Moves with respiration
Bilateral	Unilateral

Table 13.3 Radiographic localization of a mediastinal mass

Location of mass	Signs associated with mass in this compartment
Anterior mediastinum	Deformation of anterior junctional line Hilum overlay sign (hilar vessels seen through mass)
Middle mediastinum	Distortion of the paratracheal stripe Convexity of the AP window
Posterior mediastinum	Distortion or displacement of the following: <ul style="list-style-type: none"> • Azygo-oesophageal recess • Posterior junction line • Paraspinal lines

The mediastinum is divided into three arbitrary compartments to aid in the differential diagnosis of a mediastinal mass (see Table 13.3). As there are no anatomical planes separating these divisions, disease can spread from one compartment to the next.

Based on the location of the mediastinal abnormality, possible pathologies include:

- *Superior mediastinum*: thymoma, retrosternal thyroid, and lymphoma.
- *Anterior mediastinum* (anterior line formed by the anterior trachea and the posterior border of the heart and great vessels): lymphoma (Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL)), germ cell tumours, thymoma, retrosternal goitres, and Morgagni hernias (low).
- *Middle mediastinum* (extends behind the anterior mediastinum to a line 1cm posterior to the anterior border of the thoracic vertebral bodies): aortic aneurysm, bronchial carcinoma, foregut duplication cysts (including bronchogenic/oesophageal), and hiatus hernia.
- *Posterior mediastinum* (posterior to line described above): neurogenic tumours, Bochdalek hernia, dilated oesophagus, or aorta.

Pneumomediastinum

Mediastinal air may be due to a number of sources.

Intrathoracic

- Trachea and bronchi.
- Oesophagus.
- Lung.
- Pleural space.

Extrathoracic

- Head and neck.
- Intraperitoneum and retro-peritoneum.

Signs include: subcutaneous emphysema, pneumopericardium, elevated thymus (sail sign), or air around major structures such as the PmA, bronchial wall.

Enlarged lymph nodes

May be seen in any compartment.

Pleural disease

- Pleural and extra-pleural masses generally form obtuse angles with the adjacent pleura.
- Pulmonary or intra-parenchymal masses form acute angles with the pleura.

Common pleural abnormalities

Effusion

- The lateral view is more sensitive, as accumulation of fluid occurs first in the posterior recess.
- May cause mediastinal/tracheal shift to the contralateral side or adjacent atelectasis.
- US invaluable (and better than plain film) in evaluation of small effusions and guiding thoracocentesis.
- If blunting of the costophrenic angles is present, it indicates the presence of fluid of at least 200mL (P-A) or 75mL (lateral view) or may be 2° to thickening of the pleura.
- Pleural thickening most commonly a sequela of inflammatory change.
- Asbestos exposure results in a spectrum of pleural abnormality, ranging from benign plaques to fibrosis and malignant mesothelioma (obtain occupational history).
- Pleural effusion can be divided into either a transudate or an exudate.
 - Transudate: ultrafiltrate of plasma, low in protein, no inflammatory cells.
 - Exudate: rich in protein, cells, and debris.

Pneumothorax

- On an erect film, the partially collapsed lung is delineated from pleural air as a curvilinear line (visceral pleura) paralleling the chest wall.
- On a supine film, the changes are more subtle; look for the deep (costophrenic) sulcus sign, the double diaphragm sign (the dome and anterior portions of the diaphragm outlined by the lung and pleural air, respectively), hyperlucent thorax, and sharpening of mediastinal structures.
- Subtle pneumothorax will be more readily apparent on an expiratory film or a lateral decubitus film (accumulation of air superiorly).
- If the air is under tension, there may be mediastinal shift (tension pneumothorax). This can result in vascular compromise. On imaging:
 - The lung appears over-expanded.
 - Depression of the diaphragm.
 - Mediastinal shift and of the heart to contralateral side.

Thoracic intervention

Diagnostic thoracocentesis

- *Indication:* exclude malignancy; obtain a sample for culture.
- *USS used to determine skin entry site:* 18–22G needle advanced into pleural fluid. Angle over the superior border of the rib to avoid inadvertent neurovascular injury.
- *Complications:* pneumothorax (when blind, 1–3%).

Therapeutic thoracocentesis

Usually if respiratory compromise from a large effusion. Similar technique as above, but place a 7–10Fr catheter. Potential risk of expansion pulmonary oedema if evacuate in excess of 2–3L or aspirate both lungs in one sitting. Also potential for pneumothorax—always obtain post-aspiration CXR.

Percutaneous lung biopsy

True +ve rate of 90–95%. False +ve results usually related to malplacement of biopsy needle, necrotic tumour. Contraindications (relative) include severe COPD, pulmonary hypertension, coagulopathy, and contralateral pneumonectomy. Tumour seeding is extremely rare (1:20,000).

Complications include pneumothorax (25%, of which 5–10% need a chest tube) and haemoptysis (3%).

Hila

Density should be equal; the left is higher than the right by 5–15mm. If more disparity, consider elevation due to fibrosis (e.g. TB, radiotherapy) or depression by lobar or segmental collapse. Hilar enlargement may be vascular (e.g. pulmonary arterial or venous hypertension) or due to lymphadenopathy (e.g. sarcoidosis, lymphoma, or TB). Hilar calcification is seen in silicosis, sarcoidosis, and treated lymphoma.

Management of pneumothorax

Indications

- Symptomatic pneumothorax.
- Pneumothorax >20%.
- Enlarging pneumothorax on subsequent CXR.
- Tension pneumothorax.
- Poor lung function of contralateral lung disease.

Technique

Either:

- 1 Second to fourth anterior intercostal space, mid-clavicular line, or
- 2 Sixth to eighth intercostal space; mid-axillary line or posterior.

Use 8–12Fr catheters using a trocar technique. After the lung is fully re-expanded for 24h, the catheter is placed on a water seal for 6h and then removed if no residual pneumothorax.

Lung disease

Lung opacities may be subdivided into several basic patterns.

Alveolar

Air space shadowing: ill-defined, non-segmental, and with air bronchograms. No associated volume loss. Large variety of causes:

- Fluid → pulmonary oedema (cardiogenic and non-cardiogenic).
- Fat → fat embolism.
- Haemorrhage → trauma, coagulopathies, pulmonary haemosiderosis.
- Cells → pulmonary alveolar proteinosis, sarcoidosis, bronchoalveolar cell carcinoma, lymphoma, and infection (bacterial, fungal, and viral).

Reticular

Linear opacities: associated obscuration of vessels and late appearance of CXR signs:

- Collagen disorders.
- Extrinsic allergic alveolitis.
- Sarcoidosis, pneumoconiosis.
- CFA.
- Early LVF.
- Malignancy (lymphangitis carcinomatosis).

Nodular shadows

Characterize according to their size and distribution:

- If solitary, exclude tumour.
- Multiple:
 - Granulomata (TB, histoplasmosis, hydatid).
 - Immunological (Wegener's, RhA).
 - Vascular (AVMs).
 - Inhalational (progressive massive fibrosis (PMF), Caplan's syndrome).

Primary signs of a malignancy

- Mass or nodule with spiculated or irregular borders.
- Unilateral hilar enlargement or mediastinal widening.
- Cavitating nodule with thick rind of soft tissue.
- Cavitation commonest in squamous cell carcinoma (SCC).
- Malignancy can simulate air space disease (e.g. bronchoalveolar carcinoma, lymphoma).

Secondary signs of malignancy

- Atelectasis.
- Obstructive pneumonia.
- Pleural effusion.
- Interstitial patterns (lymphangitic spread of disease).
- Hilar and mediastinal adenopathy.
- Metastatic disease (including to ipsilateral or contralateral lung parenchyma).

Further reading

Corne J, Carroll M, Delaney D. *Chest X-ray Made Easy*, 2nd edn. Edinburgh: Churchill Livingstone, 2002.

Hansell DM, Lynch DA, McAdams HP, Bankier AA. *Imaging of Diseases of the Chest*, 5th edn. London: Mosby, 2010.

Patterns of lobar collapse

Lobar collapse may be complete or incomplete. The commonest cause is obstruction of a central bronchus. The 1° signs are opacification due to lack of aeration and displacement of the interlobar fissures. Typical patterns of lobar collapse are illustrated in Fig. 13.7.

Secondary signs include

- Elevation of the hemidiaphragm (more prominent in lower lobe atelectasis than upper).
- Mediastinal displacement (tracheal displacement with upper lobe and cardiac displacement with lower lobe atelectasis).
- Hilar displacement more prominent with upper lobe atelectasis than lower.
- Crowded vessels in the affected lobe.
- Compensatory hyperinflation of remaining lung.

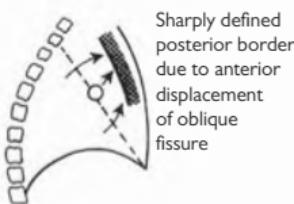
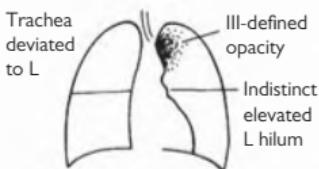
Silhouette sign

- In a normal CXR, the interface between the diaphragm and the mediastinum are visible due to a difference in density between the lung and these structures.
- The silhouette sign refers to loss of normal interfaces, implying there is opacification due to consolidation (the commonest cause), atelectasis, or a mass in the adjacent lung.
- Silhouetting helps to localize the site of the pathology, and both pleural and mediastinal disease produce the silhouette sign (see Table 13.4).

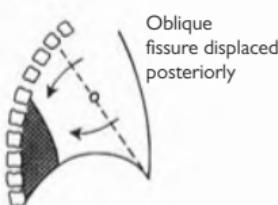
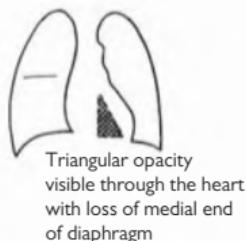
Table 13.4 Localization using the silhouette sign

Interface lost	Location of lung pathology
SVC	Right upper lobe
Right heart border	Right middle lobe
Right hemidiaphragm	Right lower lobe
Aortic knob/left superior mediastinum	Left upper lobe
Left heart border	Lingula
Left hemidiaphragm	Left lower lobe

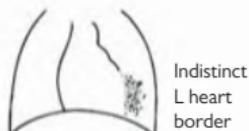
(a) Left upper-lobe collapse



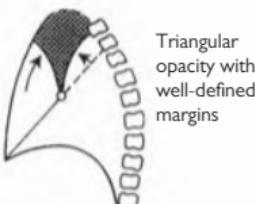
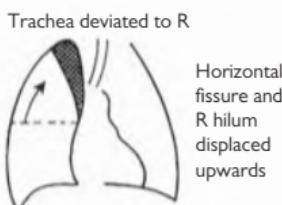
(b) Left lower lobe collapse



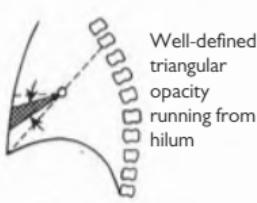
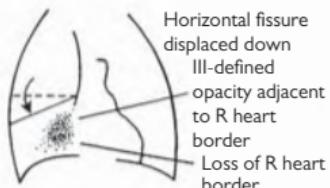
(c) Lingular consolidation



Right upper lobe collapse



(d) Right middle lobe collapse



(e) Right lower lobe collapse

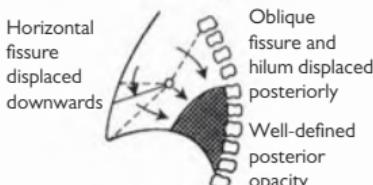
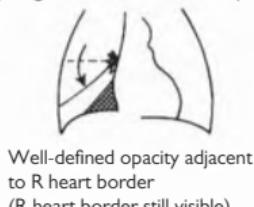


Fig. 13.7 (a) Left upper-lobe collapse. (b) Left lower lobe collapse. (c) Right upper lobe collapse. (d) Right middle lobe collapse. (e) Right lower lobe collapse.

Cardiac enlargement

↑ of the cardiothoracic ratio beyond 50% is considered abnormal if the P-A film is of good quality.

Aetiologies to consider

- Cardiomegaly (hypertrophy or dilatation of cardiac chambers).
- Pericardial effusion (globular heart).
- Poor inspiratory effort/↓ lung volumes.
- Pectus excavatum.

The location of cardiac valves may be relevant when determining the location of calcification. On a lateral projection, draw a line from the xiphisternum to the carina and divide the heart into thirds. The location of the cardiac valves will be as demonstrated in Fig. 13.8.

Normal plain film anatomy

Figure 13.9 shows a P-A view. The right cardiac margin comprises three segments:

- SVC.
- RAt.
- IVC.

The left cardiac margin comprises four segments:

- Aortic arch (AA) (becomes more prominent with age).
- Main PmA at level of the left main stem bronchus.
- Left atrial appendage (may not be visible in normal hearts).
- LV.
- RV is not usually seen in frontal projection.

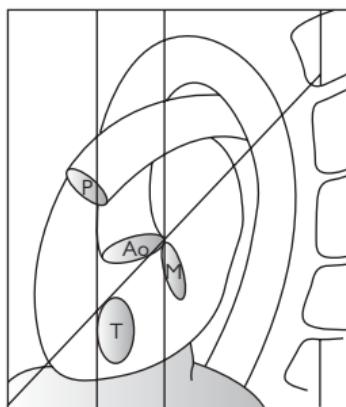
Line positions

Endotracheal tube

- Tip of the ETT should be above the carina and below the thoracic inlet.
- The inflated cuff should not bulge the tracheal wall.
- Neck position can change the impact location of the tip.
- *In neutral*: the tip should be 4–6cm above the carina.
- *Flexed*: moves the tip inferiorly by 2cm.
- *Extended*: moves the tip superiorly by 2cm.
- *Complications*: malpositioned results in atelectasis or collapse due to bronchial obstruction. Tracheomalacia if over-inflated cuff; tracheal rupture may result in pneumothorax.
- *Pearl*: in situations where there is low pulmonary compliance (e.g. acute respiratory distress syndrome (ARDS)), a tip position closer to the carina may reduce barotrauma.

Nasogastric tube

- Tip should be in the stomach.
- Tip and side port should lie distal to the oesophagogastric junction and proximal to the gastric pylorus.
- Potential complications include placement in airway or gastric/duodenal erosion.



P = pulmonary valve Ao = aortic valve
M = mitral valve T = tricuspid valve

Fig. 13.8 Schematic diagram showing the relations of the cardiac valves on a lateral film of the chest.

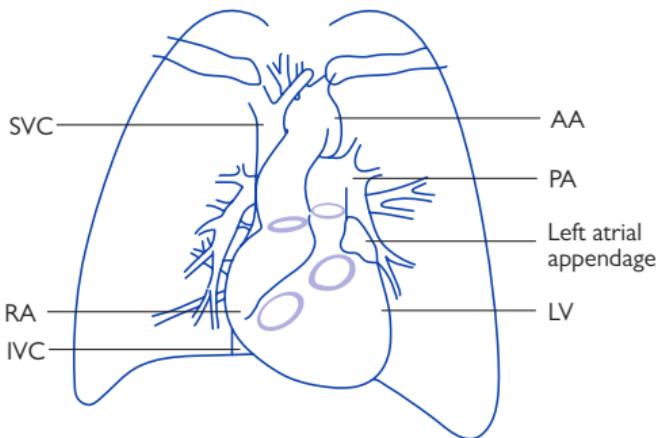


Fig. 13.9 Schematic diagram depicting a normal cardiac silhouette as seen on a plain CXR.

Swan–Ganz catheter

- Tip should be located in the left or right PmA within 1cm from the hilum. Loops in the RA or RV may cause arrhythmias. There are two types of Swan–Ganz catheters:
 - 1 Used to measure wedge pressure.
 - 2 With an integrated pacemaker.
- Complications include pulmonary infarct, haemorrhage, PmA pseudo-aneurysm, and infection. If the tip is distal to the proximal interlobar PmA, there is a potential risk of PmA rupture or pseudo-aneurysm.

Dialysis catheter

- Should be located in the RAt.

Epicardial pacing wire

- Typically anchored in the anterior mediastinum. Multiple wires may be present and usually exit through the anterior chest wall.

Automatic intracardiac defibrillation device (AICD)

- Newer systems have intraventricular electrodes with pin sensors.

Intra-aortic balloon pump

- Tip should be located just distal to the origin of the left subclavian artery and be 2–4cm below the aortic knuckle.
- Complications include aortic dissection, low position associated with mesenteric or renal ischaemia, and high position with CVA.

Central venous line

- Tip should end in the lower SVC or the cavoatrial junction below the anterior first rib. Azygos malposition is seen in 1% and is associated with risk of venous perforation or catheter-associated thrombosis.

Pacemaker

- Typical position should be in the apex of the RV. Can be located in the atrial appendage for atrial pacing and in the coronary sinus for atrial left ventricular pacing.
- Complications include electrode displacement, perforation, infection, or venous thrombosis.

Chest tube

- Side port should lie within the thoracic cavity.
- Tip of tube should not abut the mediastinum.

CT angiography of coronary vessels (coronary computed tomography angiography, CCTA)

CT is very sensitive at:

- Detecting and quantitating calcification in coronary arteries.
- Non-invasively depicting the entire coronary tree.
- Determining the luminal diameter.

Improved hardware has resulted in less blooming artefact which could potentially overestimate the degree of stenosis seen with calcified plaques. It is very sensitive to haemodynamic stenosis (>50% of the luminal diameter). Multiple meta-analyses have shown NPV in high 90s.

For calcium scoring, the examination is performed without IV contrast and there are various algorithms available that provide a measure of the total coronary plaque burden. ECG gating is used to minimize cardiac motion; the type of gating employed has significant impact on patient radiation dose.

Magnetic resonance coronary angiography

Also has potential for non-invasive diagnosis of coronary artery disease. The in-plane image resolution for current MR techniques is about 0.5mm, sufficient for assessment of large coronary vessels as well as in venous grafts following coronary artery bypass grafting, but inadequate for detecting disease in smaller side branches.

Cardiac magnetic resonance imaging

Provides a high-resolution dynamic study using predominantly steady-state free precession (SSFP) and/or gradient echo cine sequences.

SSFP is a 'white blood sequence' that provides excellent contrast between the myocardium and the blood pool. It is suited to cardiac imaging due to its high temporal resolution and excellent contrast.

Cine MRI provides quantitative assessment of cardiac morphology and function. Real-time images can be used for subjective analysis when gating is poor. Tissue characterization is usually performed with double or triple inversion fast spin echo sequences. These 'black blood' sequences null signal from flowing blood.

First-pass contrast-enhanced perfusion MRI is performed pre- and post-vasodilator stress to assess myocardial perfusion. Delayed contrast-enhanced MRI is used to assess myocardial viability, inflammation, and fibrosis.

Further reading

Miller SW, Abbara S, Boxt LB. *Cardiac Imaging: The Requisites*, 3rd edn. Mosby, 2009.

Raff GL, Abidov A, Achenbach S, et al. SCCT guidelines for the interpretation and reporting of coronary computed tomographic angiography. *J Cardiovasc Comput Tomogr* 2009; 3: 122–36.

Computed tomography of the thorax

Indications include

- Evaluation of an abnormal finding on plain film.
- Staging of 1° or metastatic malignancies.
- Evaluation of suspected mediastinal or hilar mass.
- Detection of thromboembolic disease by CTPA (see Figs 13.10 and 13.11).
- Detection and assessment of aortic dissection.
- Distinguishing empyema from lung abscess.
- CT-guided percutaneous needle biopsy of focal lung lesion or mediastinal abnormality.
- CT-guided pleural biopsy.

High-resolution computed tomography (HRCT) comprises thin section images that are reconstructed using a special algorithm.

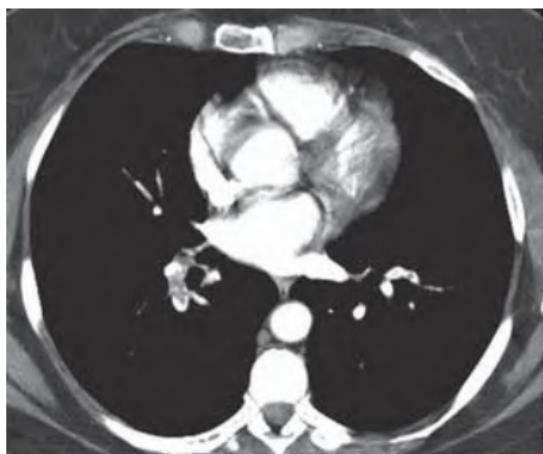


Fig. 13.10 Axial image from CTPA showing the presence of thrombus within the lower lobe PmA.

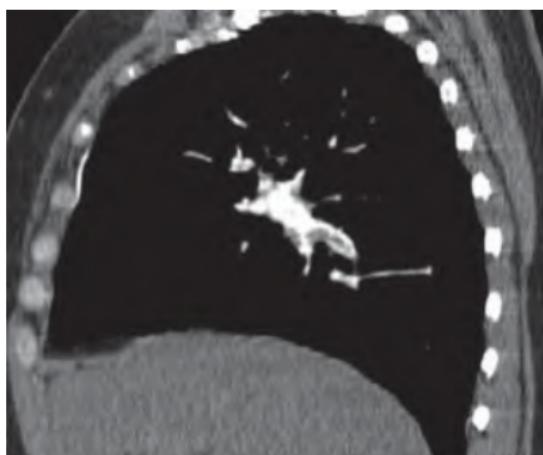


Fig. 13.11 Sagittal reformatted image confirms the extent of the thrombus and its relationship to the lumen.

Indications include

- Evaluation of a diffuse lung disease (see Fig. 13.12).
- Characterization of a solitary pulmonary nodule.
- Haemoptysis.
- Lung disease in a patient with abnormal pulmonary function tests but apparently normal CXR (see Table 13.5).
- Assessment of emphysema.

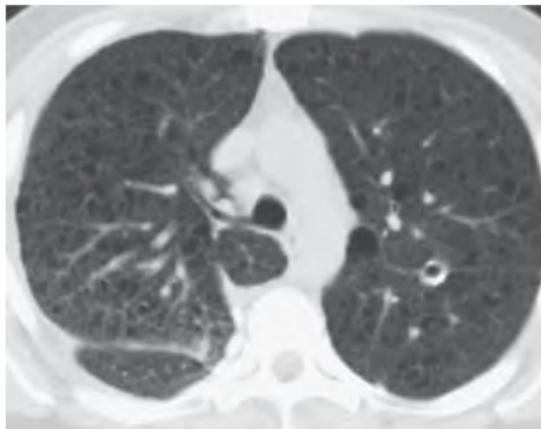


Fig. 13.12 HRCT image of the chest in a patient with cystic lung disease due to tuberous sclerosis.

Table 13.5 HRCT patterns of interstitial lung disease

Pattern	Description	Causes
Ground glass opacity	↑ haze Vessels can be seen through hazing	Allergic hypersensitivity Acute interstitial disease (DIP, active IPF), viral PCP, BOOP, COP, pulmonary oedema, eosinophilic pneumonia
Reticulonodular	Peri-bronchovascular thickening (equivalent of cuffing on CXR) Thickened interlobular septal (Kerley) lines	Pulmonary fibrosis, viral, <i>Mycoplasma</i> pneumonia, PCP Pulmonary oedema, IPF Lymphangitic spread of tumours, fibrosis due to drugs, radiation, asbestos, collagen vascular disease
Nodular opacities	1–2mm interstitial nodules, often seen in conjunction with reticular opacities	Haematogenous infection, metastases, sarcoid, pneumoconiosis, histiocytosis, silicosis, and CWP
Cystic spaces	May or may not have walls	Lymphangioleiomyomatosis Histiocytosis, honeycombing IPF, LIP, cystic PCP End-stage interstitial disease

BOOP, bronchiolitis obliterans organizing pneumonia; COP, cryptogenic organizing pneumonia; CWP, coalworker's pneumoconiosis; DIP, desquamative interstitial pneumonia; IPF, idiopathic pulmonary fibrosis; LIP, lymphocytic interstitial pneumonitis; PCP, pneumocystis pneumonia.

Abdominal X-ray: useful landmarks

Interpretation of the AXR, like the CXR, requires experience. In order to make things slightly easier, we have provided a rough guide to the various bony, soft tissue, and gas shadows seen on a 'typical' AXR (see Fig. 13.13). Figure 13.14 provides a normal AXR for correlation with the diagram. Figure 13.15 shows diffuse sclerotic metastases.

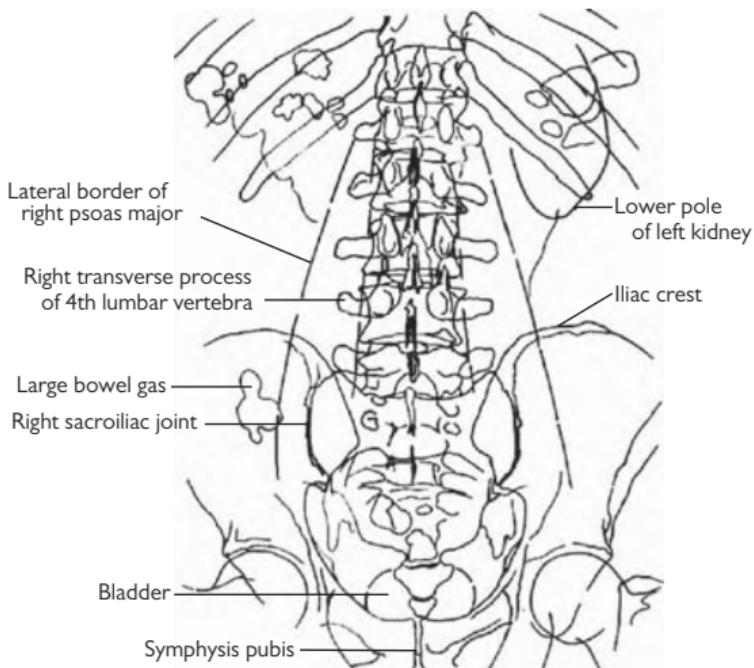


Fig. 13.13 P-A AXR landmarks.



Fig. 13.14 P-A AXR.



Fig. 13.15 Diffuse sclerotic metastases in a patient with a prostatic carcinoma. Note the presence of bilateral ureteric stents.

Plain abdominal X-ray

The standard plain film is a supine AXR. Erect views have largely been superseded and in the acute setting have been replaced by the erect chest to show free subphrenic air. Furthermore, chest diseases, such as MI or pneumonia, may simulate an acute abdomen. If there is doubt regarding the presence of a pneumoperitoneum, consider a lateral decubitus film (displays as little as 1mL of air).

Indications

Suspected obstruction, perforation, renal colic, and toxic megacolon and bowel ischaemia.

Contraindications

None, but where abdominal pain is non-specific and not attributable to the conditions listed above, an AXR is unlikely to be helpful.

Interpretation of the plain abdominal X-ray

A normal patient will have variable amounts of gas in the stomach, small bowel, and colon. You can identify the stomach as it lies above the transverse colon, has an air–fluid level in the erect view, and has rugae in its lumen. Large bowel calibre is variable; 5.5cm is considered the upper limit for the transverse colon in toxic megacolon and 9cm for the caecum in obstruction. Short fluid levels are normal. Fluid levels are abnormal when seen in dilated bowel or if numerous. If the bowel is dilated, distinguish between small and large bowel by the features listed in Table 13.6. Thickening of the bowel wall may be seen in a variety of aetiologies, most notably ischaemia, but also in IBD.

Causes of bowel dilatation include mechanical obstruction, paralytic ileus, or a localized peritonitis (meteorism), e.g. adjacent to pancreatitis or appendicitis (see Table 13.7).

Table 13.6 Distinguishing features between small and large bowel

	Small bowel	Large bowel
Haustra	Absent	Present
Valvulae conniventes	Present in jejunum	Absent
Number of loops	Many	Few
Distribution of loops	Central	Peripheral
Diameter of loops	30–50mm	>50mm
Solid faeces	Absent	May be present
Maximum diameter	3cm	6cm (9cm in caecum)
Maximum fold thickness	3mm	5mm

Table 13.7 Distinguishing mechanical obstruction from a paralytic ileus

Feature	Ileus	Obstruction
Bowel calibre	Normal or dilated	Dilated
Air–fluid levels	Same level in a single loop	Differential levels (stepladder)
Other distinguishing features	Air seen throughout the GIT (diffuse ileus) or in localized ileus may be confined to a short segment	Distension seen to level of transition. Beyond this level, no air in bowel

Look for extraluminal gas

- *Gas in the peritoneal cavity:* look for air under the hemidiaphragm, outlining the falciform ligament or both sides of the bowel wall (Rigler's sign). If there is any doubt, consider a lateral decubitus film. Causes include perforation (ulcer, neoplasm), post-operative, following peritoneal dialysis, or tracking down from the mediastinum.
- *Air in the biliary tree:* following sphincterotomy, gallstone ileus, or following anastomosis of the CBD to the bowel. This has a linear morphology and is seen centrally within the liver.
- *Portal vein gas:* pre-morbid sign in the context of bowel infarction, but less sinister in neonates with necrotizing enterocolitis (NEC) or following umbilical catheterization. In contrast to air in the bile ducts, its location is peripheral.
- *Intramural gas:* linear streaks of air in the bowel wall again usually a sinister finding implying ischaemia, but may be seen due to benign causes such as in patients with COPD where its configuration is more rounded and cystic (pneumatosis cystoides).
- *Air in the retroperitoneum:* delineates the renal shadows and psoas muscle; common causes are trauma, iatrogenic (e.g. after colonoscopic perforation), and after perforation of a duodenal ulcer.
- *Gas in an abscess:* look for displacement of adjacent bowel and an air–fluid level. Other causes include air in the urinary tract and within necrotic tumours. In this scenario, the gas is mottled and does not display features consistent with bowel.
- *Look for any soft tissue masses or ascites:* the latter is detectable on plain films if gross. There will be displacement of the ascending and descending colon from the side walls, with loops of small bowel seen centrally.
- *Look for abdominal or pelvic calcification:* firstly, localize the site. This may require another view. The vast majority are clinically insignificant, i.e. vascular calcification, pelvic phleboliths, and calcified mesenteric nodes. In the abdomen, there may be pancreatic calcification (chronic pancreatitis) or hepatic calcification (old granulomata, abscesses, or less commonly hepatomas and metastases from mucinous 1°s). Gallstones are less commonly calcified and may contain central lucency (e.g. Mercedes Benz sign), whilst renal and ureteric calculi commonly calcify.

Renal tumours and cysts rarely calcify, and more widespread renal calcifications may be seen in nephrocalcinosis due to a wide variety of causes. In the pelvis, ovarian calcifications (less common with malignant masses and seen more often in association with benign pathologies such as dermoids) is uncommon, whilst uterine calcifications due to fibroids commonly occur. Bladder wall calcifications may be seen with bladder tumours, TB, and schistosomiasis. Prostatic calculi and calcifications are common and of no significance. Vas deferens calcifications is seen in patients with diabetes.

- **Soft tissues:** look at renal outlines (normally smooth and parallel to the psoas; should be between 2–3 vertebral bodies). Absence of psoas margins may indicate retroperitoneal disease and haemorrhage.
- **Bones of the pelvis and lumbar spine:** look for OA, metabolic bone disease (hyperparathyroidism, sickle-cell anaemia), the rugger jersey spine of osteomalacia, and Paget's disease (➡ Spinal imaging, pp. 836–7; Pelvis, p. 838). Bony metastases may be lytic or sclerotic.

Barium studies

Barium suspension is made up of small particles of barium sulfate in a solution. Due to its high atomic number, it is highly visible on X-rays. The constituents of individual suspensions vary, depending on the part of the GIT being examined. The particles are coated to improve flow and aid mucosal adhesion. When made up, it comprises a chalky (sometimes unpalatable!) suspension. Advantages include low cost, easy availability, and good assessment of the mucosal surface.

Risks are commoner in the context of

- **Perforation:** if leakage occurs into the peritoneal cavity, it can produce pain and hypovolaemic shock (50% mortality). Long-term sequelae include peritoneal adhesions.
- **Aspiration:** in small amounts, unlikely to have any clinical significance, but if pre-existing respiratory impairment or aspiration of larger amounts (i.e. more than a few mouthfuls), the patient will need physiotherapy.
- **Obscuration:** CT examination in the presence of a recent barium examination will result in a poorly diagnostic study, as high-density barium results in streak artefacts.
- **Barium impaction:** rarely may exacerbate obstruction if barium collects and is concentrated above a point of obstruction.

Water-soluble contrast media

These are more expensive and provide inferior coating and contrast. They include iodinated agents such as Gastrografin®.

Indications for their use include

- Suspected perforation, especially into the peritoneal cavity.
- Meconium ileus.
- To opacify bowel during CT examinations.

Risks include pulmonary oedema if aspirated and hypovolaemia, especially in children. Both are a result of hyperosmolar effects. If aspiration is likely, use water-soluble non-ionic contrast, which causes less shift of body fluid compartments. Non-ionic contrast should be used in all infants (especially neonates) and preoperative patients requiring water-soluble contrast.

Contrast agents

(See Table 13.8.)

For studies other than when barium is being utilized, the contrast media are non-ionic iodinated agents that are given PO, endoluminally, or IV. Non-ionic contrast agents are higher in cost than their ionic counterparts (rarely used currently) but have a lower incidence of adverse reactions (by a factor of 9 for severe reactions). They are typically excreted by glomerular filtration. Half-life is dependent on the dose given, distribution, and renal function. Contrast reactions can be idiosyncratic or anaphylactoid or non-idiosyncratic.

Table 13.8 Pharmacological agents used in barium studies

Agent	Dose	Advantages	Disadvantages
Hyoscine butylbromide	20mg IV	Reduced bowel peristalsis due to smooth muscle relaxant action Immediate onset Short duration of action (15min)	Anticholinergic side effects Contraindicated with CVD and glaucoma
Glucagon	0.3mg IV for barium meal 1.0mg IV for barium enema	More potent smooth muscle relaxant than hyoscine butylbromide Short duration of action, no interference with small bowel transit	Contraindicated with insulinomas or phaeochromocytomas Relatively expensive
Metoclopramide	20mg PO or IV	Gastric peristalsis enhances barium transit during a follow-through study	Possible extrapyramidal side effects

High-risk patients are those with prior contrast reactions, a history of allergy or atopy, sickle-cell disease, phaeochromocytoma, and multiple myeloma (to name a few). Contrast-induced nephropathy is commoner if creatinine is elevated at time of administration or if the patient has a pre-existing renal impairment, e.g. DM.

Pre-medication can be administered to patients with prior contrast reactions. Protocols vary but typically include a corticosteroid and an antihistamine. Consider the use of alternate modalities if risk is high, e.g. USS or MRI, instead of CT with contrast.

Extravasation of contrast is typically treated symptomatically with ice pack and elevation. Plastic surgery consult should be obtained if concern regarding compartment syndrome in patients who have in excess of 100mL in extremity or severe pain/discoloration or altered perfusion.

Gadolinium is an MR-based contrast agent that is paramagnetic. It is also excreted by glomerular filtration.

Its safety profile is favourable in that it does not have any of the nephro-toxicity associated with the iodinated contrast media and is more commonly associated with minor reactions such as headaches. Since 2006, there is an established association between the use of gadolinium-containing contrast agents and NSF. NSF involves fibrosis of the skin, joints, eyes, and other viscera. Subsequent to this finding, the use of gadolinium is contraindicated in patients with an eGFR of under 60 and particularly if below 30.

Barium swallow

Plain films do not usually demonstrate the oesophagus, unless it is very distended, e.g. achalasia. They may be useful in identifying an opaque foreign body within the lumen. The barium swallow is the usual contrast examination to visualize the oesophagus (see Fig. 13.16). Rapid-sequence films are taken with a fluoroscopy unit, whilst the patient swallows barium (usually in an erect position). Films are taken in an AP and oblique projection (to throw the oesophagus clear of the spine), with the oesophagus distended with barium (to demonstrate its outline) and empty to show the mucosal folds.

Normal anatomy

The oesophagus commences at C5/6. There are normal indentations on its outline by the cricoid cartilage, aortic arch, left main bronchus, and left heart.

Indications

These include the assessment of dysphagia, pain, reflux disease, tracheo-oesophageal fistulae (in children), and post-operative assessment where there has been gastric or oesophageal surgery.

Contraindications

No absolute contraindications exist, but in all barium studies, the quality of the study relies heavily on patient co-operation, and therefore immobile patients who are unable to weight-bear may only be suitable for limited studies. The post-operative oesophagus is usually assessed with Gastromiro® or a non-ionic contrast.



Fig. 13.16 Lateral oblique projection during a barium swallow series shows a feline oesophagus. This can be a normal variant but is often associated with GORD.

Common disorders and patterns

- **Diverticulae:** these include pharyngeal pouches (a midline diverticulum), traction diverticulae (due to adhesions), or pseudodiverticulae; dilated mucous glands seen in reflux or infective oesophagitis.
- **Luminal narrowing:** strictures may be benign (e.g. oesophagitis—shown in Fig. 13.17, scleroderma, pemphigus, corrosives, or infection) or malignant.
- **Webs:** mucosal structures, which may be seen anywhere in the oesophagus; seen with skin lesions, e.g. epidermolysis or pemphigus, GVHD, and Plummer–Vinson syndrome.
- **Mega-oesophagus:** can be with associated obstruction, as in malignant strictures, or without as in achalasia, diabetic neuropathy, or Chagas' disease (see Fig. 13.18a–c).
- **Ulceration/oesophagitis:** may be due to GORD, infection, corrosives, or iatrogenic. Findings include lack of distensibility, fold thickening, and mucosal irregularity.
- **Oesophageal tears:** spontaneous, neoplastic, post-traumatic, iatrogenic, and following prolonged emesis. Look for pneumomediastinum, left pleural effusion, and features of mediastinitis.
- **Filling defects:** foreign bodies, varices (proximal due to SVC obstruction), distal (in association with portal hypertension), neoplasms that may be benign (as in leiomyoma) or malignant. Most commonly SCC (95%).
- **Fold thickening:** may be due to oesophagitis, varices, or infiltration by lymphoma.
- **Air–fluid level:** commonest in hiatal hernias, but also seen with a pharyngeal pouch.

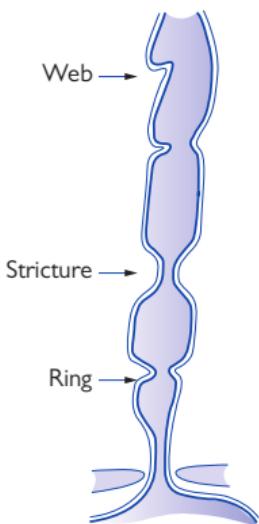


Fig. 13.17 Benign strictures.

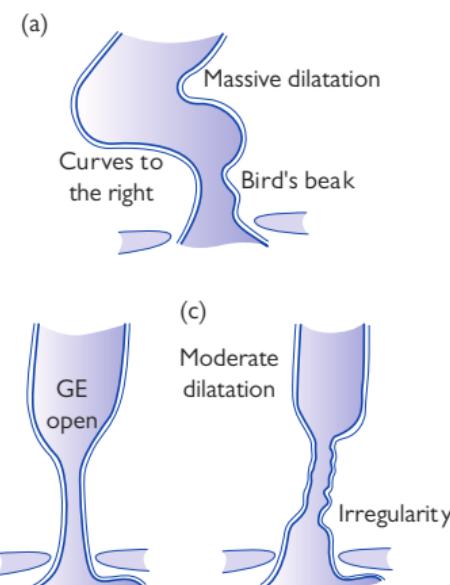


Fig. 13.18 Obstruction in: (a) achalasia, (b) scleroderma, and (c) cancer.

Barium meal

About 200mL of a high-density (85% w/v barium sulfate), low-viscosity barium is used for a double contrast study, which gives good coating without obscuration of mucosal detail. An effervescent agent is given to provide adequate luminal distension. The gastric mucosa is characterized by rugae (parallel to the long axis, 3–5mm thick) and area gastricae (nodular elevations, 2–3mm wide). The patient is fasted for about 6h to avoid food residue, which may cause diagnostic uncertainty. The techniques for coating the stomach and projections are variable. A smooth muscle relaxant may be given as part of the routine, particularly to assess the pylorus and duodenum.

Indications

Dyspepsia, weight loss, abdominal masses, iron deficiency anaemia of uncertain cause, partial outlet obstruction, and previous GI haemorrhage.

Contraindications

Complete large bowel obstruction.

Abnormal findings

- **Filling defects:** these may be intrinsic or extrinsic. Carcinoma remains the commonest cause of a filling defect in an adult (irregular, shouldered with overhanging edges). If there is antral involvement, there may be associated outlet obstruction. Diffuse mucosal thickening and failure to distend is seen with linitis plastica. Other causes include gastric lymphoma, polyps (histology difficult to predict), and bezoars. Smooth filling defects are seen in conjunction with leiomyomas, lipomas, and metastases. Extrinsic indentation by pancreatic tumours or an enlarged spleen may cause an apparent filling defect.
- **Fold thickening (>5mm):** seen in association with hypersecretion states such as Zollinger–Ellison syndrome, gastritis, and Crohn's disease. It may also be 2° to infiltration by carcinomas, lymphomas, or eosinophilia.
- **Outlet obstruction:** may be diagnosed by failure of the stomach to empty <50% of the barium ingested at 4h. This may be seen in carcinomas, but also in scarring caused by chronic duodenal ulceration.
- **Hiatal hernia:** herniation of the stomach into the chest occurs via the oesophageal hiatus in the diaphragm. There are two types—in a sliding hernia (commoner), there is incompetence of the sphincter at the cardia, often associated with reflux. Other sequelae include oesophagitis, ulceration, or stricture. In a rolling hernia, the fundus herniates through the diaphragm, but the gastro-oesophageal junction remains competent and lies below the diaphragm.
- **Gastritis and ulceration:** gastritis is characterized by small, shallow barium pools with surrounding lucent rings due to oedema. There are features that may be used to distinguish benign from malignant ulcers on barium studies. Ulcers are seen either as a crater or as a projection from the luminal surface (see Fig. 13.19). Benign ulcers



Fig. 13.19 Double contrast barium meal showing an ulcer crater.

are commonly seen on the lesser curve with smooth radiating folds, which reach the edge of the ulcer crater. Malignant lesions may have an associated mass and have a shallow crater and an irregular contour. With the ease of availability of endoscopy, the use of barium meals in diagnosing ulceration has declined. Endoscopy has the advantage of being able to diagnose gastritis more accurately, assess ulcer healing, make a histological diagnosis, and more accurately assess the post-operative stomach. However, early assessment of the post-operative stomach is radiologically performed to exclude complications such as anastomotic leaks. A water-soluble contrast agent is preferred in the early post-operative phase.

Small intestine

Small bowel studies are performed for indications such as occult bleeding, recurrent obstructive symptoms, and malabsorption, and to confirm and define the extent of small bowel disease in Crohn's.

- *Small bowel follow-through:* the patient drinks 200–300mL of barium (with metoclopramide to speed transit time). The single contrast column is followed by films at regular intervals until the barium reaches the colon. Transit time is variable, but the entire process may take 1–6h, depending on the adequacy of bowel preparation. Films are taken at intervals of ~20min initially, in the prone position, which aids separation of the loops. When the barium reaches the caecum, spot views of the terminal ileum are taken.
- *Small bowel enema (enteroclysis):* this technique provides better demonstration of mucosal detail, as there is rapid infusion of a continuous column of barium directly into the jejunum. Methylcellulose is administered following the barium to provide double contrast. This is achieved via a weighted nasogastric tube which is positioned at, or distal to, the duodenojejunal (DJ) flexure. Disadvantages include poor patient tolerance (related to intubation) and a relatively high screening dose.

Both techniques require the patient to be on a low-residue diet beforehand.

Indications

The indications are the same for both techniques and include pain, diarrhoea, bleeding, partial obstruction, malabsorption, overgrowth syndromes, assessment of Crohn's disease activity and extent, and suspected masses. The small bowel enema may be preferred for assessment of recurrent Crohn's disease or complex post-operative problems, but the small bowel follow-through is otherwise routinely used.

Contraindications

Complete obstruction and suspected perforation.

Normal findings

The small intestine measures ~5m and extends from the DJ flexure to the ileocaecal valve. The proximal two-fifths is the jejunum; the distal three-fifths is the ileum. Normal calibre is 3.5cm for the jejunum and 2.5cm for the ileum (up to 1cm more on enteroclysis). The valvulae conniventes are circular in configuration, and ~2mm thick in the jejunum and 1mm thick in the ileum (see Fig. 13.20).

Abnormal findings

- *Dilatation* is indicative of malabsorption, small bowel obstruction (SBO), or paralytic ileus. There may be accompanying effacement of the mucosal pattern. When seen with fold thickening, it may be due to Crohn's, ischaemia, or radiotherapy. Mucosal thickening may be due to infiltration by lymphoma or eosinophilia, adhesions, ischaemia, or radiotherapy.
- *Strictures* are seen in Crohn's disease and lymphoma. There is usually dilatation of the bowel proximally. Crohn's disease causes skip lesions, ulceration, strictures of variable length, and a high incidence of terminal ileal involvement. There may be associated ulceration, fold thickening, and fistulation (see Fig. 13.21).
- *Malabsorption:* radiological investigation may reveal an underlying structural abnormality. The findings in malabsorption include dilatation, fold thickening, and flocculation of barium.

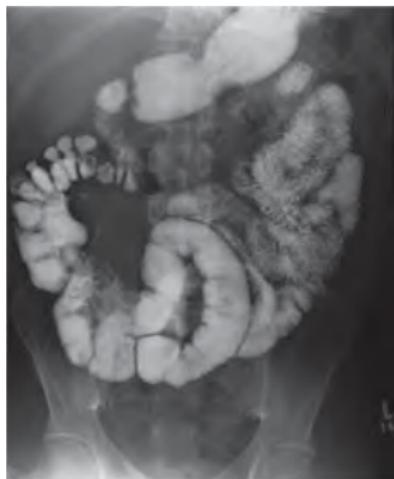


Fig. 13.20 A 30min film in a small bowel follow-through series showing a normal mucosal fold pattern.

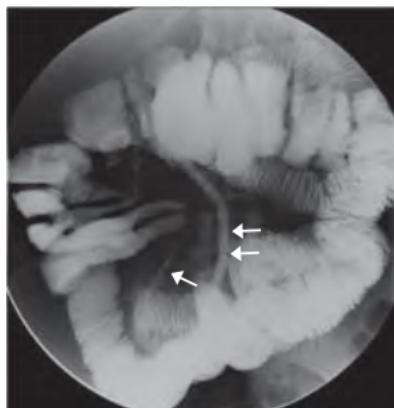


Fig. 13.21 Small bowel enema showing at least two strictures (arrows) in this patient with Crohn's disease. Compare the mucosal detail with the small bowel follow-through.

CT enteroclysis

This is a hybrid technique that combines fluoroscopic intubation and small bowel infusion with an abdominal CT. This can be performed with +ve enteral contrast or neutral enteral contrast.

The advantage of this technique is that both intra- and extraluminal/extra-enteric information is obtained, rendering it superior to many of the per-oral small bowel studies. Disadvantages include the minimally invasive nature and the radiation associated with a CT examination. CT enteroclysis is complementary to capsule endoscopy in the elective investigation of small bowel disease and should be particularly considered in Crohn's disease, small bowel obstruction, and unexplained GI bleeding. There has been a paradigm shift to CT enterography for the assessment of small bowel disease. This is most relevant in the IBD population who are on disease-modifying drugs.

Cholangiography

Ultrasound

This is the 1° modality for assessment of the biliary tree and for exclusion of pancreatic pathology. With the current scanner resolution, the sensitivity for stone disease is in the range of >95% for stones exceeding 2mm in diameter. For choledocholithiasis (stones in the CBD), the sensitivity falls to around 50%, and MRCP or ERCP is more helpful.

Intravenous cholangiography

This is rarely performed but may be useful in patients with biliary symptoms post-cholecystectomy or with a non-functional gall bladder. It is contraindicated in the presence of severe hepatorenal disease, as the side effects related to the contrast media are considerable. CT cholangiography uses a similar contrast agent but offers the advantage of cross-sectional assessment of the bile ducts. It is often used when MRCP has not helped delineate the anatomy in donors prior to liver transplantation or when MR is contraindicated or simply not available.

Endoscopic retrograde cholangiopancreatography

The biliary and pancreatic ducts are directly filled with contrast, following endoscopic cannulation and during X-ray screening. This has both a diagnostic and therapeutic role. It is particularly of value in the demonstration of ampullary lesions and to delineate the level of biliary tree obstruction in patients with obstructive jaundice. It allows sphincterotomy to be performed to facilitate the passage of stones lodged in the CBD.

Percutaneous transhepatic cholangiography

The biliary tree is directly injected with contrast, following percutaneous puncture of the liver. This is both diagnostic in defining a level of obstruction and therapeutic in biliary duct obstruction, as it may be used as a precursor to a biliary drainage procedure or prior to insertion of a stent. Contraindications include bleeding diatheses and ascites.

Other cholangiographic techniques

- *Per-operative cholangiogram:* in which the CBD is filled with contrast during cholecystectomy to exclude the presence of CBD stones.
- *T-tube cholangiogram:* after operative exploration, a T-tube is left in the CBD for a post-operative contrast study to exclude the presence of retained stones.
- *MRCP:* this is a non-invasive technique where heavily T2-weighted (T2W) images are obtained without contrast administration. The bile acts as an intrinsic contrast agent, and stones are visualized as filling defects. The entire biliary and pancreatic ductal system can be visualized (see Fig. 13.22). Common indications for this technique include unsuccessful ERCP, a contraindication to ERCP, as well as evaluation of the post-surgical biliary tree.

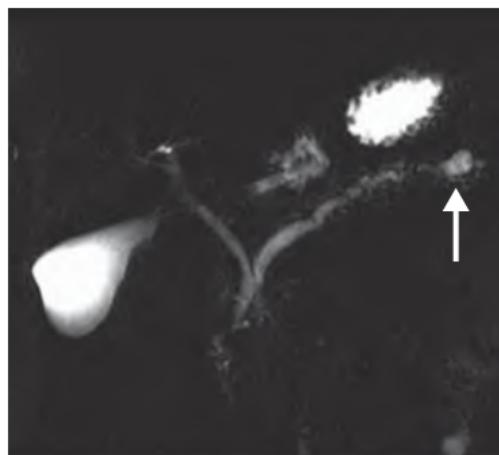


Fig. 13.22 Heavily T2-weighted slab image showing an irregular beaded pancreatic duct in this patient with chronic pancreatitis. There is a small pseudocyst in the tail (arrowhead).

Magnetic resonance cholangiopancreatography

- Non-invasive.
- Cheaper.
- Uses no radiation.
- Requires no anaesthesia.
- Less operator-dependent.
- Allows better visualization of the ducts proximal to the level of obstruction.
- When combined with conventional sequences, allows detection of extraductal disease.

Disadvantages

- ↓ spatial resolution for peripheral intra-hepatic ducts and for pancreatic ductal side branches (e.g. as in pancreatitis).
- Subtle ductal lesions may be difficult to appreciate, as ducts are imaged in the non-distended physiological state.
- Inability to perform therapeutic endoscopic or percutaneous intervention of obstructing bile duct lesions.
- MRCP is comparable with ERCP in the detection of obstruction, with a sensitivity and specificity of 91 and 100%, respectively.
- Causes of filling defects are usually stones, air, tumours, blood, or sludge.

Contrast-enhanced MRCP can also be performed with fat-saturated T1-weighted (T1W) imaging after injection of gadolinium contrast agents that have biliary excretion. These cause T1 hyperintensity with bile but require a 20–45min delay prior to imaging to allow for biliary excretion.

Liver disease

US is the modality of choice for initial screening, whether assessing the parenchyma for diffuse disease or trying to evaluate and/or characterize focal liver lesions. Although US is sensitive in depicting focal lesions, it is not specific and there can be overlap in the imaging characteristics of benign and malignant lesions. CT, and particularly MRI, are more tissue-specific in characterizing liver lesions. The liver is supplied predominantly by the portal venous system (80%). On CT, the differing phases of enhancement are utilized to assess a lesion.

Arterial phase images (20–30s after injection) ↑ the conspicuity of lesions that are hypervascular such as hepatocellular carcinoma or focal nodular hyperplasia. Portal venous phase images are acquired at 50–70s and provide maximum enhancement of background hepatic parenchyma. Lesions that are relatively hypovascular on this phase stand out such as metastases.

Delayed imaging (equilibrium phase) minutes after contrast administration allows lesions that demonstrate relative washout of contrast (i.e. appear hypo-attenuating) relative to background liver, such as hepatocellular carcinomas, to stand out. Lesions that are relatively fibrotic (e.g. in tissue content or scars) conversely exhibit ↑ enhancement on delayed images.

MRI displays the same patterns of contrast enhancement but has superior lesion-to-liver contrast and imparts no ionizing radiation. Multiple dynamic post-contrast sequences can thus be obtained. Tissue characterization allows for detection of intralesional lipid (as in adenomas), and advanced techniques such as diffusion-weighted imaging (DWI) improve sensitivity for lesion detection.

Further characterization of lesions can be obtained by hepatocellular-specific MRI contrast agents which allow definitive assessment of lesions with hepatocytes such as focal nodular hyperplasia (FNH), precluding the need for biopsy.

Barium enema

This is used for evaluation of the large bowel. Increasingly, many institutions are replacing this technique with CT colonography (CTC;  Virtual colonoscopy, p. 807) or conventional CT, depending on the clinical indication. Barium is run into the colon under gravity via a tube inserted into the rectum. The column of barium is followed by air (room air or CO₂) to achieve double contrast. The CO₂ is better tolerated and more readily absorbed. Hyoscine butylbromide (a smooth muscle relaxant) may be given to minimize spasm and optimize mucosal relief. Bowel preparation prior to the examination (low-residue diet and aperients) is vital to ensure that there is no faecal material, which may mask mucosal abnormalities or be mistaken for small polyps. Remember the examination is uncomfortable and requires reasonably good patient co-operation and mobility.

► Do not request this in frail or elderly patients, unless there is a good clinical indication.

A rectal examination or sigmoidoscopy is essential to avoid abnormalities being missed.

Single vs double contrast

If evaluation of the colonic mucosa is not the 1° aim, then a single contrast technique will suffice. This is applicable in children where the patient is uncooperative and where gross pathology is being excluded, and in the evaluation of obstruction/volvulus or in the reduction of an intussusception.

Indications

Change in bowel habit, iron deficiency anaemia, abdominal pain, palpable mass of suspected colonic origin, and weight loss of unknown cause.

Contraindications

Suspected perforation, recent rectal biopsy, toxic megacolon, or pseudo-membranous colitis.

Common findings

- *Solitary filling defect:* polyps are classified according to histology. The commonest are hyperplastic (no malignant potential); adenomatous polyps are premalignant with the risk of malignancy ↑ with size (<5mm = 0%, >2cm = 20–40%). Also found are adenocarcinoma (↑ risk in ulcerative colitis, polyposis syndromes, villous adenoma) and less commonly metastases and lymphoma.
- *Multiple filling defects:* polyps (polyposis syndromes or post-inflammatory pseudopolyps), pneumatisis coli, metastases, and lymphoma.
- *Ulceration:* IBD, ischaemia, infection, radiation, and neoplasia.
- *Colonic narrowing:* neoplasms (apple core lesion), metastases, lymphoma, diverticular disease, IBD, ischaemia, and radiation.
- *Dilatation:* mechanical, e.g. proximal to neoplasm, volvulus or non-mechanical, post-operative ileus, metabolic, and toxic megacolon.
- *Diminished haustration:* cathartic colon, IBD, and scleroderma.

- ↑ *hastration* (thumbprinting): ischaemia, haemorrhage, neoplasm, and IBD.
- *Widening of the pre-sacral space* (>1.5cm at S2): normal in up to 40%, but also seen in association with IBD, neoplasms, infection, and sacral/pelvic lipomatosis.

Colonoscopy

Remains a complementary technique and has the advantage of being both therapeutic and diagnostic (e.g. biopsy, polypectomy, etc.). In elderly patients, CT with prior bowel preparation and air insufflation is less invasive and less arduous.

Virtual colonoscopy

Helical CT images of distended colon taken during a breath-hold are used to obtain 2D or 3D images of the colon. Images are acquired in the supine and prone positions to assess lesional mobility (and thus distinguish stool from polyps). No IV contrast is administered for routine screening studies, and the examination is often performed utilizing a low-dose technique. Recent studies using 1° 3D interpretation, as well as national studies, such as the ACRIN II trial, have shown sensitivities in the range of 94% for polyps of at least 1cm in diameter and around 88% for lesions measuring at least 6mm. Current refinements in this technique include the use of computer-assisted detection (CAD) to improve performance, as well as the use of prepless techniques (i.e. the patient does not have to undergo prior bowel cleansing).

(See Figs 13.23 and 13.24.)



Fig. 13.23 Standard supine projection from a double contrast barium enema (DCBE) showing a normal large bowel.



Fig. 13.24 Supine DCBE showing a tight stricture in the transverse colon in this patient with IBD.

Further reading

Pickhardt PJ, Choi JR, Hwang I, et al. Computed tomographic virtual colonoscopy to screen for colorectal neoplasia in asymptomatic adults. *N Engl J Med* 2003; 349: 2191–200.

Radiology of the urinary tract

Plain abdominal film

- *Look for any urinary tract calcification:* 90% of stones are radio-opaque. Other causes include hyperparathyroidism, medullary sponge kidney, and RTA.
- *Renal outline:* between T12 and L3 and 10–15cm. Left bigger and higher than the right.
- *Assess bones of spine and sacrum:* for bony metastases or spina bifida (may be relevant in enuresis).

Intravenous urogram

This provides a good overview of the urinary tract and, in particular, the pelvicalyceal anatomy. Fluid restriction and laxatives are no longer necessary and, in particular, the former is to be avoided in diabetics, renal failure, and myeloma. Following the preliminary plain film, 300mg/kg of contrast media is injected IV. The film sequence is varied according to the clinical scenario. An immediate film shows the nephrogram phase and displays the renal outlines. An increasingly dense delayed nephrogram is seen in acute obstruction, acute hypotension, ATN, and renal vein thrombosis. A faint persistent nephrogram is seen with acute glomerulonephritis and it may be delayed in RAS. Later films show the pelvicalyceal systems (pyelogram), ureters, and bladder.

Common abnormalities

(See Fig. 13.25.)

- *Loss of renal outline:* congenital absence, ectopic kidney, tumour, abscess, or post-nephrectomy (look for absent twelfth rib).
- *Small kidney (unilateral):* ischaemia (RAS), radiation, or congenital hypoplasia.
- *Small kidney (bilateral):* atheroma, papillary necrosis, or glomerulonephritis.
- *Large kidney (unilateral):* duplex, acute pyelonephritis, tumour, or hydronephrosis.
- *Large kidney (bilateral):* polycystic kidneys and infiltrative disease such as myeloma, amyloid, and lymphoma. Acute inflammation such as glomerulonephritis, ATN, and collagen vascular disease.
- *Pelvicalyceal filling defect:* smoothly marginated (clot, papilloma), irregular margins (tumour, e.g. renal cell or transitional carcinoma), intraluminal (sloughed papilla, calculus, or clot), extrinsic (vascular impression or cyst), irregular renal outline (scarring, e.g. in ischaemia, TB, pyelonephritis, or reflux nephropathy).
- *Dilated ureter:* >8mm in entire length. May be due to obstruction (functional as in 1° megaureter) or mechanical stenosis as in ureteric or urethral stricture and in reflux disease.
- *Ureteric stricture:* wide differential; determine the length. Differentials include tumour (transitional cell carcinoma, metastatic), inflammatory (TB, schistosomiasis), congenital, trauma (radiation or iatrogenic).

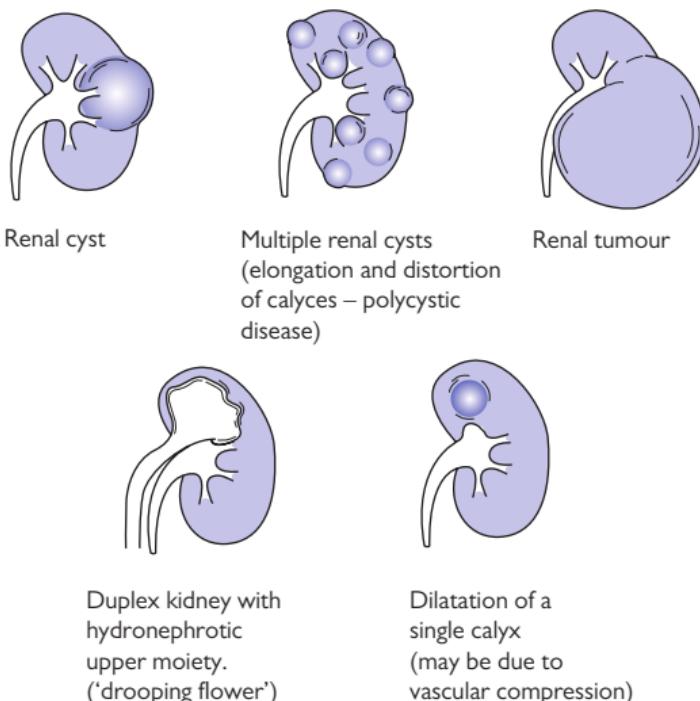


Fig. 13.25 Common patterns of abnormalities seen on IVU.

- *Deviated ureters*: normal course of ureters in close proximity to transverse processes of vertebral bodies.
- *Lateral deviation*: seen with retroperitoneal nodes, tumours, and aortic aneurysm.
- *Medial deviation*: posterior bladder diverticulum, retroperitoneal fibrosis (can be idiopathic or related to various aetiologies, including malignancy).

Computed tomography in genitourinary pathology

CT is the preferred method for assessment of many pathologies within the genitourinary (GnU) tract, including trauma, complex infections, renal and adrenal masses, neoplastic disease, retroperitoneal processes, renovascular hypertension, and in renal colic.

Computed tomography urography

In many institutions, IVU has been replaced by its CT counterpart (CT urography (CTU)). MDCT has had an impact on slice thickness and speed of scanning, such that the urinary tract mucosa can be assessed in exquisite detail. Depending on institutional protocol, the examination is performed as a 2- or 3-part study. Typically, it includes a non-contrast phase (to assess for stones, acute blood) and then an excretory/delayed phase to assess

the collecting systems and ureters. It has a high sensitivity (95%) in detecting upper urinary tract uroepithelial malignancies. Common indications for usage include:

- Haematuria (with –ve cystoscopy and USS having excluded parenchymal causes).
- Unexplained hydronephrosis on USS.
- Evaluation of the upper tract in patients with known lower urinary tract transitional cell carcinoma or following trauma or iatrogenic ureteric injury.

Ultrasound

May be used as an alternative or complementary examination with IVU and may be used to:

- Demonstrate or exclude hydronephrosis, especially in ARF.
- Evaluate renal tumours, cysts, and abscesses.
- Follow up transplant kidneys and chronic renal disease.
- Assess renal blood flow using Doppler.
- Perform serial scanning in children with recurrent UTIs.
- Assess bladder morphology and volume, and the prostate.
- Provide guidance for interventional techniques, e.g. renal biopsy and nephrostomy placement.

Computed tomography and magnetic resonance imaging

CT is more accurate for staging renal tumours, assessing retroperitoneal pathology, staging bladder and prostatic tumours, and assessing renal vascular pathology (such as RAS). In many centres, unenhanced CT is replacing IVU as a gold standard for assessment of renal stone disease. It is more accurate at depicting stone burden than IVU and precisely demonstrating the level and cause of obstruction in the acute setting. Evaluation of ureteric pathology in the context of haematuria is also being performed with contrast-enhanced CT.

MRI is valuable in staging vascular involvement by renal carcinomas. Dedicated pelvic coils and endoluminal coils show excellent results in staging pelvic and gynaecological malignancies.

Micturating cystourethrogram

Following catheterization of the bladder, contrast is introduced till bladder capacity is reached. This is the technique of choice for defining the urethral anatomy and gauging the presence/degree of vesicoureteric reflux in children. It is also used if there are recurrent UTIs or suspected lower urinary tract obstruction.

Ascending urethrography

Contrast is injected directly into the urethra in ♂ in the assessment of urethral trauma, strictures, and congenital anomalies such as hypospadias.

Retrograde pyelography

The ureters are catheterized (usually following cystoscopy in theatre) and contrast injected under X-ray screening. Of value in urothelial tumours and to define the site of obstruction, e.g. non-opaque calculi. Useful if IV techniques have failed to demonstrate the intra-renal collecting system or ureters due to impaired renal function or a high-grade obstruction.

Angiography

A femoral approach with selective catheterization of renal vessels. Main uses include haematuria (look for AVMs), hypertension (RAS), in transplant donors (to define anatomy), and in renal cell carcinoma (where embolization is being contemplated).

Nephrostomy

➔ Interventional radiology, pp. 844–7.

Breast imaging

Breast cancer is a common problem (1 in 12 women). The average ♀ has a 1 in 8 chance of being diagnosed with breast cancer during her lifetime. Mammography is the first-line tool for detection of breast cancer; however, sensitivity of screening mammogram is variable and is influenced by variables such as density of breast tissue. Sensitivity is between 68 and 90% and is higher if the patient is symptomatic (93%).

Screening mammography detects 2–8 cancers per 1000 women screened. Since 1990, mortality from breast cancer has steadily declined, and this has been attributed to advances in adjuvant therapy as well as to mammographic screening.

Mammography

Technical factors

Breast tissue has a narrow spectrum of inherent densities, and in order to display these optimally, a low-kilovoltage (kV) beam is used. It enhances the differential absorption of fatty, glandular, and calcific tissues. Dedicated mammographic units provide low-energy X-ray beams with short exposure times. The breast is compressed to minimize motion and geometric unsharpness. High resolution is paramount in order to detect microcalcification (as small as 0.1mm). The breast is a radiosensitive organ, so doses need to be kept to a minimum.

Standard projections

These are the mediolateral oblique (MLO) and craniocaudal (CC) views (see Fig. 13.26). The CC view is trans-axial. The MLO image plane is ~45–60° from the axial plane and parallels the pectoralis major. Adequacy of the lateral oblique view may be gauged by the pectoralis major muscle, which should be visible to the level of the nipple, inclusion of the axillary tail,

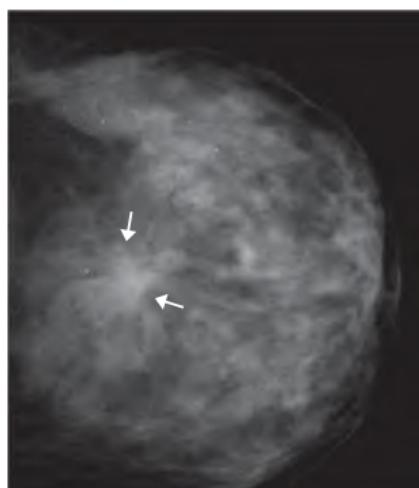


Fig. 13.26 CC mammographic view of the breast. The arrows depict a stellate mass consistent with a carcinoma.

and inclusion of the inframammary fold. The CC view detects posteromedial tumours that may be missed on the MLO view and is better at breast compression. Additional projections, such as true lateral, cleavage, exaggerated CC, spot compression, and magnification views, may be used to clarify abnormalities. These techniques provide better detail and disperse any overlapping tissue to avoid obscuration of lesions.

Mammographic signs

The breast parenchyma is made up of glandular tissue in a fibrofatty stroma. Cooper's ligaments form a connective tissue network. The amount of glandular tissue ↓ with age; as it is dense on mammography, the suitability of the technique for detecting pathology ↑ with age.

Systematic evaluation of a mammogram

- Adequacy of study; are additional views required?
- Adequate penetration of fibroglandular tissue.
- Skin, nipple, trabecular changes.
- Presence of masses.
- Calcifications.
- Axillary nodes.
- Asymmetry (may be a normal variant).
- Architectural distortion.

Comparison with prior imaging is imperative, as changes can be subtle and progressive.

Additional technical adequacy

- There should be adequate tissue demonstrated on both CC and MLO views. The posterior nipple line is a line drawn from the posterior nipple to the pectoralis muscle. On each view, the posterior nipple line should be within 1cm of each other.
- Image should be free from blur and artefacts.
- Nipple should be in profile on at least one view.
- Blurring can cause benign calcification to look amorphous, and subtle calcification may be missed.

Primary signs of a malignancy

- A mass with ill-defined or spiculate borders (see Fig. 13.27).
- Clustered, linear, or irregular calcification (which may occur in the absence of a mass).
- 2° signs include distortion of adjacent stroma, skin thickening, and nipple retraction.

Ninety-four per cent of breast carcinomas is ductal in origin.

The breast imaging and reporting data system (BIRADS) is a standardized way of reporting mammography and includes a lexicon, a structured report, and clear categories for follow-up and reassessment of imaging findings.

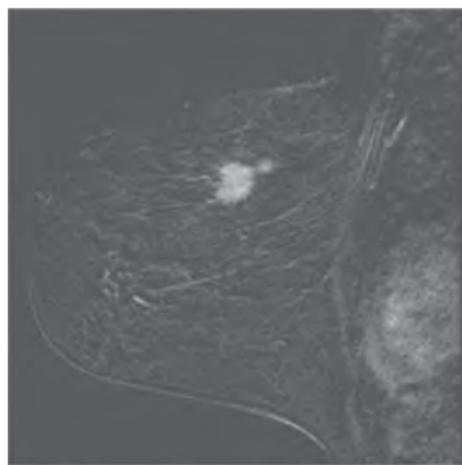


Fig. 13.27 Subtraction sagittal MR image of the breast following gadolinium, showing an enhancing spiculated mass consistent with malignancy.

Breast ultrasound

This largely forms a modality for assessment, not diagnosis or detection, and is a valuable adjunct and problem-solving tool. It can be used to evaluate non-palpable masses and palpable masses not seen on mammography, to determine the internal architecture (solid vs cystic), to assess asymmetric density, to assess breast implants, and as a 1° imaging modality in young women (<35 years), as well as pregnant and lactating women. It is also used as a tool to guide intervention, i.e. drainage of cysts and biopsy of suspicious lesions.

Magnetic resonance imaging

MRI remains a problem-solving tool in breast imaging. Widespread implementation of MRI (for instance, for screening) is hampered by its low specificity (37–97%). It can ↑ the number of benign biopsies that are performed. This clearly has resource implications, as well as generating unnecessary patient anxiety. Both MRI and US may be used to evaluate implants and their integrity, but MRI is the only modality that is sensitive in the evaluation of intracapsular implant rupture. Contrast-enhanced MRI of the breast is also a sensitive method for detection of malignancy, with reported sensitivities in the region of 93%. It is especially useful to detect recurrent breast carcinoma and where conventional techniques are unable to help in the distinction from more benign lesions. Breast MRI is also being advocated for screening young patients with a family history/genetic risk of breast carcinoma.

Breast MRI is increasingly being used for staging breast carcinomas, to look for synchronous 1° lesions, and to evaluate the breast in patients found to have malignant nodes in the axilla. It can also be used to evaluate tumour response to neo-adjuvant chemotherapy. In problematic mammographic patients, it can be useful in distinguishing dense breast tissue or fibrosis from malignancy. In the post-operative setting, it can be used in patients with +ve surgical margins or to assess post-operative scar vs disease recurrence. The selection of pulse sequences and IV contrast administration is based on the clinical indication.

The patient lies prone on the scanner, and a specialized coil surrounds the breast. The entire scan varies in duration from 20min to 1h. Most protocols for exclusion of malignancy rely on a dynamic enhanced sequence. Cancers typically enhance more rapidly than benign lesions.

Ultrasound

US is a high-frequency mechanical vibration produced by piezoelectric materials, which have the property of changing thickness when a voltage is applied across them. It is an important cross-sectional modality and has widespread applications in the abdomen, neck, pelvis, and extremities. At diagnostic levels, there are no known damaging sequelae to tissues, and therefore it is safe for use in obstetrics, providing invaluable imaging of the developing fetus.

Probe selection is dependent on the area being imaged. High-frequency probes provide greater resolution but have limited depth of penetration and may therefore be suitable for assessment of superficial structures (e.g. extremity US, thyroid, testicular US). Doppler USS is based on the principle that sound reflected by a moving target has a different frequency to the incident sound wave. The frequency shift is proportional to the velocity of the flowing material. Doppler therefore not only enables detection but also quantification of velocity.

Indications

USS is cheap, readily available, and non-invasive, and has high patient acceptability. It has a wide range of applications as listed below. There are also no radiation implications. Again, advances in technology have resulted in vast improvements in the resolution of this modality, such that subtle pathology is more readily identifiable. The portability of USS also lends itself to use in the setting of emergency and critically ill patients, as well as providing guidance for intra-operative procedures.

Contraindications

None, but remember that USS is operator- and patient-dependent and should be used as a problem-orientated modality, not as a total body survey. It cannot be used to image air-containing structures or bone. The resolution of the USS image is inversely related to the depth of penetration. Therefore, image quality in obese patients is suboptimal.

Applications

- *Head and neck:* may be used for evaluation of the salivary glands, thyroid, lymph nodes, and palpable or clinically suspected masses. Doppler is used to assess the carotid vessels and quantify the degree of stenosis/occlusion.
- *Chest (excluding breast):* the use here is limited to palpable chest wall lesions, assessment of pleural abnormalities, biopsy, and drainage of pleural effusions, and is occasionally of use in directing a biopsy of peripheral lung or mediastinal masses.
- *Abdomen and pelvis:* this is the main use of USS. Useful for assessment of solid organs, e.g. kidneys, spleen, gall bladder (see Figs 13.28 and 13.29), liver (see Fig. 13.30), pancreas, uterus/adnexae, and bladder. A full bladder is used as an acoustic window in the pelvis. Retroperitoneal masses and lymph nodes may be visible, depending on patient habitus. USS is useful for directing biopsy of solid organs/masses and for drainage of ascites, abscesses, and collections.



Fig. 13.28 Trans-axial view of the gall bladder on US demonstrating a soft tissue mass in the lumen, suspicious for carcinoma. There is cholelithiasis, a common coexistent entity. The arrow shows an abnormal interface with the liver parenchyma, suggestive of local infiltration.

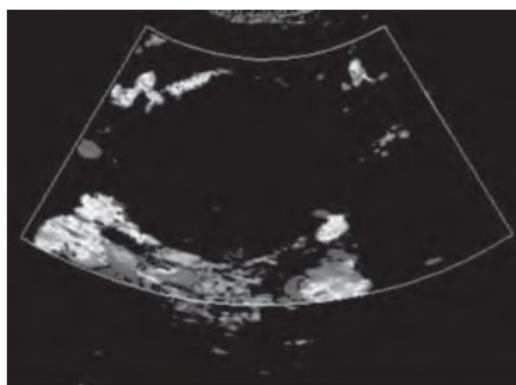


Fig. 13.29 Colour Doppler image in the same patient showing abnormal vascularity in the wall of the gall bladder (→ Colour plate 5.)



Fig. 13.30 Dilated intra-hepatic ducts seen in this axial view of the liver.

- *Limbs:* musculoskeletal USS has been revolutionized by advances in high-frequency probes, which enable characterization of soft tissue masses, tendon-related pathology, rotator cuff lesions, masses, effusions, and collections. The dynamic nature of the examination allows the diagnosis of functional pathological conditions and is also used to guide aspiration and lavage. Assessment of superficial lumps and masses can be performed as a first line before triaging to other modalities such as MRI. It is also used for vascular assessment and the diagnosis of DVT.
- *Intracavitory transducers:* these place the transducer as close as possible to the area of interest. They include transvaginal, transrectal, urethral, oesophageal, and intravascular probes. They are usually high-frequency transducers that produce detailed high-resolution images. Transvaginal USS is more invasive than transabdominal scanning but is used in the routine assessment of gynaecological disorders. It can also be used for infertility monitoring, egg retrieval, and the exclusion of suspected ectopic pregnancy. Transrectal scanning is used for screening, assessment, and biopsy of suspected prostatic pathology, as well as rectal pathology including staging rectal cancers. Endo-anal probes may be used to assess morphology and characterize tears of the anal sphincter.
- *Contrast agents:* US contrast agents are available as an additional tool in diagnosis, although currently used primarily in academic centres. These are micro-bubbles, which are stable over a period of time, and may be used to improve anatomical detail, assess tubal patency (hysterosalpingography), assess tumour vascularity, and characterize focal masses (e.g. within the liver), and for contrast enhancement.

Obstetric imaging

US is the 1° imaging modality in obstetric imaging, with MRI being used for problem-solving. USS is performed via both the transabdominal and transvaginal approach. Imaging is never performed in isolation and should always be performed in conjunction with clinical information, such as the patient's menstrual status (including date of last menstrual period), presence/absence of pain and vaginal bleeding, as well as knowledge of biochemical parameters including serum β-hCG when available.

Role of imaging

First trimester

- Confirm an intrauterine pregnancy (IUP).
- Confirm the presence of a gestational sac and date the pregnancy.
- Determine the fetal number and placenta.
- Exclude ectopic gestation.
- If there is bleeding, assess the viability of pregnancy (possible aetiologies in this scenario include a normal IUP, impending abortion (missed, incomplete, or impending), ectopic pregnancy, or a subchorionic bleed).
- 8- to 12-week gestational age dating US (most accurate form of pregnancy dating).
- Measurement of crown–rump length (margin of error \pm 5 days).
- Change the estimated date of confinement (EDC) to US date if >5 days discrepancy from EDC based on last menstrual period.
- 11- to 14-week gestational age nuchal translucency US (assess fluid behind the fetal neck); early screen for trisomy 21.

Second trimester

- Determine the fetal number and viability.
- Locate and assess placental morphology.
- Estimate the volume of placental fluid.
- Assess gestational age and evaluate growth (margin of error \pm 10 days).
- Fetal survey.
- Assess the cervix and look at adnexae.

Third trimester

- Fetal presentation (cephalic, breech).
- Type of placenta.
- Assess the cervix and os.
- Biophysical profile and serial growth.

Amniocentesis

Typically performed at 15–16 weeks with US guidance. Indications include advanced maternal age, abnormal biochemical markers (triple screen or AFP), and a history of genetic/chromosomal disorders. CVS typically performed earlier (10–12 weeks) and also under imaging guidance using the transabdominal or transcervical approach.

Table 13.9 Transvaginal scan landmarks (accuracy \pm 0.5 week)

Age	β -hCG	Gestational sac	Yolk sac	Heartbeat	Embryo (fetal pole)
5 weeks	500–1000	+	–	–	–
5.5 weeks	>3600	+	+	–	–
6 weeks	>5400	+	+	+	–
>6 weeks		+	+	+	+

- **Gestational sac:** the product of implantation and is usually visible within the uterus at 2–3mm.
 - Normal mean sac diameter (MSD, mm) + 30 = days of pregnancy.
 - Normal landmarks (transvaginal scan) (see Table 13.9).
 - MSD >8mm: yolk sac should be visible.
 - MSD >16mm: heartbeat should be present (crown–rump length >5mm).
- **Other criteria worrisome for abnormal pregnancy:**
 - Irregular sac contour.
 - Crown–rump length of <7mm and no heartbeat.
 - Mean sac length of 16–24mm and no embryo.
 - Absence of embryo \geq 6 week after last menstrual period.
 - Empty amnion.
 - Enlarged yolk sac of >7mm.
- **Small gestational sac** also has a high risk of subsequent pregnancy loss (>90%). MSD (mm) to crown–rump length (mm) <5mm indicates loss of pregnancy
- **Empty sac:** one without a yolk sac or embryo. May represent a very early IUP (if MSD <8mm) or an embryonic pregnancy (if MSD >8mm), or a pseudo-gestational sac as seen in ectopic pregnancy.

A full review of obstetric imaging is beyond the scope of this chapter.

Gynaecological imaging

USS remains the modality of choice for initial assessment of pelvic pathology in ♀ and can be used to assess uterine morphology and endometrial thickness, to exclude focal uterine pathology such as leiomyomas (fibroids), and for initial assessment of adnexal pathology.

Normal endometrium is echogenic with a surrounding hypo-echoic halo. Thickness is variable, depending on the stage of the menstrual cycle and the patient's menstrual status (e.g. pre- or post-menopausal). Typical values range from <4mm in the menstrual phase, 4–8mm in the proliferative phase (up to day 14 of the cycle) to 7–14mm in the secretory phase.

The post-menopausal uterus is typically atrophic and may be modified by the administration of exogenous hormones (hormone replacement therapy (HRT)), which will also influence endometrial thickness. Normal thickness <5mm if no HRT usage.

Hysterosalpingogram

- *Indications:* for assessment of infertility, to define uterine anatomy, and to evaluate tubal patency as a precursor for *in vitro* fertilization or for evaluation of congenital anomalies.
- Procedure performed at days 6–12 of menstrual cycle. Foley catheter inserted into the cervical canal, and contrast hand-injected to define the above. Complications include pain and infection. Contraindications are active infection, pregnancy, or recent uterine surgery.

Pelvic magnetic resonance imaging

Indications

Include locating and confirming the presence of leiomyomas (often pre- and post-uterine fibroid embolization (UFE);  Interventional radiology, pp. 844–7), confirming the presence of adenomyosis, endometriosis, in the assessment of congenital uterine anomalies, as well for the assessment of complex pelvic or adnexal masses.

T2W imaging of the uterus defines the zonal anatomy and is invaluable in staging neoplasms. MRI is the modality of choice for tissue characterization and in this setting will demonstrate small quantities of blood products (as in endometriosis plaque), as well as showing tissue content such as intra-lesional fat (dermoids).

Computed tomography

This technique differs from conventional radiography in that it is able to visualize a vast spectrum of absorption values, and therefore tissue densities. Furthermore, being a tomographic technique, the resultant image is essentially 2D and overcomes the problem of confusing overlap of 3D structures on plain film. The image is a grey-scale representation of the density of tissues (attenuation), as depicted by X-rays. Each image is made up of a matrix of squares (pixels), which collectively represent the attenuation values of tissues within that volume (voxel). With conventional CT, separate exposures are made for each slice. Current scanners can acquire data in a continuous helical or spiral fashion, shortening the acquisition time and reducing artefacts caused by patient movement. This improves the overall throughput and ↑ the likelihood of a diagnostic scan, particularly in unco-operative patients. The volumetric data that are acquired may be manipulated by image processing and displayed in a variety of techniques, including 3D reformats and 'virtual' endoscopy.

The attenuation values are expressed on an arbitrary scale (Hounsfield units), with water being 0, air being -1000 units, and bone +1000 units. The range of densities displayed on a particular image can be manipulated by altering the window width and level. This also allows discrimination of tissues that differ in density by as little as 1%.

Prior to scanning the abdomen or pelvis, dilute oral contrast is given to opacify the bowel. IV contrast is given to aid the problem-solving process and differentiate vascular-enhancing lesions from surrounding tissue.

Multislice CT scanners are third-generation scanners with helical capabilities and low-voltage slip rings, which acquire anywhere between 64 and 320 slices (and counting!) per X-ray tube rotation.

Dose management has become more of a concern with the ↑ utility of CT across a spectrum of pathologies. The dose is dependent on a number of patient- and scan-related variables, including patient habitus, the volume (area) scanned, the number(s) of acquisitions, as well as the desired resolution and image quality.

To address this problem, there have been innovations in reconstruction to minimize the dose without impacting on noise and including instances where data are incomplete. These include adaptive statistical iterative reconstruction. There is minimal, if little, impact on spatial or contrast resolution.

Indications

There are a wide variety as detailed below. CT is often the most diagnostic cross-sectional examination and more definitive than USS in many instances.

Contraindications

Due to the relatively high radiation dose, CT should be avoided in pregnancy. Artefact from indwelling, high-density foreign material, e.g. hip prosthesis, dental amalgam, and barium, may limit the diagnostic quality of the examination. Claustrophobia is less of a problem, compared to MRI.

Applications

- **CNS/spine:** CT remains the tool for 1° diagnosis, pre-surgical assessment, treatment monitoring, and detection of relapse in many CNS disease conditions. MRI is superior in the posterior fossa and parasellar region and for assessment in MS, epilepsy, and tumours. Where MRI is not available, it is useful for assessment of degenerative spinal and disc disease. It is superior to MRI in the assessment of head injury. In the context of trauma, MRI is only utilized when CT is –ve despite strong clinical suspicion. CT is also used as the 1° modality in the evaluation of acute stroke and in the emergency settling prior to LP of patients suspected of CNS infection such as meningitis.
- **Orthopaedics/trauma:** uses include diagnosis and staging of bony and soft tissue neoplasms and assessment of vertebral, pelvic, and complex extremity trauma (e.g. tibial plateau fractures). It is also used in the detection of loose bodies, in the assessment of acetabular dysplasia, and in providing an answer in joint instability (especially in shoulders, wrists, and elbows where it may be performed as an adjunct to/in conjunction with conventional arthrography).
- **Oncology/radiotherapy:** staging of solid tumours, treatment planning, and the detection of relapse. CT is of particular value in obtaining whole body scans in oncology due to the speed and ease of use with the advent of multislice CT. CT is used for radiotherapy treatment planning to allow more precise targeting of treatment.
- **Chest:** indications include the staging of bronchogenic carcinomas, characterization of solitary nodules, diffuse infiltrative lung disease, widened mediastinum/mediastinal masses, and pleural abnormalities. With multislice CT, pulmonary angiography has advanced the diagnosis of PE, particularly when V/Q scanning is indeterminate or equivocal. Helical CT is equivalent to formal angiography in the detection of emboli within proximal arteries of < fifth/sixth generation. Sensitivity (80–100%, specificity 78–100%).
- **Abdomen:** applications include the diagnosis of abdominal pathology, which may be of traumatic, neoplastic, inflammatory, or infective origin (see Figs 13.31–13.34). CT is particularly useful for masses, pancreatic and hepatic disease, detection of the site and nature of obstructive jaundice, and the assessment of abdominal trauma. It is also used in the pre-surgical assessment of abdominal aneurysms (see Fig. 13.35) and as an aid to interventional techniques (➔ Interventional radiology, pp. 844–7).



Fig. 13.31 Direct coronal reformat through the liver showing findings consistent with 1° sclerosing cholangitis. Note the markedly thickened bile duct wall (arrows).



Fig. 13.32 Free intraperitoneal air 2° to perforation of a gastric ulcer. Note the thickened antrum of the stomach.



Fig. 13.33 Virtual barium enema image reconstructed from CT colonoscopy. The arrow shows the location of the polyps.

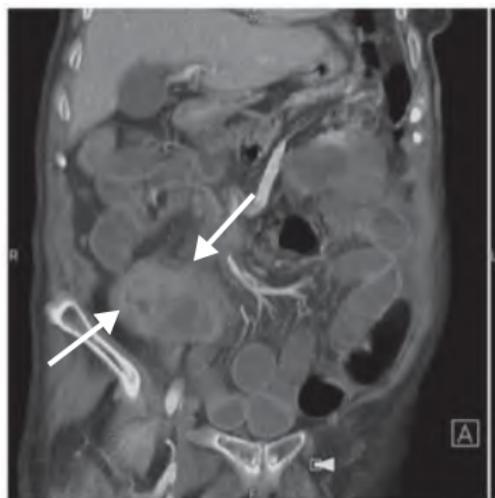


Fig. 13.34 Reformatted maximum intensity projection MIP image of an abdominal CT showing small bowel obstruction due to the presence of a caecal carcinoma (arrows).

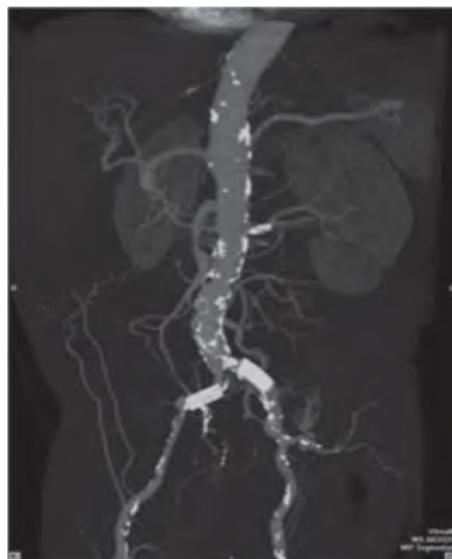


Fig. 13.35 Reformat of a CT angiogram to assess the extent of RAS in this patient who has bilateral common iliac stents.

Magnetic resonance imaging

This is a non-invasive technique, which displays the internal structure, whilst avoiding the use of ionizing radiation. The nuclei of certain elements align with the magnetic force when placed in a strong magnetic field. These are usually hydrogen nuclei in water and lipid (at clinical field strengths), which resonate to produce a signal when a radiofrequency pulse is applied. When the radiofrequency pulse is switched off, the protons return to their pre-excitation axis, giving off the energy they absorbed. The energy can be measured with a detector and used by a computer to display anatomical information. The T1 and T2 signal characteristics of common tissue types are seen opposite (see Table 13.10). Further discussion of the physics is beyond the scope of this chapter. Inherent tissue T1 and T2 characteristics depend on the longitudinal relaxation (T1) and transverse relaxation (T2) times of the protons in that tissue. Pathologic processes alter the relaxation times of the tissue and will produce signal abnormalities.

MR image weighting (e.g. T1W or T2W images) depends on the repetition time (TR) and echo time (TE) used to obtain images.

Table 13.10 Examples of sequences used in clinical practice

Pulse sequence	Acronyms	Clinical applications
Conventional spin echo	SE	Workhorse sequences. Therefore, integral to most protocols
Multi-spin echo	TSE, HASTE, RARE	Reduced scan time, but similar applications to SE
Inversion recovery	FLAIR, STIR	Used to suppress certain tissues by nulling their signal Therefore, high net signal results in ↑ conspicuity of pathology, e.g. infarcts, perilesional oedema, MS plaque STIR invaluable in marrow imaging to show oedema, to assess optic nerves
Gradient echo	FLASH, SPGR, FISP, FIESTA, many more	Uses gradient to rephase signal Reduced scanning time Useful for showing acute blood, in cardiac imaging with ECG gating Can also be used in functional imaging Blood O ₂ level (BOLD)-dependent sequences
Echoplanar imaging	EPI	Generates multiple quantities of data (k-space) in short time, so potential for real-time/interventional MRI Applications include cardiac, abdominal imaging

T1-weighted images

- Contrast is due to inherent T1 relaxation.
- Provides good anatomical information.
- Fat is displayed as high signal (white).
- Distinction between cystic (black) and solid structures is possible.
- Good evaluation of marrow signal.
- The sequence of choice when evaluating enhancement, as gadolinium administration makes structures of even higher signal intensity on T1W images (see Tables 13.10 and 13.11).

T2-weighted images

- Technique of choice for evaluating pathology.
- Fluid is of high signal and therefore optimally displays oedema.
- Improved soft tissue contrast allows evaluation of zonal anatomy of organs such as the uterus and prostate.

Magnetic resonance angiography

MRI principles are used to exploit the properties of flowing blood. Images generated display structures containing flowing blood, with suppression of all other structures. These principles can be further modified, so that only vessels with flow in a specific direction (i.e. arteries vs veins are) visualized. MRA is currently being used in the evaluation of suspected cerebrovascular disease, renovascular disease, and peripheral vascular disease.

Functional imaging

MRI techniques have evolved, such that diffusion imaging utilizes the diffusion of water protons in the diagnosis of evolving ischaemia. This technique shows how movement of water molecules is impeded by cytotoxic oedema of ischaemic cells. These are manifested by signal changes that show early evidence of cerebral ischaemia prior to structural changes becoming apparent.

Diffusion MRI comprises two separate components—DWI and apparent diffusion coefficient (ADC) which are interpreted together to evaluate the diffusion characteristics of tissue. DWI has had a profound impact in the

Table 13.11 MR signal intensity of common tissues

Tissue or body fluid	T1 signal	T2 signal
Air	Nil	Nil
Bone or calculi	Nil	Nil
Fat	High (bright)	Medium to high
Proteinaceous fluid (e.g. abscess or complex cyst)	Medium	High
Muscle	Low	Low to medium
CSF, urine, bile, or oedema	Low	High
Blood (depends on age), hyperacute (oxyHb), chronic (haemosiderin)	Low/low	High/low

assessment of cerebral infarcts and is ~95% sensitive and specific for infarcts within minutes of onset of symptoms. The ADC map shows pure diffusion information without any T2 weighting. Reduction in diffusion is therefore of low signal (hypointense) on the ADC map. The *b*-value is an important concept for impacting sensitivity for the detection of diffusion abnormalities. The higher the *b*-value, the more contrast the image provides for detection of reduced diffusion. DWI is also increasingly used in the oncological setting both at the time of staging but also for evaluation of treatment response.

Similarly, early ischaemia of the myocardium is detectable on MRI. This has great therapeutic potential, as early treatment may prevent the establishment of ischaemia and result in overall improvement of ventricular function and survival. MRA is also being used to depict coronary vessel disease non-invasively.

Perfusion imaging

An advanced technique where the brain is repeatedly imaged as a bolus of gadolinium (contrast) is injected. Gadolinium causes a magnetic field disturbance which results in a transient reduction in image intensity. As these are echo-planar T2*images, they can be acquired quickly and are invaluable in imaging of strokes and tumours. The signal changes associated with gadolinium can be plotted over time for a selected brain volume. The time signal plots can be manipulated to obtain parameters related to cerebral perfusion.

Indications

The indications are legion and continue to grow. There are a wide variety of indications, summarized below (☞ Applications, pp. 830–2). MR is especially useful in imaging the brain, spine, peripheral limbs, and joints, neck, and pelvis. Again, improvements in scanner hardware and software have had huge impact on clinical practice. The prior limitations of long scan times have been overcome by robust sequences that can be performed in a breath-hold. This means that respiratory and peristaltic artefacts are no longer an issue when imaging in the chest and abdomen.

Contraindications

These largely apply to patients with magnetically susceptible devices or materials whose movement or loss of function can have deleterious consequences. These include cardiac pacemakers, metallic fragments, and prosthetic heart valves. Relative contraindications include pregnancy (especially the first trimester) and claustrophobia. MRI magnets are relatively confined and even those who are not normally claustrophobic may be provoked.

Applications

- **The spine:** MR imaging is superior to other techniques in displaying anatomy and is the technique of choice in assessing disc disease and the post-operative back, in evaluating neural compression (benign or malignant), in imaging acute myelitis and infection (such as discitis or osteomyelitis), and in excluding marrow infiltration. Contrast is helpful in assessing the post-operative back to distinguish scar from residual herniation, as well as for confirming extruded fragments. It also helps confirm the presence of neuritis 2° to a disc herniation.

- **CNS:** imaging of the CNS is used to evaluate mass lesions, hydrocephalus, white matter disease, leptomeningeal pathology, cerebrovascular disease (see Figs 13.36, 13.37, 13.38,), degenerative disorders, and visual and endocrine disorders such as pituitary dysfunction. In trauma/acute haemorrhage, CT is the preferred technique (Neuroradiology, pp. 856–9).
- **Paediatric:** the uses here include assessment of perinatal trauma/anoxic injury, congenital anomalies, and developmental delay. Within the spine, it is invaluable in the assessment of spinal dysraphism and progressive scoliosis.

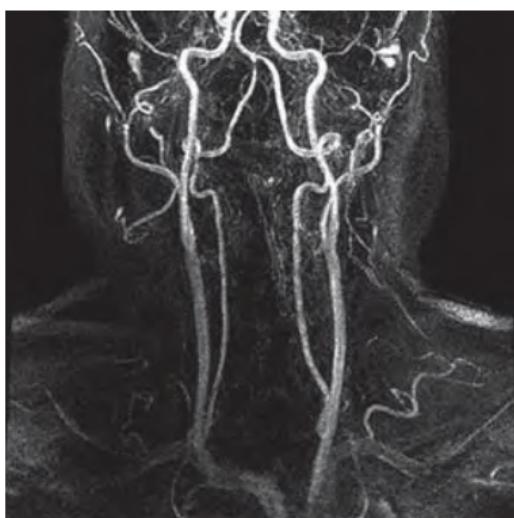


Fig. 13.36 Axial T2W shows acute infarction in the right cerebellar hemisphere.

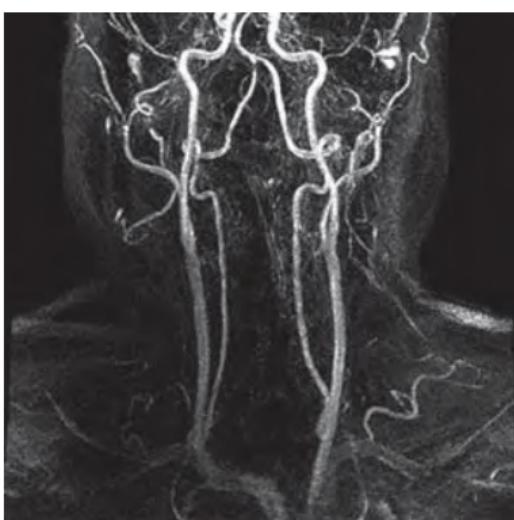


Fig. 13.37 Coronal reformatted MRI image showing a tight stenosis of the distal vertebral artery on the right.

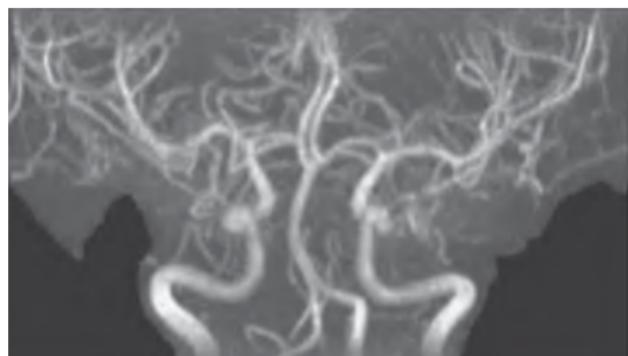


Fig. 13.38 AP coronal MRA showing mild irregularity of the carotid siphon on the left. The images are examined in multiple projections to exclude anomalies such as stenosis or small aneurysms.

- **Musculoskeletal:** along with CNS disease, this is a major component of the MRI workload. It has revolutionized musculoskeletal imaging and is used to characterize meniscal pathology, ligamentous injury, and degeneration and sequelae of trauma in the knee, shoulder, wrist, and ankle. Further uses include imaging mass lesions, assessing the extent of infection, and diagnosing early avascular necrosis (AVN).
- **Chest/cardiac:** within the thorax, MRI is useful for assessment of apical lesions such as Pancoast's tumours (see Fig. 13.39), chest wall and brachial plexus lesions, and mediastinal masses. Cardiac applications are legion and fast-evolving: they include imaging of the great vessels to exclude congenital/acquired aortic disease (including dissection) and the diagnosis of PE.
- **Abdominal/pelvic MRI:** within the abdomen, MR is often a problem-solving tool and can be used to more confidently characterize focal liver and pancreatic lesions, as well as assess diffuse liver disease. It is also of use in evaluating indeterminate adrenal masses. Within the pelvis, uses include imaging of congenital anomalies, as well as staging tumours such as cervical (see Figs 13.40 and 13.41), prostate, and rectal tumours. There have been rapid advances in techniques for imaging bowel-related pathology.
- **Interventional MRI:** open MRI units image patients in large-bore or C-shaped units, rather than the closed narrow tunnel used in conventional units. They can therefore be used for claustrophobic patients and to provide imaging guidance for interventional procedures. Disadvantages include a low magnetic field strength (0.1–0.3T vs 1.5T) and a limited anatomical and spatial resolution due to their basic construct.

Recently, short-bore magnets have been developed that combine the accuracy of a tunnel scanner and the comfort of an open MRI scan. Although they are not completely open, they are much less constrictive because of the short-bore magnet (shorter tunnels) but can produce a high field.

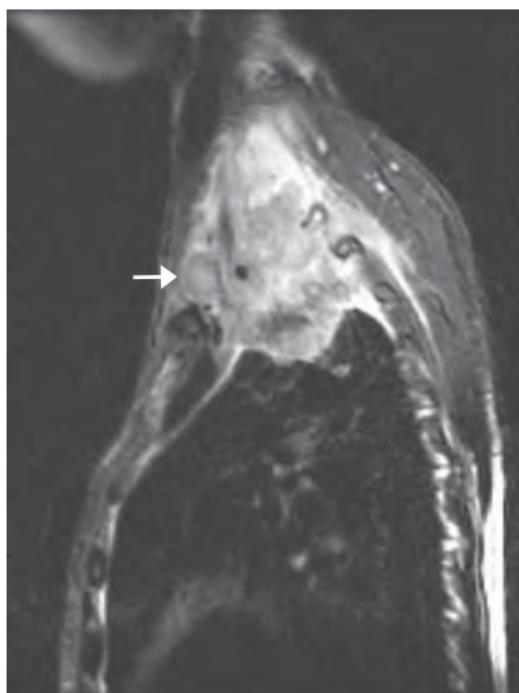


Fig. 13.39 Sagittal T2W MRI image in patient with a superior sulcus (Pancoast's) tumour showing invasion into the chest wall.

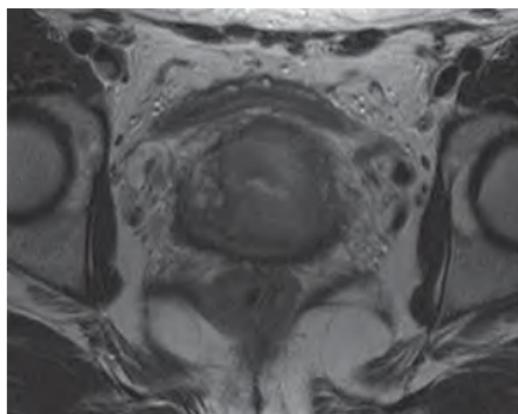


Fig. 13.40 Axial T2W sequence showing bilateral parametrial infiltration in this patient with a cervical mass.

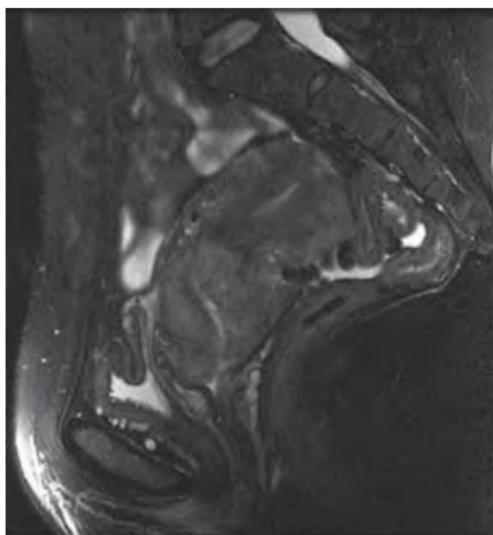


Fig. 13.41 Sagittal and axial T2W images showing a large cervical carcinoma. MRI is the modality of choice for local staging.

Spinal imaging

Basic principles of bony trauma imaging

- Obtain two films at right angles to one another (most commonly an AP and a lateral to rule out a fracture).
- Image proximal and distal joints.
- Bone scanning is more sensitive, but less specific, than plain films to rule out a fracture. (*Not useful in the acute setting.*)
- CT is invaluable for complex injuries (e.g. spine, calcaneus, and sacrum).
- MRI is used to assess ligaments, tendons, joint capsules, menisci, and cartilage.

In each case, evaluate the following

- Site of fracture: assess if proximal/distal and intra- vs extra-articular.
- Type of fracture: simple (transverse, oblique, spiral) vs comminuted.
- Degree of displacement: usually described with reference to the distal fragment.
- Soft tissue involvement: exclude foreign bodies, presence of gas. Open (compound) vs closed.

Cervical spine

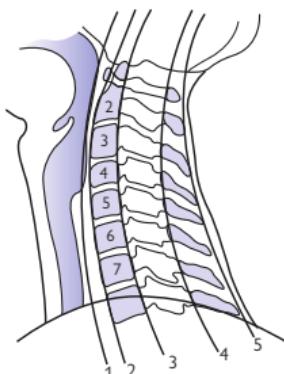
- Trauma: obtain a cross-table lateral first (this has the highest yield), and then perform the remainder of the cervical spine series (AP and open mouth peg views), if patient mobility allows and a high index of suspicion. All seven cervical vertebral bodies should be visualized (a large number of cervicothoracic injuries are missed because of inadequate views). If not seen, request a specialized lateral view (swimmer's) or a CT. Then sequentially evaluate:
 - Alignment: assess the following lines shown in Fig. 13.42. They should be parallel with no step-offs.
 - Bones: inspect C1 and C2. The anterior arch of C1 should be 3mm from the dens in adults (5mm in children). The vertebral bodies should be intact, and they should be uniform in size and shape. Check disc spaces for any inordinate narrowing or widening which may be post-traumatic.

Cartilage

- Soft tissues: look for abnormal widening or a localized bulge; 50% of patients with a bony injury will have soft tissue thickening. The soft tissues should be no more than one-third of a vertebral body until C4, and a vertebral body width thereafter.
- The peg views: do not mistake a superimposed arch of C1 or the incisors as a fracture. Important points to remember are:
 - The lateral margins of C1 and C2 should align.
 - The spaces on either side of the peg should be equal (see Fig. 13.42).

Remember normal plain films do not exclude ligamentous injury

In the routine setting, cervical spine films are taken to exclude spondylosis (disc space narrowing and osteophytes) and atlantoaxial subluxation, which results in long tract signs and cord compression (RhA, ankylosing spondylitis, Down's syndrome). (See Fig. 13.43 for image showing a large osteophyte.)



- 1—Soft tissue line closely applied to posterior aspect of the airway widens at level of laryngeal cartilage
- 2—Anterior border of vertebral bodies
- 3—Posterior border of vertebral bodies
- 4—Spino laminar line = posterior limit of spinal canal
- 5—Tips of the spinous processes

Fig. 13.42 Schematic diagram depicting the lines that need to be assessed when looking at a lateral film of the cervical spine.



Fig. 13.43 Sagittal T2W image showing a large osteophyte at the C6/7 level.

Thoracic and lumbar spine

Degenerative disease is common with disc space narrowing, end-plate sclerosis, and osteophyte formation. Wedge compression fractures are common in the osteoporotic spine and need to be distinguished from the more sinister causes (absence of paraspinal mass, posterior elements spared). Multiple collapsed vertebrae are found in osteoporosis, neoplastic disease, trauma, and histiocytosis X. Bone density may help narrow the differential, which includes ↑ (sclerotic metastases, lymphoma) and ↓ (acute infection, osteoporosis).

Spondylolisthesis is the subluxation of one vertebral body on another and may be degenerative or due to bilateral pars defects (spondylosis). This is a fracture/defect of the posterior elements of the vertebrae. In an oblique view, the posterior elements form a Scottie dog (with the pars making up the collar). This may be a purely incidental finding; however, if severe, it can result in neuroforaminal stenosis. Plain films are insensitive in the evaluation of disc disease. MRI is the investigation of choice for disc disease and its neurological complications.

Fractures typically occur at the thoracolumbar junction (90% at T11–L4). CT typically indicates other than in stable compression fractures, isolated spinous or transverse process fractures, and spondylolysis.

Plain film findings include paraspinal haematoma and widened interpedicular distance. An unstable injury may be accompanied by disruption of the posterior elements and widened interlaminar space, and is seen in the context of all fracture dislocations or if there is a compression fracture of >50%.

Pelvis

Pelvic fractures are complex, and there are many classification systems around. The pelvis should be regarded as being made up of three bony rings. The sacroiliac joints and the pubic symphysis are part of the main bony ring. A fracture of one ring is frequently associated with a second ring fracture (see Fig. 13.44).

- Sacroiliac joints should be equal in width.
- The superior surfaces of the pubic rami should align. The joint width should be no more than 5mm.
- The sacral foramina should form a smooth arc.
- Acetabular fractures are subtle—look for symmetry.

Stable fractures (single break of the pelvic ring or peripheral fractures) commoner. These include avulsion injuries (e.g. anterior superior iliac spine (ASIS), pubis, and ischial tuberosity), as well as sacral fractures and those of the ischiopubic rami.

Unstable fractures (pelvic ring interrupted in two places)—less common. All require CT for clarification of the extent of injury, as a plain film may underestimate the extent of posterior ring disruption (includes Malgaigne and bucket handle fractures).

- **Bone texture:** the pelvis is a common site for metastatic involvement, especially with urological malignancies, e.g. prostate (sclerotic metastases) and myeloma (multiple lytic lesions). Paget's disease of the pelvis may mimic sclerotic metastases but tends to be confined to one hemipelvis and may expand or thicken bone.
- **Sacroiliitis:** sacroiliac joint involvement is common in seronegative arthropathies and is usually symmetrical in conditions such as IBD, ankylosing spondylitis, and hyperparathyroidism. More asymmetrical change is seen in Reiter's disease and RhA. It is characterized by initial erosion and widening of the joint, resulting in chronic sclerosis, which has a preferential involvement of the lower one-third of the joint (iliac > sacral side).
- **AVN of the femoral heads:** an important finding but is often advanced when plain film findings are seen. Radiographically occult AVN may be detected on MRI or a bone scan. On plain X-ray, it is characterized by sclerosis, flattening, and fragmentation of the femoral head. Subchondral crescents are pathognomonic. AVN can also be a sequel of trauma, but bilateral AVN is seen in conjunction with steroid therapy, sickle-cell disease, and as part of Perthe's disease.

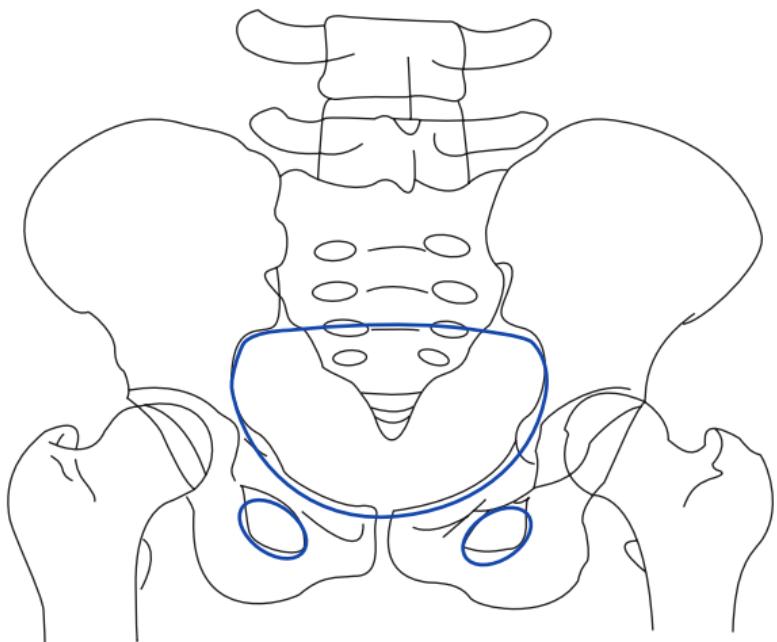


Fig. 13.44 The pelvis is made up of bony rings: the main pelvic ring and two smaller rings made up of the pubic and ischial bones.

Vascular intervention

Angiography is catheterization of a vessel followed by subsequent opacification with a water-soluble iodine-containing contrast medium. Catheterization is usually performed using the Seldinger technique.

An approach to interpretation of an angiogram

- What: is the type of study—angiogram, digital subtraction angiogram (DSA), venogram?
- Where is the catheter and which vessel? Is it a large vessel or a selective/super-selective angiogram?
- When: what phase is it (early/late arterial, parenchymal, or venous)?
- Vessels: is there contrast in unexpected vessels (extravasation or neovascularity)? Is the vascular contour normal (look for irregularity, stenosis, dissection, or encasement)?
- Intervention (previously placed stents, filters, coils, clips, or drains).

Catheter selection

Catheters are sized in French (Fr) where 1 Fr = 0.33mm. Sheath vs catheter. A sheath has a defined luminal diameter; however, the overall diameter will generally be larger.

High-flow (flush) catheters have multiple side holes and are used for large vessel angiography.

Selective catheters have a single hole at the end. The distal portion has numerous shapes tailored to a specific or general purpose.

Indications include

- Demonstration of arterial anatomy prior to surgery where this is likely to influence surgical management.
- To elucidate the nature of arterial disease, e.g. occlusions, stenoses, thrombi, aneurysms, and vascular malformations in coronary, carotid, and cerebrovascular disease. Also in the setting of endovascular procedures (aneurysm repair, thrombolysis, stenting, and angioplasty).
- To identify the source of bleeding in the GIT or following trauma.
- To demonstrate tumour circulation (often prior to embolization).
- Due to advances in technique, non-invasive assessment of vessels by techniques such as colour Doppler US, CT angiography, and MRA is often used as first line.

Potential complications include

Puncture site haematoma, infection, pseudo-aneurysm, AV fistula, dissection, thrombosis, embolic occlusion of a distal vessel.

Contrast

Volumes used are variable, depending on the area being imaged. The contrast agents are iodinated, non-ionic, and of low osmolarity, resulting in reduced toxicity. Nevertheless, potential side effects include anaphylaxis, hypotension, urticaria, and bronchospasm. Patients particularly at risk include those with a history of a previous reaction, iodine allergy, and atopy. Nephrotoxicity is a potential risk and may be exacerbated by dehydration.

Contrast reactions are seen in 1/1000 patients. Risk of anaphylaxis is 1/400,000. Pre-medication with corticosteroids may reduce the incidence of reactions if contrast administration is essential, but this is not universally accepted.

Specific applications

These include pulmonary angiography (gold standard for detection of PEs), which is highly invasive, and therefore reserved for when thrombolysis or embolectomy are being considered. Cerebral angiography is useful in the diagnosis of aneurysms, AVMs, tumour vascularity, and both intra- and extracranial vascular disease. Renal angiography is selectively performed to diagnose RAS and prior to embolization of tumours.

- **DSA:** a technique whereby there is subtraction of the contrast-containing shadows from the initial plain films (mask), resulting in an image containing opacified structures only. The resulting images may be digitized and manipulated. The overall advantage is smaller doses of contrast and smaller catheters may be used.
- **Angioplasty:** percutaneous transluminal angioplasty (PTA) is a method used to fracture the vascular intima and stretch the media of a vessel by a balloon. Atherosclerotic plaques are very firm and are fractured by PTA. Healing occurs by intimal hyperplasia. Indications include claudication or rest pain. A common alternate to surgical bypass, with 5-year patency similar to that of surgery.
- **Intravascular stents:** typically, metallic stents used when there has been unsuccessful PTA or in cases of recurrent stenosis, venous obstruction/ thrombosis or as transjugular intra-hepatic portosystemic shunt (TIPS) shunts (干预放射学, Transjugular intrahepatic portosystemic shunt, p. 846). They can either be balloon-expandable or self-expandable. Aortic stent grafts used to treat aortic aneurysms or dissections are typically a combination of a metallic stent with synthetic graft material. Stents can also be used in revascularization procedures when there is long segment stenosis, total occlusion, or ineffective PTA.
- **Therapeutic embolization:**
 - Used to selectively occlude vessels by introducing a variety of materials via a catheter. Permanent materials used include metallic coil, balloons, and cyanoacrylate glue. Temporary embolic materials include gel foam and autologous blood clots. This technique is used at active bleeding sites and to reduce tumour vascularity preoperatively in resectable tumours. It can also be used to treat AVMs, for symptomatic uterine fibroid embolization (干预放射学 Figs 13.49 and 13.50, p. 848), and in varicocele embolization for infertility. Proximal occlusion of a vessel is equivalent to surgical ligation and does not compromise collateral flow.
 - Distal embolization usually infarcts tissue and is followed by necrosis.
 - Complications include post-embolization syndrome (fever, pain, elevated WBC), infection of embolized area, and reflux of embolic material (non-target embolization).

- **Vascular catheterization:** is also used to selectively infuse vessels, as with thrombolytic treatment or rarely with cytotoxics. Vascular stenting is of ↑ use in coronary and peripheral vascular disease. IVC filters (see Fig. 13.45) are metallic umbrellas used to mechanically trap emboli and prevent venous thromboembolic disease. They are percutaneously placed via the femoral, jugular, or antecubital vein. In the treatment of patients with recurrent PEs despite anticoagulation or where anticoagulation is contraindicated. IVC filters can be temporary (retrievable) or permanent. They are placed infrarenally to avoid renal vein thrombosis.
- **Thrombolytic therapy:**
 - Is the infusion of a fibrinolytic agent (urokinase, streptokinase, TNK, tissue plasminogen activator (tPA)) via a catheter inserted directly into a thrombus. This can restore blood flow in a vessel obstructed with a thrombus or embolus.
 - Indications include treatment of an ischaemic limb, early treatment of MI or stroke to reduce end-organ damage, and treatment of venous thrombosis (DVT) of the leg or PE.

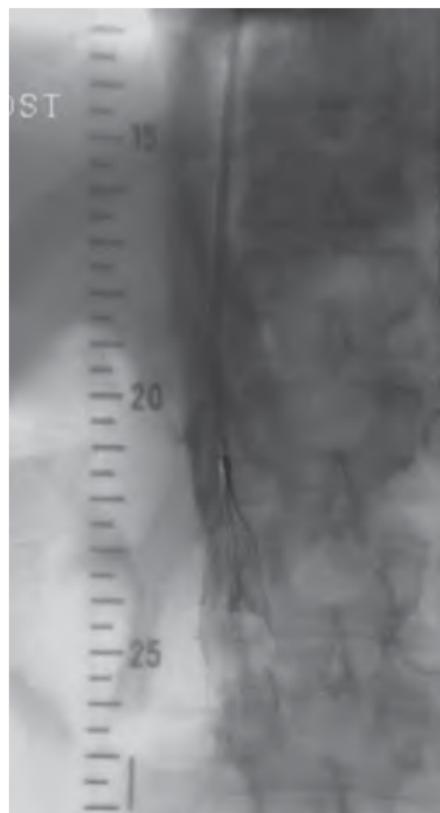


Fig. 13.45 Cavogram showing infrarenal deployment of an IVC filter (Gunther-Tulip).

- Contraindications include active bleeding, recent intracranial event (CVA, tumour, or recent surgery), non-viable limb, and infected thrombus.

Central venous access: there are a variety of devices available—peripherally inserted central catheters (PICCs), external tunnelled catheter (Hickman), subcutaneous port (Portacath™). Indications include chemotherapy, total parenteral nutrition (TPN), long-term antibiotics, administration of fluids and blood products, and blood sampling. Potential complications are venous thrombosis, infection, and pneumothorax.

Interventional radiology

Interventional radiology is a sub-speciality where a variety of imaging modalities are used to guide percutaneous procedures. This may obviate alternative surgical procedures and consequently result in lower morbidity. Interventional procedures are usually carried out under LA or using conscious sedation (➡ Interventional radiology, Conscious sedation, p. 846), and on an outpatient basis, thereby considerably reducing bed occupancy. There is a huge range of procedures that are currently performed. The following is a limited list of some of them (see also Table 13.12).

Percutaneous biopsy

Biopsy needle placement may be done under fluoroscopy, CT, MRI, and US. This provides a non-operative confirmation of tissue diagnosis and, in the case of a suspected malignancy, it is possible to accurately plan treatment. For histology, a 14–18G needle is used. With a fine-aspiration needle (20–22G), material may be obtained for cytology. Using imaging guidance, there is avoidance of damage to vital structures such as blood vessels, solid organs, and bowel loops. With chest biopsy, there is a 30% risk of pneumothorax. Other potential complications include false –ve samples (due to sampling error or tissue necrosis). Bleeding is more likely to occur in patients with underlying coagulopathies (vigorous pre-biopsy screening important for safety) and in patients with ascites.

Percutaneous drainage

With image guidance, surgical intervention may be avoided by accurate placement of a drainage catheter. Calibre varies from 8 to 14F, depending on the nature of the underlying fluid. Regular irrigation of the catheter may be necessary to ensure successful drainage. Successful resolution may be impeded in the more complex and multiloculated collections. There are a variety of routes of access other than the conventional percutaneous, including transvaginal, transrectal, and transgluteal. Potential complications include injury to overlying structures and sepsis.

Drainage of the urinary system

Can be via double J stents, which are placed into an obstructed collecting system, with the distal catheter tip lying in the bladder. More short-term drainage is achieved via a percutaneous nephrostomy. Here, the obstructed kidney is punctured under fluoroscopic or US guidance, and a catheter placed in the renal calyx (preferably the lower pole). This is the technique of choice in the acutely obstructed or infected kidney. Nephrostomy insertion is also performed in the setting of ureteric injury either with or without accompanying peritonitis. Potential risks include septic shock, haematuria (due to pseudo-aneurysm or AV fistula), or injury to regional structures.

Biliary system drainage

Surgical outcome in patients with malignant bile duct obstruction is often poor. This may be due to carcinoma of the pancreas or cholangiocarcinoma. Biliary stenting alleviates obstruction and improves quality of life. Stenting may be performed at ERCP or percutaneously via antegrade puncture

Table 13.12 Established and newer interventional applications

Established techniques	Newer interventional applications
Arterial and venous sampling	AVM embolization
Biliary drainage, stenting, angioplasty, biopsy, and stone extraction	Chemoembolization
Diagnostic angiography	Cryoablation of liver tumours
Embolization	
Oesophageal dilatation	Endovascular repair of abdominal aortic aneurysms with stent grafts
Fallopian tube recanalization	Radiofrequency ablation of tumours
Feeding tube placement	Uterine artery embolization for symptomatic fibroids
IVC filter placement	Varicocele embolization
Nephrostomy placement	Vertebroplasty
Pain management (e.g. coeliac ganglion block, selective and non-selective nerve block)	
Percutaneous biopsy, cholecystostomy, and drainage of fluid collections	
1° gastrostomy, gastrojejunostomy, or jejunostomy tube placement	
Thrombolysis	
TIPS insertion	
Ureteral stent placement	
Vascular angioplasty and stent placement	
Venous access and foreign body retrieval	

through the liver (PTC to delineate the anatomy, being performed first). Other than alleviating biliary obstruction, PTC can provide biliary diversion in the case of ductal injury (post-traumatic or post-surgical). Often there is unilateral obstruction which requires only treatment of the affected side. Hilar obstruction, however, due to a hilar (Klatskin) tumour requires two biliary drains.

Percutaneous drainage of the gall bladder (cholecystostomy) is indicated for the treatment of acute calculous or acalculous cholecystitis in patients who are not surgical candidates or as a temporizing measure prior to definitive surgery.

Other GI interventions include stenting/balloon dilatation of oesophageal strictures, stenting of obstructive colonic neoplasms (see Fig. 13.46), and percutaneous gastrostomy or gastrojejunostomy insertion. Oesophageal, head and neck, and neurologic disease may necessitate percutaneous gastrostomy. In some instances, it may be used for long-term bowel decompression, for instance in a prolonged ileus.

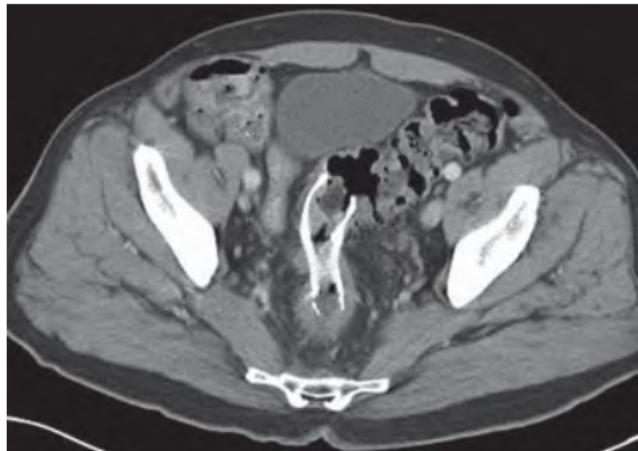


Fig. 13.46 Obstructing sigmoid carcinoma has been decompressed with a colonic stent.

Transjugular intra-hepatic portosystemic shunt

A procedure whereby a connection is made between the hepatic and portal veins to reduce portal pressure in patients with portal hypertension. The mortality is considerably lower than in acute shunt surgery, particularly in the context of an acute variceal bleed, which has failed to respond to sclerotherapy.

Conscious sedation

Form of sedation whereby the patient is given sedation and analgesic medication but remains conscious and easily arousable. At all times, the following are monitored—BP, pulse oximetry, ECG, and heart rate. Typical drugs used include a short-acting benzodiazepine (e.g. midazolam) and a narcotic analgesic (e.g. fentanyl), administered in small aliquots.

Endovascular repair of abdominal aortic aneurysms

With stent grafts, this is a new image-guided, catheter-based approach that provides a valuable alternative to standard open surgical repair. Radiological imaging plays an essential role in pre-procedure evaluation, the procedure itself, and patient follow-up. The ultimate goal remains the same—complete exclusion of the aneurysm sac to prevent rupture. Advantages include lower blood loss, shorter ITU stay, and rapid recovery. Complications include graft thrombosis, kinking, pseudo-aneurysm caused by graft infection, and endoleak. Bifurcation grafts are used mainly for abdominal aortic aneurysm (AAA) repair and aortoiliac occlusive disease. Tube grafts are used mainly for AAA repair.

Radiofrequency ablation

Not all patients with tumours are eligible for surgical intervention because of unfavourable location of the tumour, adverse clinical conditions, or advanced disease. In addition, the cost, morbidity, and mortality associated with surgical resection have led to the search for other forms of therapy. Newer, minimally invasive treatments include intra-arterial chemoembolization, injection of ethanol, and radiofrequency ablation. Among the thermal ablative procedures, radiofrequency ablation has evolved into an established technique for non-invasive management of tumours in patients

where underlying medical co-morbidities or tumour burden preclude definitive surgery or as a bridge to further therapy. For this procedure, US, CT scanning (see Figs 13.47 and 13.48), or MRI is used by the radiologist to guide percutaneous placement of long, thin (usually <18G), insulated needles into the tumour. The distal end (1–3cm) of each needle is not insulated and emits radiowaves. Electrodes are attached to a generator, and the electrical energy is converted to heat that kills cells through coagulation necrosis. The radiologist can vary the amount of current used, thereby tightly controlling the treatment radius. The entire treatment session usually lasts 1h and can be performed in the outpatient setting. The procedure is typically performed with conscious sedation and LA. Radiofrequency ablation has been used to treat a variety of tumours, but the commonest utility is for hepatocellular carcinoma, liver metastases, and renal tumours.

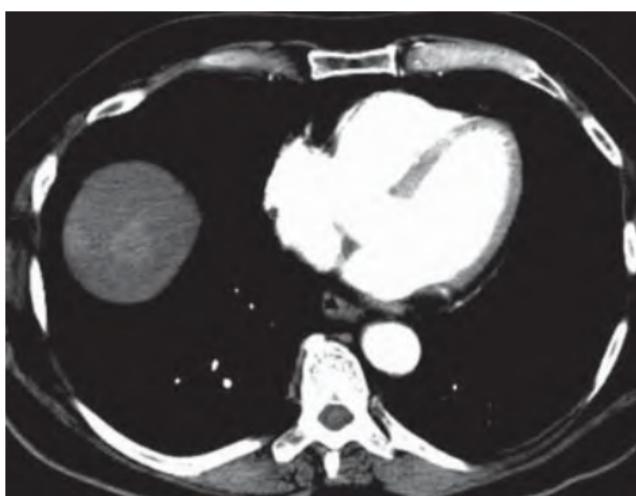


Fig. 13.47 Contrast-enhanced CT showing several high-attenuation metastases overlying the dome of the liver.

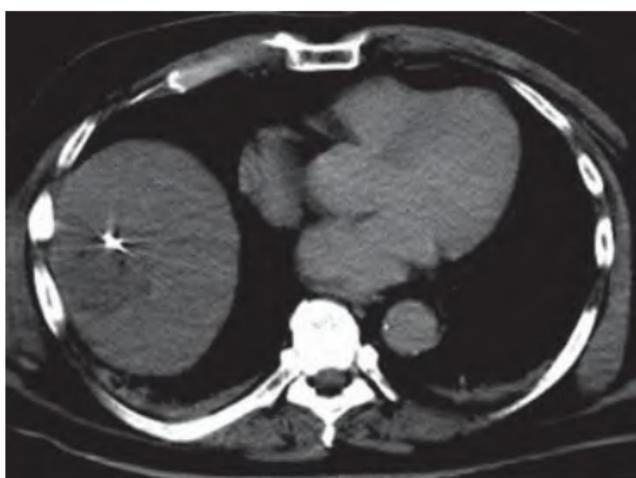


Fig. 13.48 Radiofrequency ablation of the metastasis shows the electrode *in situ*.

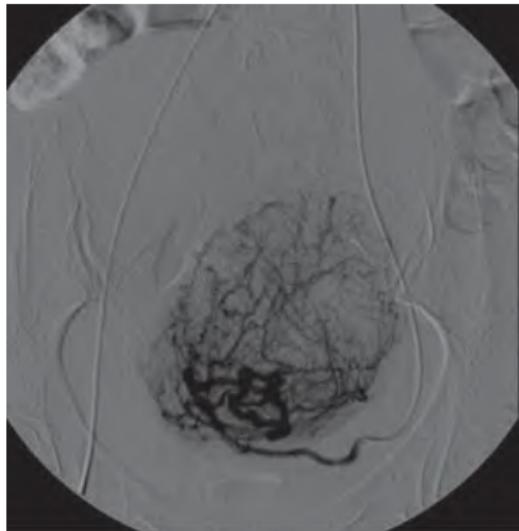


Fig. 13.49 Pre-uterine artery embolization (UFE). Selective catheterization shows the supply to a large left-sided fibroid.

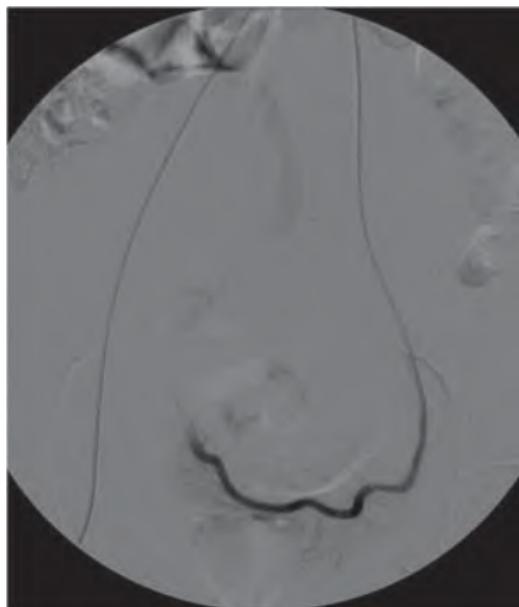


Fig. 13.50 Post-embolization with polyvinyl alcohol (PVA) showing no flow in the previously demonstrated fibroid.

Musculoskeletal imaging

Fracture terminology

Anatomical site

- In long bones, divide the shaft into thirds.
- Use anatomical landmarks for description.

Pattern of fracture

- *Simple fracture*: no fragments. Describe the orientation of the fracture plane, e.g. transverse, spiral, and oblique.
- *Comminuted fracture*: >2 fragments: T-, V-, and Y-shaped patterns, butterfly fragments.

Apposition and alignment

Defined in relation to distal fragments.

Other important descriptors include:

- Displacement (e.g. medial, lateral, anterior, posterior).
- Angulation (direction of fracture apex, e.g. valgus or varus).
- Rotation (internal vs external).
- Overlap (of fragments).
- Distraction (refers to degree of separation of fragments).
- Impaction: fracture fragments are compressed, resulting in shortened bone.

Adjacent joints: are they normal? Is there dislocation, subluxation, or any intra-articular extension of the fracture line?

Also consider the following

- Soft tissue involvement (foreign body, gas, calcification).
- Type of fracture (stress vs pathological).
- Stress fractures are of two types:
 - Insufficiency-related (if abnormal underlying architecture, as in osteoporosis).
 - Fatigue: when abnormal stress is placed on normal bone (as in march injuries).
- Pathological fracture occurs when fracture occurs in the setting of underlying bone disease or discrete lesion.

Types of orthopaedic hardware

- Intra-medullary rods: usually hollow, closed nails. Rotation and shortening of bone fragments is avoided by distal interlocking screws.
- Kirschner wires (K-wires): these are used to temporarily fixate or treat fractures, particularly with small fragments or paediatric metaphyseal fractures. These are drilled into cancellous bone and can be used to maintain rotational stability if >1 is used. The ends are typically folded over to avoid any soft tissue or other injury.
- Circumferential wires can be used to contain bone fragments.
- Other hardware includes staples, plates, nails, and screws.

Types of joint replacements

- Polyethylene is a radiolucent substance used for joints with concave articular surfaces (e.g. the hip). Typically backed with metal as a support. Ultra-high molecular weight versions are also available.
- Silastic is also radiolucent and is used for hand and foot arthroplasty implants.
- Methylmethacrylate is used as a cement or can be directly injected into the medullary space. Radio-opaque.
- Variety of metal alloys.

Constrained prostheses are intrinsically stable and are therefore more likely to suffer loosening. Unconstrained prostheses rely on extra-articular structures to provide stability and are therefore less likely to dislocate.

A discussion of the types of prostheses is beyond the scope of this chapter. Potential complications of prostheses include, but are not limited to, the following.

- Polyethylene wear, dislocation, heterotopic bone formation, and infection can be seen. Small particle disease is seen as areas of osteolysis around the prosthesis due to macrophage-mediated reaction to particle debris. Cement leakage can cause necrosis and vascular and neurological injury, depending on location.
- Acute loosening occurs in the setting of infection. More chronic loosening is seen due to mechanical factors.

Magnetic resonance imaging in musculoskeletal imaging

Musculoskeletal neoplasm imaging

Critical role in evaluating disease extent, staging, and treatment planning. Lack of mobile protons, and an acellular matrix render cortical bone, ligament, tendon, and fibrous signal of low signal intensity on all sequences. Tissues like muscle, fat, osteoid, and chondroid matrix have differing signal intensities, which can be used to differentiate tumours. Marrow involvement can be excluded by T1W and STIR sequences (MRI Magnetic resonance imaging, pp. 828–32). The bone involved should be imaged in its entirety to exclude skip lesions. IV contrast is mandatory to assess lesional margin and tumour vascularity.

Joint imaging

MRI is the mainstay in assessment of joint pathology and, in particular, is excellent in depiction of ligaments, cartilage, and joint effusions. It is therefore the modality of choice for infection, neoplasm, trauma, and arthritis. STIR shows marrow oedema, fluid collections, and bursal inflammation. Dual-echo steady state (DESS) is a sequence used for specific evaluation of articular cartilage. MRI is also the gold standard for marrow imaging. It is able to differentiate red from yellow marrow. T1W and STIR sequences are used for evaluating marrow pathology. Marrow oedema, as well as early involvement of the marrow by pathologies such as infection, neoplasia, and subtle trauma, is seen on STIR sequences when not easily evident on plain radiographs.

Hands

There are specific patterns that may be seen in the hand as an indicator of the underlying disorder. Some patterns are pathognomonic, whereas others are more non-specific.

- **Generalized osteopenia:** osteoporosis, multiple myeloma, and RhA.
- **Coarsening of the trabecular pattern:** common in haemoglobinopathies, especially thalassaemia and Gaucher's disease.
- **Periosteal reaction:**
 - HPOA associations include carcinoma of the bronchus, IBD, and coeliac disease.
 - Thyroid acropachy, commonest on the radial side of the thumbs.
 - Juvenile chronic arthritis seen in about 25%.
- **Carpal abnormalities:** include short metacarpals (Turner's syndrome, pseudo- and pseudopseudohypoparathyroidism), carpal fusion (inflammatory arthritis, RhA and juvenile chronic arthritis, post-trauma), and look for syndactyly and polydactyly.
- **Soft tissue changes:** e.g. ↑ in soft tissue thickness/size seen in acromegaly, localized ↑ seen in gouty tophi, nodes as in OA, soft tissue calcification seen in CREST (calcinosis, Raynaud's syndrome, oesophageal motility dysfunction, sclerodactyly, and telangiectasia), and scleroderma.
- **Joint disease:** the hand X-ray above all may help in sorting out the type of arthropathy and aid rheumatological management (see Fig. 13.51). The ABCS approach is invaluable (see Table 13.13).



Fig. 13.51 Asymmetric oligoarthritis with dominant involvement of the DIP joints. The pencil-in-cup appearance is typical.

Table 13.13 An ABCS approach to interpretation of the hand radiograph

A: Alignment	Subluxation/dislocation common in RhA and SLE
B: Bone	<p><i>Osteoporosis:</i> mineralization is usually normal, except in RhA. <i>Juxta-articular osteopenia</i> can be seen in any arthropathy. Diffuse osteopenia is seen in RhA.</p> <p><i>Erosions:</i></p> <ul style="list-style-type: none"> • Aggressive (i.e. no sclerosis of margins) seen in RhA and psoriasis • Non-aggressive (with sclerotic margins) seen in gout; inflammatory erosions are marginal (OA erosions are central) (Distinguish from subperiosteal resorption (radial border; seen in hyperparathyroidism) <p><i>Bone production:</i> periosteal new bone formation, (psoriasis)</p> <p><i>Reiter's syndrome:</i></p> <ul style="list-style-type: none"> • Ankylosis (bony bridging) in inflammatory arthropathies • Overhanging cortex (typical of gout) • Osteophyte formation, OA • Subchondral bone (reparative bone beneath cortex); typical of OA
C: Cartilage	<p>Joint space has uniform narrowing in all arthritis, except OA</p> <p><i>Eccentric narrowing:</i> typically seen in OA</p> <p><i>Wide joint space :</i> early arthritis, gout, and pigmented villonodular synovitis (PVNS)</p>
S: Soft tissue	<p><i>Swelling:</i></p> <p><i>Symmetrical:</i> about joint seen in all inflammatory arthropathies, but most typically RhA</p> <p><i>Asymmetric:</i> typically due to osteophytes and seen with OA</p> <p><i>Lumpy swelling</i> of soft tissues seen in gout (due to tophi)</p> <p><i>Swelling</i> of entire digit; psoriasis, Reiter's</p> <p><i>Calcification:</i> soft tissue; gout (calcified tophus)</p> <p><i>Cartilage:</i> CPPD</p> <p><i>Subcutaneous tissues:</i> scleroderma</p>

Trauma

Two views are essential for ensuring no subtle injuries are missed. On a P-A view, the spaces between the carpal bones and the carpometacarpal articulations should be roughly equal (1–2mm). If a dislocation is present, then there is obliteration/overlap. Common injuries include Bennett's fracture and the first metacarpal base injury extending into the joint surface with dislocation at the carpometacarpal joint. Scaphoid fractures are the commonest (75–90%) of carpal injuries. Because of the blood supply, there is a potential risk of osteonecrosis of the proximal pole.

Image-guided arthrography

May be performed in isolation or in conjunction with subsequent cross-sectional imaging (CT or MRI) for the following indications:

- Ligamentous and tendinous tears.
- Cartilage injuries.
- Proliferative synovitis.
- Masses and loose bodies.
- Implant loosening.

Plain films are obtained prior to the procedure. All joint fluid that is aspirated is routinely sent for culture. The contrast flows away from the needle if the tip is intra-articular.

Single contrast studies are performed to exclude non-calcified loose bodies. Double contrast studies are performed in the setting of cartilaginous injury such as a labral tear.

- *Contraindications:* allergy to contrast media, concomitant infection.
- *Complications:* pain, infection, allergic reaction (to contrast), and vasovagal reaction.

When performing MR, the examination is performed with dilute gadolinium.

Approach to diagnosis of bone lesion

- Single or multi-focal? Multiple lesions can be seen in benign disease such as enchondromas, fibrous dysplasia, as well as malignant aetiologies such as myeloma, metastases, and lymphoma.
- What is the degree of aggressiveness (type of destruction, as well as reparative pattern)? Cortical penetration implies aggressive behavior, as does an ill-defined or wide zone of transition (area between the lesion and normal bone). Moth-eaten and permeative patterns are also in keeping with aggressive disease.
- Location (within bone). Central and cortical lesions are usually benign. 1° tumours occur at sites of rapid growth, for instance the distal femur. Metastases occur at sites of high vascularity such as the spine or iliac bone.
- Tissue characterization by matrix. Matrix refers to the intercellular substance produced by tumour cells (is done in conjunction with a cross-sectional modality such as CT). Osseous matrix is seen with osteogenic tumours, and chondrogenic tumours have a cartilaginous matrix.
- Age is a very helpful way of narrowing the differential. Over 40 years, metastases are much commoner than 1° bone tumours.
- Is there a periosteal reaction? More aggressive lesions are associated with typical patterns such as sunburst, Codman's triangle, or an onion peel configuration
- Soft tissue mass: more typically seen with malignant lesions.

Neuroradiology

- CT is the initial study for evaluation of neuropathology due to its speed, availability, and lower cost.
- Excellent for evaluation of bony abnormality.
- Done both with and without IV contrast, depending on the clinical indication. The use of contrast delineates vascular structures and anomalies.
- Visualization of the posterior fossa is inferior to MRI.
- MRI is useful in assessing diffuse axonal injury and the sequelae of head injury.

Logical approach to interpretation

- Soft tissues (look for thickening which may suggest haematoma or oedema): assess the ear and orbital contents.
- Bone (use bone window): check the calvarium, mandible, and C spine for fractures and lytic lesions. Assess sinuses and mastoid air cells for opacity that may suggest the presence of fluid, pus, blood, mass, or fracture. If there has been facial trauma, the integrity of facial bones/orbit best assessed on coronal view.
- Dura and subdural space: assess symmetry of thickness (early clue to presence of blood). Look for crescentic or lentiform density.
- Cortical parenchyma: poor differentiation between grey and white matter suggestive of infarction, tumour, oedema, infection, or contusion. Hyperdense areas may suggest enhancing lesions, intracerebral haematoma, or calcification. If central grey matter nuclei (globus pallidus, internal capsule) are not visible, suspect infarct, tumour, or infection.
- Ventricular system: assess the position for midline shift or compression. High density suggests ventricular or subdural bleed. Enlargement is suggestive of hydrocephalus. High density in the cisterns may suggest blood, pus, or tumour.
- Symmetry: asymmetry of the parenchyma suggests midline shift.
- Falx shift.

Rule out skull fracture, extradural haematoma (lentiform shape), subdural haematoma (crescentic shape), SAH (see Fig. 13.52), SOLs (see Fig. 13.53), hydrocephalus, and cerebral oedema. Look for target lesions (when contrast given): metastases, abscesses, and fungal infections.

Cerebral angiography

Evaluation of vascular lesions, including atherosclerotic disease, aneurysms, vascular malformations, and arterial dissection. Supplements information obtained on CT/MRI regarding tumour vascularity.

Conventional DSA is the gold standard for the assessment of neck and intracranial vessels; however, it is an invasive technique requiring an arterial (femoral) puncture. There is also potential for vessel injury at the time of catheter manipulation (e.g. dissection, occlusion, or vasospasm). Used for guidance of interventional procedures such as embolization.



Fig. 13.52 Non-contrast CT head showing extensive SAH in a patient with underlying cerebral aneurysm (not shown).

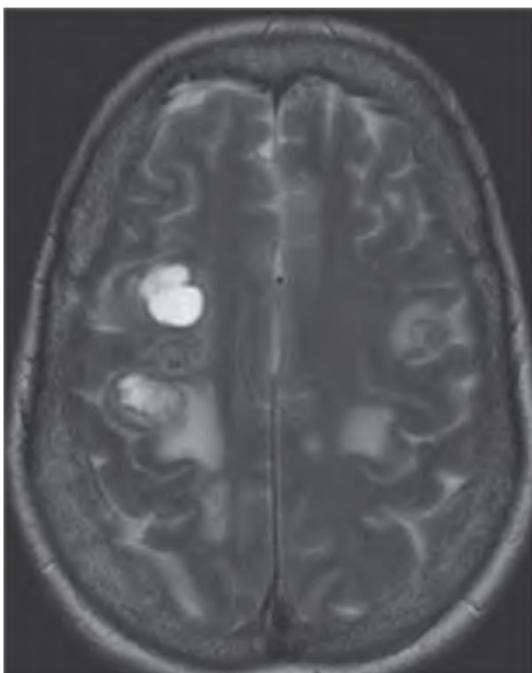


Fig. 13.53 Axial T2W MRI showing multiple high signal lesions in the deep white matter of the right frontal lobe in a patient with lung carcinoma. The anterior lesion is cystic. These are consistent with metastases. Note the surrounding oedema.

Cerebrovascular accident

Imaging may be –ve in the first 6h. Thereafter, look for:

- Oedema (loss of grey–white differentiation, sulcal effacement, and mass effect). Cytotoxic oedema develops within 6h and is seen on MRI.
- Within 24h, there will be a low-density wedge-shaped area corresponding to the vascular territory and extending to the cortical surface. Vasogenic oedema within 12–24h seen on CT.
- If ischaemic stroke, may see a hyperdense (bright) artery representing an intravascular thrombus or embolus.
- Acute haemorrhage seen if there is haemorrhagic transformation (white = acute blood). Density on NECT is 80–100HU relative to normal brain (40–50HU). There may be surrounding oedema. Will not be hyperdense if low Hct (<8g/dL). Subacute haemorrhage (3–14 days) may be hyper-, hypo-, or isointense to brain. Chronic haemorrhage (>2 weeks) is hypodense.
- Hypertensive haemorrhage typically seen in the pons in 80%.
- Prognosis depends on size, brainstem location, and intraventricular extension.
- Advances in MR sequences have revolutionized stroke imaging. Diffusion sequences (DWI) and perfusion-weighted imaging (PWI) demonstrate acute infarction, even in the context of a –ve CT.
- T2W and fluid attenuation inversion recovery (FLAIR) images show oedema with high signal.
- DWI shows high signal suggestive of cytotoxic oedema.
- Gradient echo identifies acute haemorrhage, whereas fast FLAIR can show acute subarachnoid blood. Time-of-flight angiography can non-invasively assess underlying vessels. If the infarct is thought to be venous (e.g. peripheral or haemorrhagic), then phase contrast MRV can exclude sinus thrombosis.

In the case of strokes in the posterior circulation, thin sectional axial images can exclude thrombosis or dissection within the vertebral arteries.

CT is important in the early stages of stroke evaluation to facilitate thrombolytic therapy. Highly accurate in identification of proximal occlusions in the circle of Willis and therefore aids triage to facilitate thrombolysis. Non-contrast CT is initially performed, as haemorrhage is an absolute contraindication to thrombolytic therapy.

Multiple sclerosis

MRI is the modality of choice and is highly sensitive (>90%), but with low specificity (71–74%). There can therefore be overlap with other entities, such as ischaemia, and confluent disease may simulate neoplastic mass lesions. T2W MRI shows ovoid high signal lesions in a periventricular distribution. Conventional T2 may underestimate the plaque size and overall plaque burden; advanced MRI techniques, such as DTI and MR spectroscopy, can have greater utility. FLAIR sequences show lesions in a periventricular distribution by suppressing (CSF) signal. Active lesions show enhancement following gadolinium. The spinal cord should also be screened to exclude involvement by MS.

Magnetic resonance imaging in the paediatric brain

Assessing the degree of myelination is an important part of excluding structural pathology in paediatric neurological disorders. MRI is the modality of choice in brain tumours, congenital anomalies, and hypoxic–ischaemic disorders (hypoxic–ischaemic encephalopathy (HIE)). DWI is useful in acute HIE, whilst spectroscopy is critical in metabolic disorders.

Skull X-ray

Indications

The main indication is for penetrating acute trauma, although SXR^s are of limited use in the era of widespread CT availability. Occasionally, the SXR is obtained as part of a skeletal survey in the evaluation of metabolic bone disease and endocrine disorders, and in the assessment of metastatic disease. It is still used in the assessment of sinus disease and in the evaluation of the post-operative skull or for confirmation of hardware placement (see Fig. 13.54).

Contraindications

None, but if there is suspicion of underlying intracranial injury, plain films are unnecessary (→ Fractures and associated findings, p. 861).

Normal findings

The bones of the skull vault have an inner and outer table of compact bone, with spongy diploe between the two. Sutures are visible, even after fusion, and should not be mistaken for fractures. Blood vessels may cause impressions, as can small lucencies in the inner table near the vertex caused by normal arachnoid granulations which can be mistaken for small lytic lesions.

Trauma

SXR^s are basic and widely available, and yet potentially yield the least information in the context of trauma. The presence or absence of a skull fracture does not correlate with the presence or extent of any intracranial injury. Up to 50% of films may be technically unsatisfactory due to factors such as poor patient co-operation. With the advent of CT, this is the technique of choice for evaluation in acute injury and neurological deficit. It allows a firm diagnosis to be made and excludes other alternate diagnoses.



Fig. 13.54 Lateral SXR showing 'hair-on-end' appearance in thalassaemia major.

Fractures and associated findings

Basic radiographs include a lateral projection (obtained with a horizontal beam) and a further tangential projection, depending on the site of injury.

Findings include

- A *linear fracture*: well-defined margins, no branching, and no sclerosis (cf. vascular markings or sutures that have an undulating course and sclerotic margins).
- A *depressed fracture*: ↑ density due to overlapping bone; those that are depressed by >5mm may lacerate the dura or cause parenchymal injury, and therefore need elevation.
- A *fluid level/pneumocephalus*: implies an associated basal skull fracture or a dural tear.

Note: pineal displacement is an inconstant finding and is not a reliable method of assessing the presence of intracranial injury.

Abnormal findings

Look for intracranial calcification; then examine the pituitary fossa, review the bony density, and look for focal areas of lysis and sclerosis.

- *Intracranial calcification*: the majority is normal and of no clinical significance. However, it may be of pathological significance—causes include 1° tumours, such as meningiomas, craniopharyngiomas, AVMs, and tuberous sclerosis, and infections such as toxoplasmosis.
- *Raised ICP*: in practice, plain film changes are only seen if the condition is long-standing. These include sutural widening (diastasis) and erosion of the lamina dura of the pituitary fossa.
- *Enlargement of the pituitary fossa* (normal dimensions: height 6–11mm, length 9–16mm): expansion will result in a double floor, loss of the lamina dura, and elevation/destruction of the clinoid processes. The vast majority of the lesions will be pituitary adenomas; other causes include meningiomas and aneurysms.
- *Bone lysis*: may be diffuse as in metastasis or myeloma. Large areas of bone destruction are seen in histiocytosis X and in the active phase of Paget's disease (osteoporosis circumscripta).
- *Bone sclerosis*: may be localized as in meningiomas, depressed skull fractures, or generalized as in Paget's sclerotic metastases, myeloma, and fluorosis.
- *Sutural widening*: may be due to raised ICP, infiltration by malignancy (neuroblastoma or lymphoma), or defective ossification as in rickets.

Reference section

For abbreviations, see Table 13.14. For a list of adverse reactions to contrast agents, see Table 13.15.

Table 13.14 Abbreviations used in radiology

AP	Anteroposterior
AXR	Abdominal X-ray
CT	Computed tomography
CXR	Chest X-ray
IVU	Intravenous urogram
MRI	Magnetic resonance imaging
P-A	Posteroanterior
USS	Ultrasound scan
V/Q	Ventilation perfusion scan

Table 13.15 Management of adverse reactions to intravascular contrast agents

Symptoms	Treatment
Nausea/vomiting	Reassurance
Urticaria	Antihistamine chlorpheniramine maleate 10–20mg by slow IV injection (0.2mg/kg body weight paediatric dose) Side effects: drowsiness and/or hypotension may occur
Angio-oedema/ laryngeal oedema	Adrenaline (epinephrine) 1–3mL 1:10,000 IV (slowly), 0.3–0.5mL SC or IM (1:1000) solution Protect airway and give O ₂
Bronchospasm	O ₂ by mask (6–10L/min) β2-agonists nebulized or metered-dose inhaler Adrenaline (epinephrine) 0.1–0.3 mg IM (1:1000)
Hypotension	If isolated, O ₂ by mask, IV access, and rapid IV fluids If unresponsive, adrenaline 1:1,000, 0.5mL (0.5mg) IM
Vasovagal	Hypotension and bradycardia Elevate patient legs. O ₂ by mask (6–10L/min) Atropine 0.6–1mg IV (up to maximum of 3mg) Normal saline 250–500mL IV. Rapid infusion

Data sourced from Standards for intravascular contrast administration to adult patients. 3rd edition RCR October 2014.

Order of appearance of ossification centres of the elbow

The order of appearance is more important than the absolute age of appearance, which varies widely. Remember 'CRITOE' (see Table 13.16).

Table 13.16 Order of appearance of ossification centres of the elbow

	Approximate average age (years)
Capitellum	1
Radial head	3–6
Internal (medial) epicondyle	4
Trochlea	8
Olecranon	9
External (lateral) epicondyle	10

Fracture healing rapid in children

- Periosteal new bone: 1 week.
- Loss of fracture line: 2–3 weeks.
- Hard callus: 2–4 weeks.
- Remodelling of bone: 12 months.
- Types of paediatric fractures: torus (buckle) fractures—buckled cortex only.
- Greenstick fracture: incomplete transverse fracture with intact periosteum on concave side (ruptured on side of convexity).
- Complete fracture.

Epiphyseal plate fractures: the Salter–Harris classification (SALTR)

- Type I: epiphyseal slip separates it from the physis (5–6%). S = slip of physis.
- Type II: fracture line extends into the metaphysis (50–75%). A = above physis.
- Type III: the epiphysis is vertically split, i.e. the equivalent of an intra-articular fracture (8%). L = lower than physis.
- Type IV: fracture involves the metaphysis, epiphysis, and physis (8–12%). T = through physis.
- Type V: crush injury with vascular compromise, i.e. poor prognosis for growth (1%). R = rammed physis.

Internet resources

National Radiological Protection Board

For issues regarding radiation safety: ↗ <https://www.gov.uk/government/collections/national-radiological-protection-board-nrpb-report-series>

Royal College of Radiologists

For information relating to radiological issues, guidelines in clinical management, training, and publications: ↗ <http://www.rcr.ac.uk>

MRI safety and compatibility

↗ <http://www.mrisafety.com>

Radiological Society of North America

↗ <http://www.rsna.org>

American College of Radiology

Incorporates guidelines for use of contrast media and practice guidelines, as well as information on technical standards (e.g. teleradiology): ↗ <http://www.acr.org>

British Institute of Radiology

↗ <http://www.bir.org.uk>

American Roentgen Ray Society

↗ <http://www.arrs.org>

Also includes an image library with peer-reviewed published images:

↗ <http://goldminer.arrs.org/>

General radiology resources

Educational website offering radiology cases, differential diagnoses, etc.:

↗ Learningradiology.com

CT imaging/protocols: ↗ <http://www.ctisus.com>

Nuclear medicine

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Introduction to nuclear medicine

Nuclear medicine techniques employ a carrier molecule, selected to target the organ/tissue of interest, tagged with a gamma-emitting radioisotope. The labelled drug (radiopharmaceutical) is usually given PO or IV, although it can also be administered interstitially or by inhalation. Its distribution is mapped *in vivo* using a gamma camera (scintigraphy) or, for non-imaging tests, biological specimens are assayed *in vitro* using a radiation counter. SPECT uses images collected, whilst rotating a gamma camera (usually multi-headed) around the patient, then reconstructed mathematically to produce 3D images. PET images are obtained from a ring of detectors following administration of a positron-emitting radiopharmaceutical.

Nuclear medicine procedures can detect early physiological responses to disease processes, generally before structural changes have taken place, and thus scintigraphy is often more sensitive than conventional radiology in early disease. Specificity varies depending on the radiopharmaceutical used, and characterization of abnormalities often relies upon pattern recognition within a particular clinical setting. Anatomical detail is poor, compared with conventional radiology, and increasingly single photon emission computed tomography (SPECT) and positron emission tomography (PET) images are co-registered with CT or MRI images using hybrid cameras.

Nuclear medicine procedures are non-invasive and allow the whole body to be imaged during a single examination. Absorbed radiation doses depend on the radiopharmaceutical used but are usually in the same range as diagnostic radiology. Pregnancy is an absolute contraindication to nuclear medicine examinations, except where likely clinical benefit far outweighs potential risk, e.g. lung perfusion imaging. Some radiopharmaceuticals are excreted in breast milk, and additional precautions may be advisable for lactating women.

Diagnostic radiopharmaceuticals are used in tracer quantities and toxicity is negligible. Individual hypersensitivity reactions are rare. The most widely used radionuclide is technetium-99m (^{99m}Tc), which can be obtained from an on-site generator, has a half-life of 6h, and is suitable for labelling a wide variety of radiopharmaceuticals.

Specific information required when requesting nuclear medicine tests includes

- Patient identification details.
- Examination requested.
- Relevant clinical history, including results of other investigations.
- Pregnancy/lactation details where relevant.
- Special needs—visual/hearing/learning difficulties; needle phobia.
- Some scanning beds have a weight limit.

Bone scintigraphy: bone scan

Background

Nuclear medicine investigations supplement the anatomical information obtained from radiology. Bone scintigraphy is sensitive, with changes frequently detected earlier than on plain film, but non-specific. It plays a pivotal role in the staging of patients with malignant disease and in difficult orthopaedic cases. May be restricted to 'local views' only, e.g. loose hip prosthesis or 'whole body imaging' in metastatic screening. Can include a dynamic phase image at the time of radiopharmaceutical administration, in addition to the delayed bone phase image 2–4h post-injection. Dynamic phase reflects blood flow to the site of interest—useful when there is concern over vascularity, infection, or recent trauma. Helps to differentiate soft tissue from bone pathology, e.g. cellulitis vs osteomyelitis.

The ^{99m}Tc -bisphosphonate complex (MDP/medronate, HDP/oxidronate) targets the skeleton with renal excretion of unbound activity. Patients with poor renal function may need delayed imaging to improve the bone:soft tissue ratio (see Fig. 14.1).



Fig. 14.1 Normal whole body ^{99m}Tc -bisphosphonate bone scan; anterior view on left, posterior view on right.

Indications

- Tumour staging—assess skeletal metastases (see Fig. 14.2).
- Bone pain.
- Trauma—when radiographs unhelpful.
- Prosthetic loosening, e.g. total hip replacement (THR).
- Infection.
- AVN.
- Paget's disease to assess extent (monostotic or polyostotic) (see Fig. 14.3).
- Sports injuries.

Patient preparation

Should be well hydrated and continent.

Procedure

Inject ^{99m}Tc -bisphosphonate complex IV. For suspected AVN or sepsis, image immediately to assess vascularity. Otherwise, image 2–4h later. Whole body views are required for metastatic screening. Tomography improves anatomical definition and detection of small lesions, e.g. osteoid osteoma, and is particularly useful in back pain. Fusion with CT images is being used increasingly.

Results

- Radiopharmaceutical uptake reflects osteoblastic activity.
- Focal ↑ uptake in sclerotic metastases, trauma, or infection.
- Diffuse ↑ uptake associated with advanced metastases, Paget's (local), and metabolic bone disease.
- Reduced tracer uptake in acute AVN and lytic bone metastases.

Interpretation

Sensitive, but non-specific. Interpretation relies on pattern recognition in the clinical setting.

Advantages

Sensitive—detects early changes in bone physiology, often before abnormal plain radiographs, e.g. occult trauma, metastases, and sepsis.

Pitfalls

False –ves in multiple myeloma and osteolytic bone metastases.

False +ves: artefacts due to urine contamination.

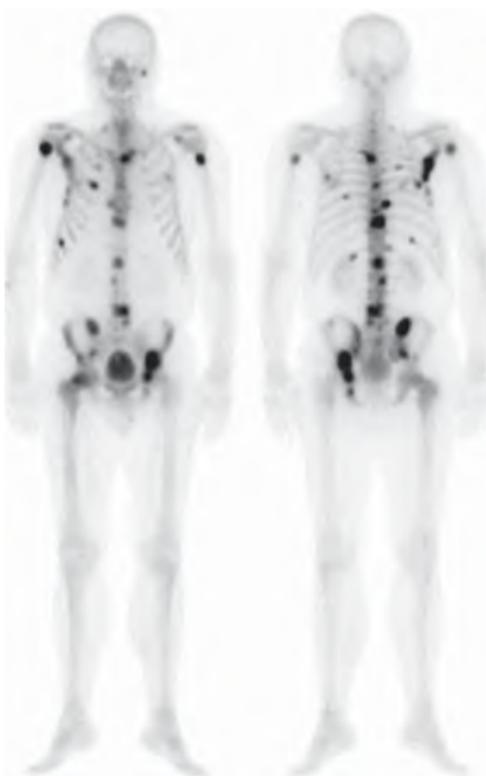


Fig. 14.2 Bone scan showing extensive metastases.



Fig. 14.3 Bone scan showing Paget's disease affecting the right hemipelvis and left distal femur.

Reticuloendothelial system: bone marrow scintigraphy

Background

Largely superseded by MRI. Colloid particles are cleared from the circulating blood pool by reticuloendothelial tissue—larger particles to the liver and spleen, smaller particles to the BM. Abnormal uptake pattern where the BM is replaced, e.g. by tumour infiltration.

Indications

- Suspected malignant marrow infiltration.
- Equivocal conventional bone imaging.
- Osteomyelitis (rarely used).

Patient preparation

None.

Procedure

- ^{99m}Tc -nanocolloid injected IV.
- Whole body gamma camera imaging at 30–45min.

Results

Normal marrow distribution in the thoracic cage, spine, pelvis, and proximal long bones. Homogeneous uptake in the liver and spleen.

Interpretation

- Focal or generalized ↓ skeletal uptake indicates marrow replacement or infiltration with marrow displacement to the distal femora and humeri.
- Heterogeneous hepatic uptake is abnormal but non-specific.

Advantages

Non-invasive. Avoids sampling errors, compared with BM biopsy.

Pitfalls

False –ves in early malignancy. Liver and spleen uptake may obscure abnormalities in the mid spine.



Cerebral blood flow imaging

Background

Used to study acute and chronic cerebrovascular disease, dementias, and epilepsy, using a neutral lipophilic ^{99m}Tc complex hexamethyl-propylene-amine-oxime (exametazime, HMPAO), which crosses the blood–brain barrier and fixes in proportion to regional blood flow, with high accumulation in cortical grey matter, compared with white matter. Images reported with CT/MRI correlation to ensure appropriate interpretation, compared with normal variants of cerebral asymmetry).

Indications

- Dementia characterization.
- Epilepsy for localization of epileptogenic focus.

Patient preparation

Secure venous access under resting conditions. Allow the patient to relax before injection of the radiopharmaceutical. Ensure that the patient can co-operate with the imaging procedure.

Procedure

- ^{99m}Tc -HMPAO (exametazime) injected IV in a quiet, darkened room, with the patient's eyes closed.
- Tomographic brain imaging undertaken immediately and may be repeated 4h later.

Results

Cortical grey matter uptake is proportional to blood flow (see Fig. 14.4a).

Interpretation

- Characteristic patterns of abnormal uptake recognized in different dementias (see Fig. 14.4b) and in cerebrovascular disease.
- ↓ uptake at epileptogenic focus on interictal scans—often changing to ↑ uptake on ictal imaging.

Advantages

Abnormalities on functional imaging should predate structural atrophy on anatomical imaging.

Pitfalls

- Tomographic image analysis degraded by movement artefact and asymmetric positioning.
- Data processing is operator-dependent.

Further reading

Kapucu OL, Nobili F, Varrone A, et al. EANM procedure guideline for brain perfusion SPECT using ^{99m}Tc -labelled radiopharmaceuticals, version 2. *Eur J Nucl Med Mol Imaging* 2009; 36: 2093–102.

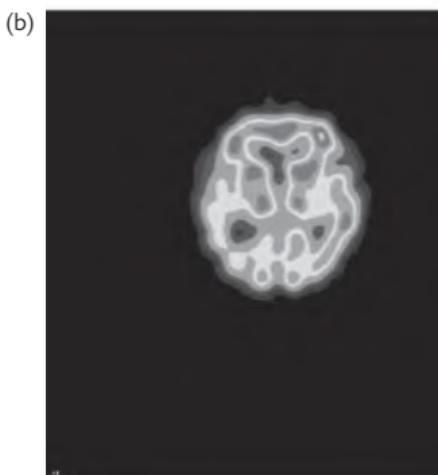
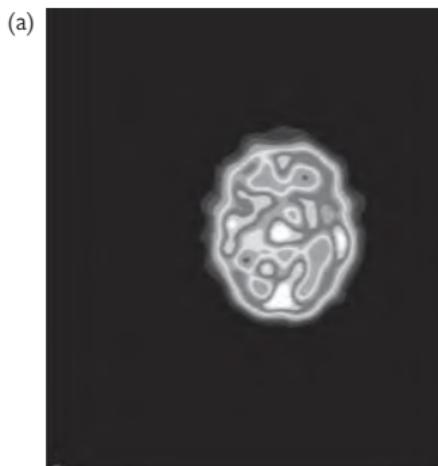


Fig. 14.4 ^{99m}Tc -HMPAO brain imaging. Trans-axial tomographic slices: (a) normal and (b) dementia. (→ Colour plate 6.)

Brain transporter imaging

Background

Reflects the role of the dopaminergic system in movement disorders, e.g. Parkinsonian syndromes, essential tremor. ^{123}I -ioflupane is a cocaine analogue, which binds to the dopamine transporter on the pre-synaptic nerve terminal. Post-synaptic receptor imaging agents are necessary to differentiate among the various Parkinsonian syndromes (not routinely available).

Indications

- Movement disorders: distinguishes Parkinson's syndrome (PkS) from benign essential tremor.

Patient preparation

- Block the thyroid—potassium iodate/iodide.
- Multiple potential drug interactions—stop:
 - Amphetamines.
 - Citalopram.
 - Cocaine.
 - Fluoxetine.
 - Fluvoxamine.
 - Mazindol.
 - Methylphenidate.
 - Orphenadrine.
 - Phentermine.
 - Procyclidine.
 - Sertraline.

Procedure

^{123}I -labelled radiotracer, e.g. ioflupane, injected IV. Tomographic gamma camera imaging 3–6h later.

Results

Intense, symmetric uptake in basal ganglia receptors—striatum, caudate, and putamen (see Fig. 14.5a).

Interpretation

↓ basal ganglia uptake in PkS (see Fig. 14.5b).

Advantages

Sensitive and specific for PkS, differentiating PkS from essential tremor. No other imaging technique currently available to demonstrate receptor status.

Pitfalls

Drug interactions (☞ Patient preparation above).



Fig. 14.5 ^{123}I -ioflupane brain transporter imaging: (a) normal dopamine transporters and (b) in Parkinson's disease. (Colour plate 7.)

Further reading

Darcourt J, Booij J, Tatsch K, et al. EANM procedure guidelines for brain neurotransmission SPECT using ^{123}I -labelled dopamine transporter ligands, version 2. *Eur J Nucl Med Mol Imaging* 2010; 37: 443–50.

Cerebrospinal fluid shunt patency

Background

Ventricular shunts are routinely used to manage hydrocephalus. Symptoms of shunt obstruction may be non-specific and do not indicate the level of obstruction. Nuclear medicine offers a straightforward means of determining shunt patency.

Indications

Suspected ventriculoperitoneal shunt obstruction.

Patient preparation

None.

Procedure

Inject ^{111}In -DTPA into the shunt reservoir using a strict aseptic technique. Image the head and abdomen immediately and 30–60min post-injection. ($^{99\text{m}}\text{Tc}$ -DTPA not recommended because difficult to ensure apyrogenicity.)

Results

Normally, rapid reservoir emptying and shunt visualization within 2–3min of injection. Free activity within the abdominal cavity by 30min (see Fig. 14.6).

Interpretation

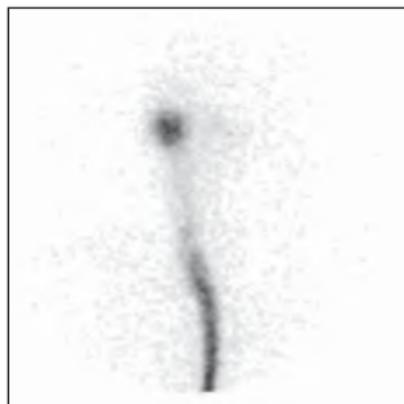
Delayed clearance implies obstruction—level usually at reservoir/proximal shunt or due to intra-abdominal kinking.

Advantages

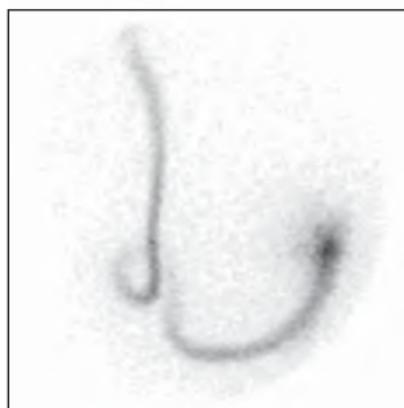
Sensitive, simple, rapid results.

Pitfalls

Infection risk.



Reservoir



Thorax

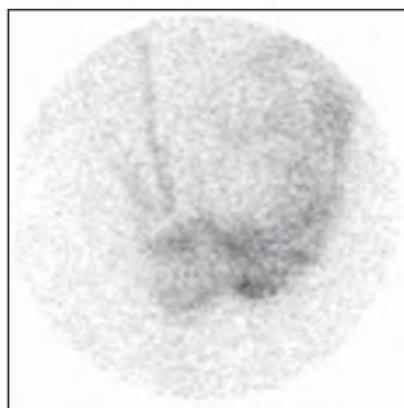
Free activity
in peritoneal
cavity

Fig. 14.6 Patent ventriculoperitoneal shunt showing reservoir, shunt, and free activity within the peritoneal cavity.

Thyroid scintigraphy

Background

Used to investigate thyrotoxicosis and thyroid ectopia. Thyroid mass or suspected malignancy should be investigated by US/CT and FNA cytology. Imaging is undertaken using ^{99m}Tc -pertechnetate, which is trapped by the thyroid by the same transporter mechanism as iodine but, unlike iodine, is not organified. ^{123}I is more sensitive and specific for the investigation of congenital hypothyroidism.

Indications

- Characterization of thyrotoxicosis—diffuse toxic goitre (Graves' disease), toxic multinodular goitre (Plummer's disease).
- Solitary autonomous nodule.
- Acute thyroiditis.

Patient preparation

Levothyroxine and iodine-rich preparations (e.g. iodine supplements, contrast media, amiodarone) will block tracer uptake by the thyroid for up to 9 months. T4 should be withdrawn for 4–6 weeks, T3 for 2 weeks. Antithyroid drugs can be continued.

Procedure

Inject ^{99m}Tc -pertechnetate IV. Image after 15–30min. Include anterior thorax views if retrosternal extension is suspected. Neck palpation essential to assess function in discrete thyroid nodules. ^{123}I may be administered either PO or IV. Imaging is carried out at 2h.

Results

- Uptake reflects function of the thyroid iodine trap (sodium iodide symporter).
- Diffuse ↑ uptake in Graves' disease (see Fig. 14.7a).
- Heterogeneous uptake with suppressed background activity indicates toxic multinodular change (see Fig. 14.7b).
- Solitary autonomous nodules show intense ↑ uptake, with complete suppression of the remainder of the gland (see Fig. 14.7c).
- Reduced tracer uptake in non-functioning nodules (see Fig. 14.7d).
- Acute thyroiditis is characterized by absent tracer uptake (see Fig. 14.7e).

Interpretation

Sensitive and specific for hyperthyroidism.

Advantages

Simple, cheap, and non-invasive. Essential to planning therapy in hyperthyroidism.

Pitfalls

Anatomical definition inferior to US, CT, etc. Superseded by US-guided FNA for diagnosis of thyroid mass lesions.

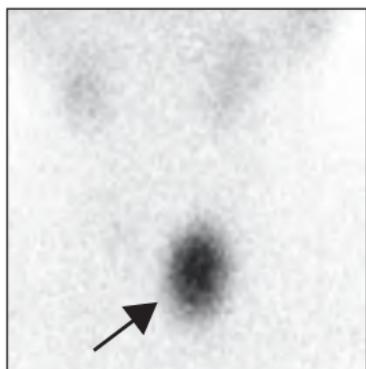
(a) Grave' disease



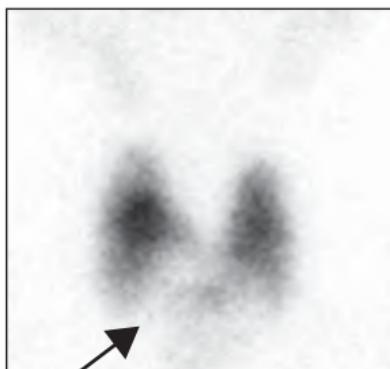
(b) Toxic multinodular goitre



(c) Toxic autonomous nodule



(d) Non-functioning nodule



(e) Thyroiditis

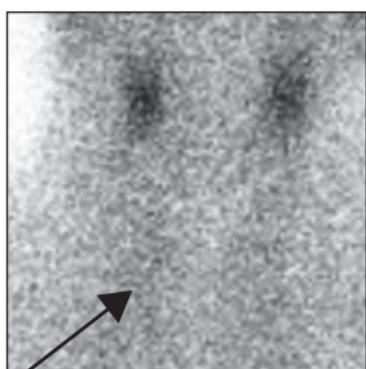


Fig. 14.7 Thyroid scintigraphy: (a) in Graves' disease (toxic diffuse hyperplasia) and (b) in toxic multinodular goitre, (c) toxic autonomous nodule (arrow), (d) non-functioning (cold nodule; arrow), and (e) thyroiditis (arrow).

Parathyroid scintigraphy

Background

Hyperparathyroidism may be 1° (i.e. functioning adenoma), 2° (where there is hypocalcaemia from chronic lowering of serum Ca^{2+} , e.g. renal insufficiency), or tertiary (i.e. hypersecretion of PTH in the presence of either normal or elevated levels of Ca^{2+}). Nuclear medicine is of value in localizing parathyroid adenomas, particularly following previous surgery.

Indications

Localization of parathyroid adenoma in proven hyperparathyroidism.

Patient preparation

Withdraw levothyroxine or iodine-containing compounds (cf. thyroid imaging).

Procedure

Three different techniques are available.

- $^{99\text{m}}\text{Tc}$ -sestamibi IV early (15 min) and delayed (2–3 h) images or
- ^{123}I -iodide IV followed by $^{99\text{m}}\text{Tc}$ -sestamibi, or
- $^{99\text{m}}\text{Tc}$ -pertechnetate followed by ^{201}Tl -thallous chloride.

Image the anterior neck and mediastinum after each administration.

Results

Normal thyroid concentrates ^{123}I , $^{99\text{m}}\text{Tc}$ -pertechnetate, $^{99\text{m}}\text{Tc}$ -sestamibi (initially), and ^{201}Tl , whereas parathyroid only concentrates $^{99\text{m}}\text{Tc}$ -sestamibi and ^{201}Tl .

Computer-assisted image subtraction [(thyroid + parathyroid) – thyroid] identifies abnormal parathyroid tissue.

Interpretation

Parathyroid adenoma shown as hyperfunctioning nodule(s) (see Figs 14.8–14.10.)

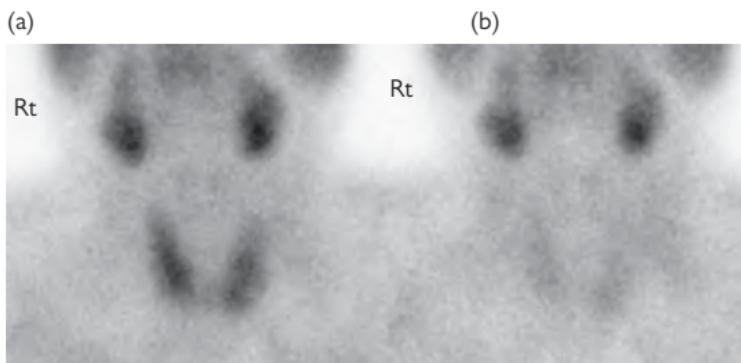


Fig. 14.8 Normal $^{99\text{m}}\text{Tc}$ -sestamibi dual-phase parathyroid scan; 5min image on left, 4h image on right.

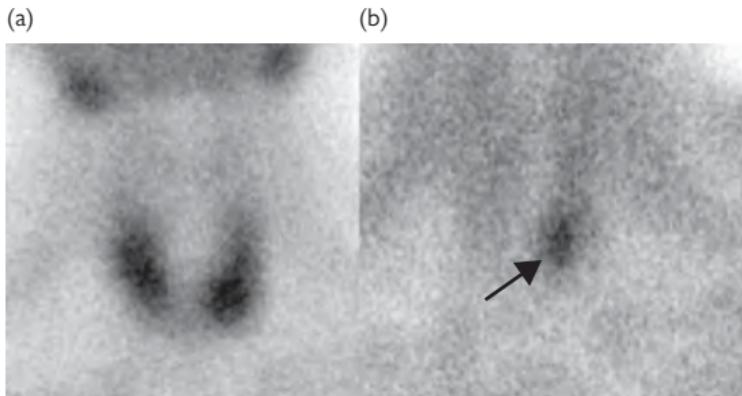


Fig. 14.9 ^{99m}Tc -sestamibi (dual-phase). Parathyroid adenoma at left lower lobe of the thyroid (arrow) evident on delayed image.

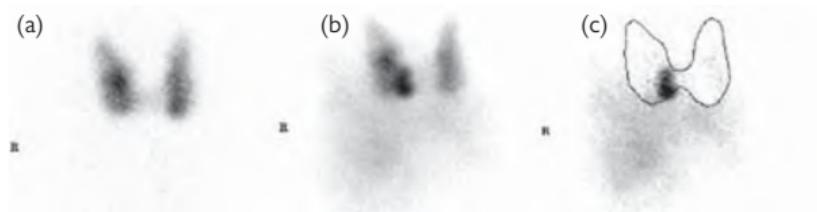


Fig. 14.10 Parathyroid scintigraphy showing images obtained with both (a) ^{123}I -iodine, (b) ^{99m}Tc -sestamibi, and (c) subtracted image showing a parathyroid adenoma.

Advantages

Good when other imaging fails, particularly ectopic adenomas and after unsuccessful neck exploration. Hybrid SPECT/CT images particularly helpful to surgeons.

Pitfalls

Multinodular thyroid prevents subtraction analysis in smaller adenomas and hyperplastic glands and thyroid nodules. Many surgeons still prefer intraoperative blue dye.

False -ves in smaller adenomas and hyperplastic glands.

False +ves in thyroid nodules.

Further reading

Hindié E, Ugur O, Fuster D, et al. 2009 EANM parathyroid guidelines. *Eur J Nucl Med Mol Imaging* 2009; 36: 1201–16.

Meta-iodobenzylguanidine (MIBG) imaging

Background

Neuroendocrine tumours (NETs) are rare. Symptoms reflect hormone hypersecretion, but intermittent secretory patterns can result in false –ve screening tests, e.g. 24h urine collections. MIBG (iomérendane) is concentrated by adrenergic tissue via the noradrenaline reuptake mechanism. Virtually all phaeochromocytomas and neuroblastomas will be demonstrated using ^{123}I -MIBG scintigraphy. The sensitivity for other NETs is variable. High-dose ^{131}I -MIBG therapy is a useful treatment for MIBG +ve NETs.

Indications

- Localization, staging, and response monitoring of neuroectodermal tumours.
- Phaeochromocytoma (imaging investigation of choice).
- Neuroblastoma.
- Carcinoid tumours.
- Medullary thyroid cancer.

Patient preparation

- Multiple known and theoretical drug interactions: *Stop for >48h:*
 - Antidepressants—tricyclics, tetracyclics, MAOIs, serotonin reuptake inhibitors.
 - Phenothiazines.
 - Labetalol (no interaction with any other α - or β -blocker or antihypertensive).
 - Levodopa, dopamine agonists.
 - Sympathomimetics—including over-the-counter nasal decongestants.
- *Block the thyroid:* potassium iodate/iodide; perchlorate.

Procedure

Inject ^{123}I -MIBG slowly IV with BP monitoring. Image the posterior abdomen at 5min to identify renal outlines, then whole body imaging at 18–24h. Tomographic imaging may improve tumour localization—not always required.

Results

Physiological uptake at 24h in the salivary glands, myocardium, liver, and normal adrenals, with gut and renal excretion.

Interpretation

- Intense ↑ uptake in phaeochromocytomas, with suppressed activity in the contralateral and normal adrenal, and myocardium. Whole body imaging identifies extra-adrenal and metastatic disease (see Fig. 14.11).
- Diffuse BM uptake common in stage IV neuroblastoma.



Fig. 14.11 ^{123}I -MIBG whole body scan. ^{123}I -MIBG positive left adrenal mass (pheochromocytoma). Excludes multi-focal, ectopic, and malignant tumour.

Advantages

Sensitive, non-invasive tumour localization preoperatively excludes multi-focal and extra-adrenal tumours. Non-invasive treatment response monitoring in neuroblastoma—avoids sampling errors, compared with BM biopsy.

Pitfalls

Drug interactions causing false -ve results. Dilated renal pelvis sometimes confused with tumour uptake. Check with 5min renal image if in doubt.

Somatostatin receptor scintigraphy: SPECT

Background

Somatostatin receptor (SSR) imaging identifies NETs, including gastroenteropancreatic tumours, e.g. carcinoids, gastrinomas, insulinomas. Many other common neoplasms also express surface SSRs. Somatostatin analogues, e.g. octreotide, bind to cell surface SSRs. Radiolabelled SSR analogues demonstrate receptor +ve disease. Sub-centimetre (i.e. below the limits of cross-sectional radiology) hyperfunctioning 1° tumours can be detected. High-dose radiolabelled SSR therapy is used to treat multi-site disease.

Indications

Localize and stage NETs, e.g. carcinoid, insulinoma, gastrinoma, phaeochromocytoma, and medullary thyroid cancer.

Patient preparation

None. Prophylactic laxatives at time of radiopharmaceutical administration accelerate gut clearance and improve image quality.

Procedure

Inject ^{111}In -pentetetreotide or $^{99\text{m}}\text{Tc}$ hynic-octreotide (SSR analogues) IV. Whole body gamma camera imaging at 4 and 24h ($\pm 48\text{h}$), with SPECT if necessary.

Results

Normal uptake in the thyroid, liver, spleen, kidneys, and RES, with gut and renal excretion.

Interpretation

↑ uptake in tumours expressing surface SSRs. SPECT improves detection of small pancreatic and intra-hepatic tumours (see Fig. 14.12).

Advantages

Tumour uptake predicts symptom response to somatostatin analogue therapy. Image co-registration with CT or MRI improves localization of occult pancreatic NETs.

Pitfalls

Interpretation often hindered by gut excretion.

Further reading

Bombardieri E, Ambrosini V, Aktolun C, et al. ^{111}In -pentetetreotide scintigraphy: procedure guidelines for tumour imaging. *Eur J Nucl Med Mol Imaging* 2010; **37**: 1441–8.

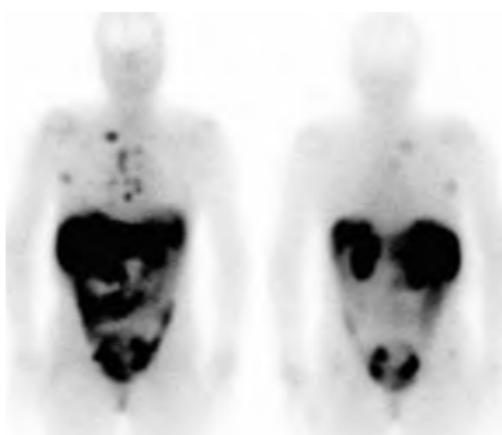


Fig. 14.12 Whole body ^{111}In -octreotide scan in a patient with a neuroendocrine tumour showing somatostatin receptor positive hepatic and extra-hepatic metastases.

Radioiodine thyroid cancer imaging

Background

The treatment for thyroid carcinoma is total thyroidectomy with lymph node dissection, depending on tumour stage. Radioactive iodine is administered post-operatively to ablate the thyroid remnant. Tg can then be used as a tumour marker—Tg is undetectable in the absence of functioning thyroid tissue. Rising Tg following ^{131}I ablation indicates recurrence. If Tg rises, a diagnostic ^{131}I imaging study localizes the site of relapse and assesses the feasibility of further radioiodine therapy. The sensitivity of imaging is ↑ by recombinant TSH stimulation.

Indications

Routine differentiated follicular thyroid cancer follow-up, after surgery and ^{131}I thyroid remnant ablation.

Patient preparation

Need high TSH drive to stimulate ^{131}I uptake—stop T3/T4 replacement for a minimum of 2 (T3) or 4–6 weeks (T4), or give recombinant TSH. Avoid cold iodine administration, IV contrast media, and amiodarone (cf. thyroid imaging).

Procedure

Give ^{131}I sodium iodide PO/IV. Obtain blood samples for Tg and TSH at the time of ^{131}I administration. Whole body gamma camera imaging 2–5 days later.

Results

Physiological uptake in salivary glands. Occasional stomach and GI retention. Renal excretion.

Interpretation

Abnormal uptake indicates functioning thyroid metastasis. Anatomical markers improve localization (see Fig. 14.13a and b).

Advantages

Detects residual tumour and identifies patients likely to benefit from ^{131}I therapy.

Pitfalls

False –ve without significant TSH drive—aim for TSH >50mU/L; undifferentiated and papillary tumours may be ^{131}I –ve.

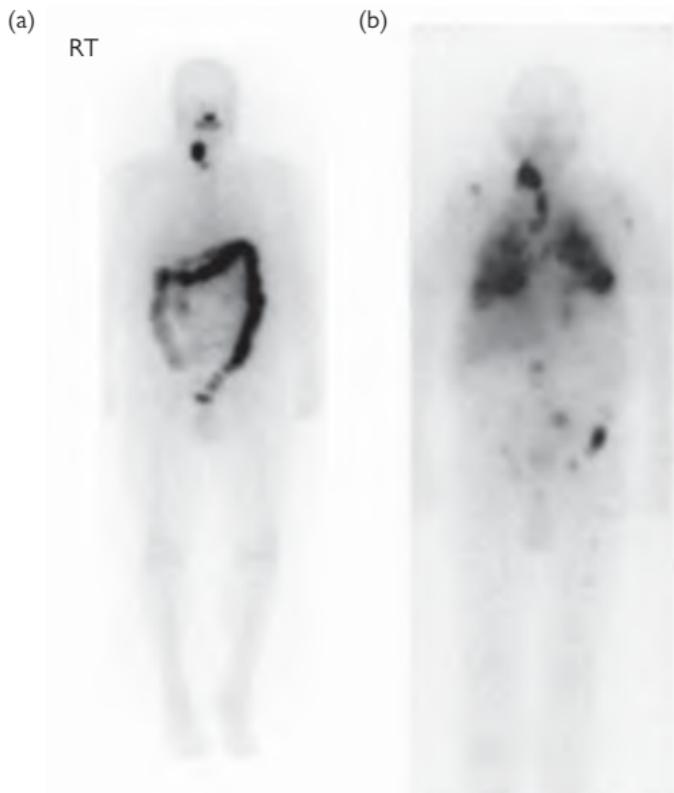


Fig. 14.13 Anterior whole body ^{131}I image showing (a) local tumour recurrence in the thyroid bed and physiological activity in the bowel and (b) local recurrence in the thyroid bed with lung, mediastinal nodal, and skeletal metastases.

Sentinel node imaging

Background

Regional lymph node dissection is performed for cancer staging to determine the need for adjuvant therapy. Lymphatic drainage can be demonstrated by radiolabelled colloid imaging, which identifies the first or 'sentinel' draining node. Staging based on the excision and histological examination of this node for evidence of metastasis is as reliable as that obtained from block dissection and avoids the morbidity of extended lymph node dissection.

Indications

Preoperative assessment in breast cancer and melanoma. May have applications in head and neck, vulval, and penile cancer staging.

Patient preparation

None. Usually undertaken within 24h of planned surgery.

Procedure

Intradermal, subcutaneous, or intratumoural injection of ^{99m}Tc -labelled nanocolloid. Gamma camera imaging of draining lymph nodes to identify sentinel node. Where surgery is undertaken within 24h, an intra-operative gamma probe can be used to identify the sentinel node for staging excision biopsy.

Results

Sentinel node usually identifiable 15min to 2h post-injection, depending on the 1° tumour location and injection technique used.

Interpretation

The sentinel node is the first lymph node identified on gamma imaging or the node with the highest radioactive count rate using the gamma probe (see Fig. 14.14a and b).

Advantages

Accurate sentinel node identification avoids block node dissection where this is undertaken solely for tumour staging.

Pitfalls

May fail if local lymphatic channels have been disrupted by previous surgery.

Further reading

Procedure guidelines for several types of cancer are available at:  <http://www.eanm.org>.

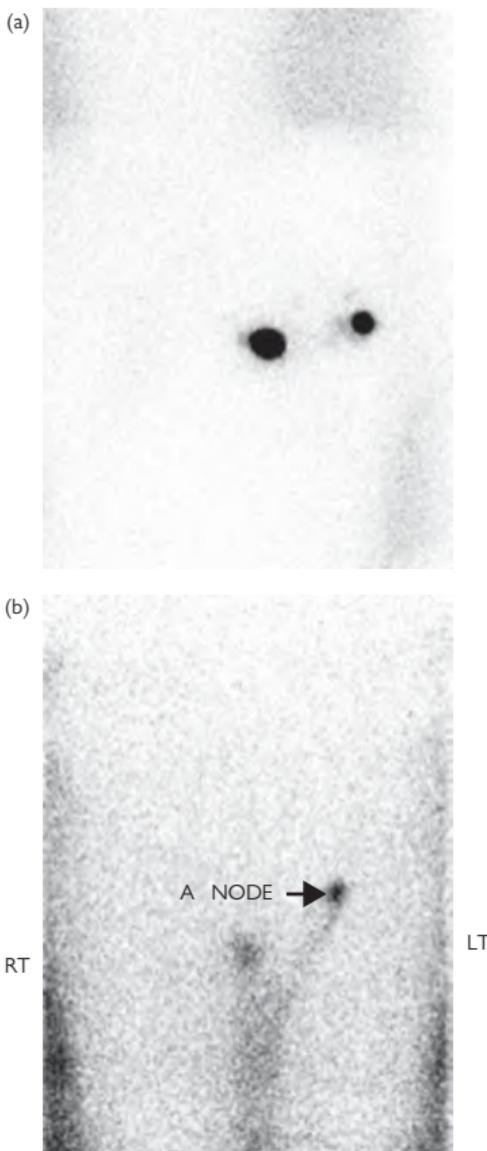


Fig. 14.14 ^{99m}Tc -nanocolloid sentinel node study. (a) Breast cancer: left breast injection and sentinel lymph node in left axilla (arrow). (b) Melanoma left thigh: left thigh injection (not shown) and sentinel lymph node in left groin (arrow).

Scintimammography

Breast cancer diagnosis relies on accurate localization (US ± mammography) and tissue biopsy (FNA) or core biopsy. Where mammography is non-diagnostic, MRI, CT, or ¹⁸F-FDG PET/CT are useful. Nuclear medicine scintimammography is as sensitive as, but more specific than, MRI and mammography in palpable lesions.

Indications

Investigation of suspicious breast lesions, in difficult-to-interpret mammograms, e.g. dense or lumpy breast tissue, calcification, breast implants, previous surgery.

Patient preparation

None.

Procedure

^{99m}Tc-sestamibi administered IV ideally into the contralateral foot, with early (5–10min) imaging post-injection. Patient is imaged prone, with the breast fully dependent, with prone and lateral views of each breast, to include the axillae.

Results

Normal distribution of ^{99m}Tc-sestamibi is to the myocardium, the liver, and occasionally the thyroid.

Interpretation

Focal accumulation in the breast and/or axilla implies the presence of a tumour. Findings should be interpreted in conjunction with other tests.

Advantages

Can demonstrate multi-focal, multicentric disease, and both ipsilateral and contralateral axillary spread. May be used to identify the most suitable site for guided biopsy.

Pitfalls

Not reliable in small (<1cm) lesions; extravasation of injection in upper limbs may result in false +ve axillary uptake.

Poor injection technique will lead to errors in analysis. The camera and supporting software require high count rate capability, and the technique requires expertise in data analysis to ensure reliable, reproducible results. Close liaison with the referring clinician is essential to maximize the value of the investigation.

Further reading

- Buscombe J, Hill J, Parbhoo S. *Scintimammography: A Guide to Good Practice*. Birmingham: Gibbs Associates Ltd, 1998.
Taillefer R. Clinical applications of ^{99m}Tc-sestamibi scintimammography. *Semin Nucl Med* 2005; 35: 100–15.

Positron emission tomography (PET)

PET has expanded rapidly over the last 20 years. Tomographic PET images are acquired using a dedicated PET scanner after administration of positron-emitting radiopharmaceuticals. Spatial resolution (~5mm) is significantly superior to conventional nuclear medicine imaging.

PET uses biologically important molecules such as radiolabelled water, ammonia, amino acids, or glucose derivatives—the glucose analogue ^{18}F -fluorodeoxyglucose (^{18}F -FDG) being used for most clinical PET studies, particularly in oncology. Malignant cells have both a higher glycolytic rate and over-expression of membrane glucose transporters, leading to high ^{18}F -FDG uptake, compared to normal tissues. ^{18}F -FDG is trapped within metabolically active cells, so that abnormalities are detected by metabolic differences, rather than anatomical size, i.e. inherently more sensitive than structure-based imaging.

The main indications for PET imaging are in oncology where ^{18}F -FDG PET is used for diagnosis, staging, and monitoring treatment response. Low-grade uptake occurs in granulomatous disease, inflammation, and sepsis.

PET allows radioactive concentrations within tissues to be measured accurately, so that physiological processes can be expressed in absolute units. The standardized uptake value (SUV) measures the concentration of tracer within a tumour, compared to the injected activity, normalized to body weight. Serial SUV measurements allow ^{18}F -FDG uptake to be followed as a marker of treatment response and may be of prognostic value.

Other major applications include nuclear cardiology where patterns of myocardial ^{18}F -FDG uptake are used to detect myocardial hibernation. In neurosciences, PET remains a largely research tool for the investigation of movement disorders, dementia, and degenerative disease.

Other oncological tracers that are available for clinical use include ^{11}C -methionine, measuring tumour amino acid transport and protein synthesis and H_2^{15}O water for blood flow measurements. ^{68}Ga -labelled somatostatin peptides are used to image NETs. Future developments will include labelled thymidine analogues (e.g. ^{18}F -FLT) to measure proliferation, hypoxia markers, and tracers capable of detecting apoptosis and angiogenesis.

The main limitation of PET imaging in oncology is limited anatomical definition. To improve attenuation correction and tomographic localization, PET imaging is usually combined with CT (or MRI). The combination of functional and anatomical data in fused images significantly improves the sensitivity and specificity of imaging.

Indications

- *Tumour diagnosis:* solitary pulmonary nodule characterization, location of carcinoma of unknown 1^o origin.
- *Tumour staging:* non-SCLC; lymphoma; oesophageal cancer; colorectal cancer; head and neck cancer; melanoma.
- *Response assessment/relapse detection:* as above.

Patient preparation

- Cellular ^{18}F -FDG uptake is glucose-dependent.
 - Non-diabetic patients—6h fast.
 - Aim for blood glucose <7mmol/L.
 - Insulin-dependent diabetic patients—allow a normal diet and insulin.
 - Avoid hyperglycaemia—use a sliding scale if necessary or defer the investigation.
- Patients should be rested: to avoid skeletal muscle uptake.
- *Diazepam administration:* 5mg PO reduces physiological brown fat uptake in young patients.
- Patients with head and neck cancer should be silent during injection and until completion of imaging to prevent vocal cord uptake.

Procedure

- ^{18}F -FDG injected IV supine in restful surroundings.
- Whole body or half-body (base of skull to proximal femora) imaging undertaken 60–90min post-injection.

Results

The normal distribution of ^{18}F -FDG is to the brain and myocardium, with GI and renal excretion (see Fig. 14.15).

Interpretation

Abnormal uptake should be correlated with cross-sectional imaging for precise anatomical localization (see Figs 14.16 and 14.17). SUV (⊕ pp. 894–7) measurement is helpful in serial studies.



Fig. 14.15 Normal ^{18}F -FDG PET scan—coronal tomogram.

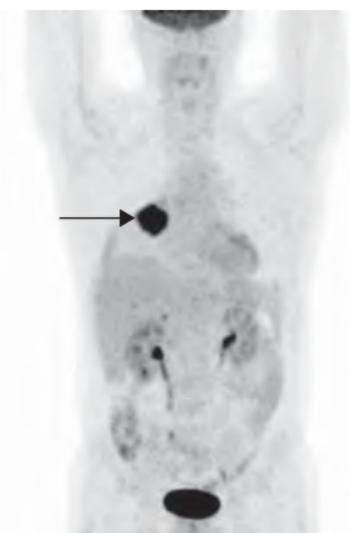


Fig. 14.16 ^{18}F -FDG PET scan—non-SCLC right lung (arrow). Whole body coronal.

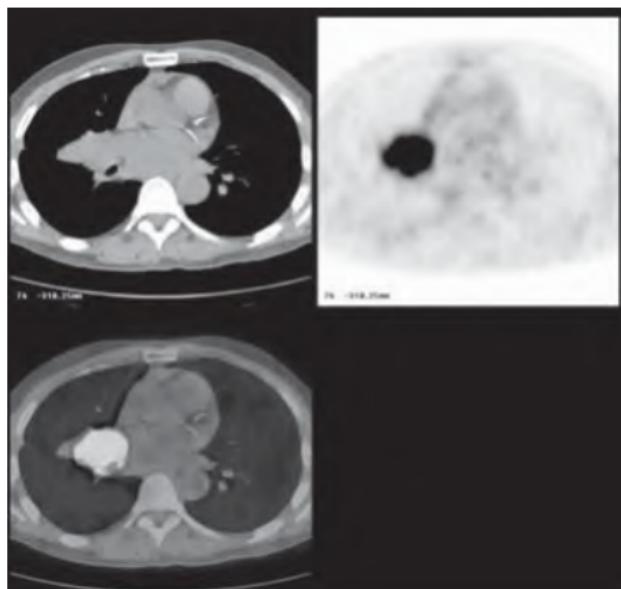


Fig. 14.17 ^{18}F -FDG PET scan—trans-axial views showing CT (top left) correlation with PET (top right). The image fusion is shown (bottom left). (Colour plate 8.)

Advantages

- Discriminates between viable and necrotic/scar tissue where a residual mass may persist on anatomical imaging post-therapy (see Figs 14.18 and 14.19).
- Non-invasive, whole body 3D imaging.

Pitfalls

- Sensitivity lower in more indolent tumours—lung carcinoid, alveolar cell carcinoma, NETs, depending on proliferative index, and occasionally in pancreatic tumours.
- Limited availability—specialist centres only.
- Relatively expensive.

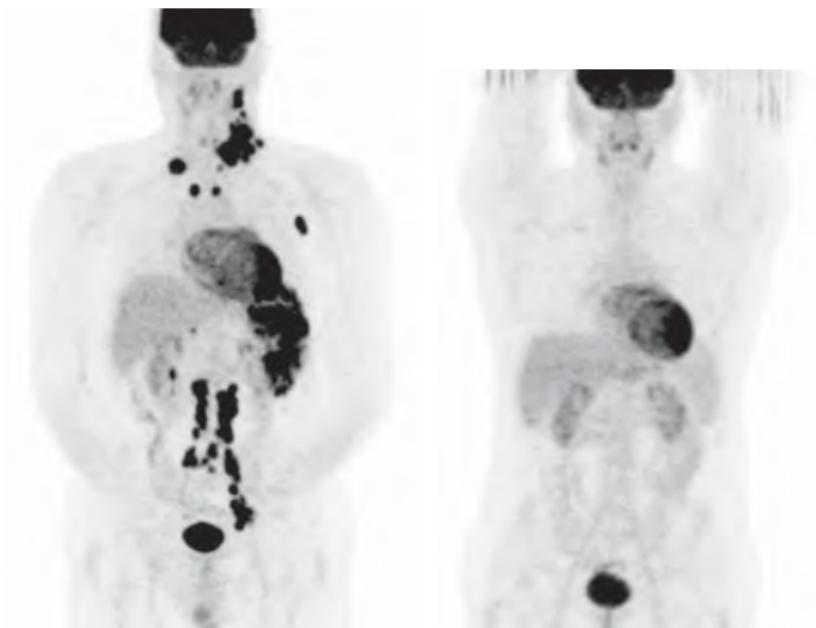


Fig. 14.18 ^{18}F -FDG PET scan—non-Hodgkin's lymphoma. Whole body coronal view of patient pre-treatment showing extensive FDG-avid adenopathy.

Fig. 14.19 ^{18}F -FDG PET scan—non-Hodgkin's lymphoma. Post-treatment showing complete metabolic response.

Somatostatin receptor scintigraphy: ^{68}Ga -DOTA-peptide PET/CT

Background

SSR imaging identifies NETs, including gastroenteropancreatic tumours, e.g. carcinoids, gastrinomas, insulinomas. Many other common neoplasms also express surface SSRs. Somatostatin analogues, e.g. octreotide, bind to cell surface SSRs. Radiolabelled SSR analogues demonstrate receptor +ve disease. High-dose radiolabelled SSR therapy is used to treat multi-site disease. Currently, given its high accuracy, compared with conventional SPECT imaging techniques, ^{68}Ga -DOTA-peptide PET/CT is considered to be the first-line diagnostic imaging technique of choice for high SSR-expressing tumours.

Indications

Localize and stage NETs, e.g. carcinoid, insulinoma, gastrinoma, phaeochromocytoma, and medullary thyroid cancer.

Patient preparation

Best option is to perform the study after discontinuing short-acting octreotide for 12–24h and perform imaging in the week before the next dose of long-acting octreotide.

Procedure

Inject ^{68}Ga -labelled SSR analogue (DOTA-TATE/DOTA-NOC) IV. Whole body (vertex to mid thigh) PET/CT imaging SSR PET/CT is performed 45–60min after radiotracer injection.

Results

Normal uptake in the spleen, adrenal glands, kidneys, and pituitary gland. Moderately intense uptake is often seen in the liver, salivary glands, and thyroid gland (see Fig. 14.20a).

Interpretation

↑ uptake in tumours expressing surface SSRs (see Fig. 14.20b).

Advantages

Short half-life of 68min (compared with 2.8 days for ^{111}In); lower radiation dose to the patient; imaging performed 45–60min after radiotracer injection; more accurate than conventional imaging; and allows identification of additional sites of disease and helps identify patients who are suitable for radiopeptide therapy (e.g. ^{177}Lu -DOTA-TATE).

Pitfalls

False –ve: octreotide therapy or the endogenous production of somatostatin by the tumour may interfere with tumour detection. False +ve: prominent pancreatic uncinate process uptake, benign meningioma inflammation, osteoblastic activity (degenerative bone disease, fracture, vertebral haemangioma), splenunculi or splenosis, etc.

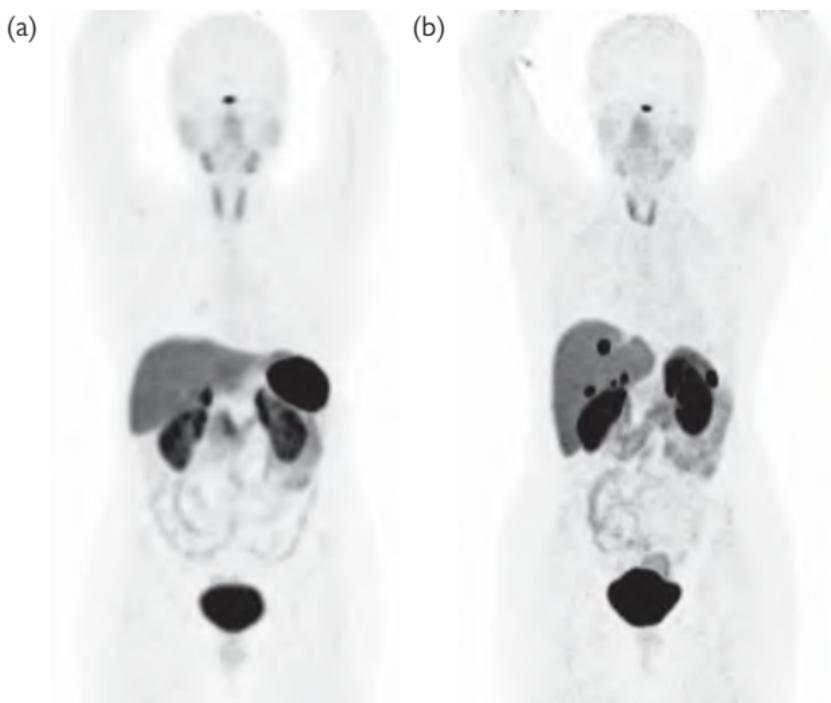


Fig. 14.20 (a) Normal ^{68}Ga -DOTA-TATE scan; (b) abnormal ^{68}Ga -DOTA-TATE scan showing liver metastases.

Further reading

Virgolini I, Ambrosini V, Bomanji JB, et al. Procedure guidelines for PET/CT tumour imaging with ^{68}Ga -DOTA-conjugated peptides: ^{68}Ga -DOTA-TOC, ^{68}Ga -DOTA-NOC, ^{68}Ga -DOTA-TATE. *Eur J Nucl Med Mol Imaging* 2010; 37: 2004–10.

Prostate-specific membrane antigen: ^{68}Ga -PSMA PET/CT

Background

Prostate-specific membrane antigen (PSMA) is a cell surface protein with high expression in prostate cancer cells, and over-expression enables targeting of prostate cancer metastases using ^{68}Ga -labelled PSMA ligands for PET/CT imaging.

Indications

High-risk prostate cancer prior to radical prostatectomy, patients with rising PSA levels after radical prostatectomy, and diagnosis of recurrent prostate cancer.

Patient preparation

None.

Procedure

^{68}Ga -PSMA is injected IV and PET/CT performed 60min post-injection.

Results

Physiological uptake in kidneys and salivary glands. ↑ uptake in prostate cancer cells expressing PSMA (see Fig. 14.21a).

Interpretation

↑ tracer uptake in tumours expressing PSMA (prostate and extra-prostatic) (see Fig. 14.21b).

Advantages

Superior to choline tracers in detecting prostate cancer cells and recurrent disease at low PSA levels.

Pitfalls

False -ve: limited detection of micrometastases. False +ve: PSMA is also expressed in tumour-associated neovasculature of most solid tumours.

Further reading

Maurer T, Gschwend JE, Rauscher I, et al. Diagnostic efficacy of $^{68}\text{Gallium}$ -PSMA positron emission tomography compared to conventional imaging in lymph node staging of 130 consecutive patients with intermediate to high risk prostate cancer. *J Urol* 2016; **195**: 1436–43.

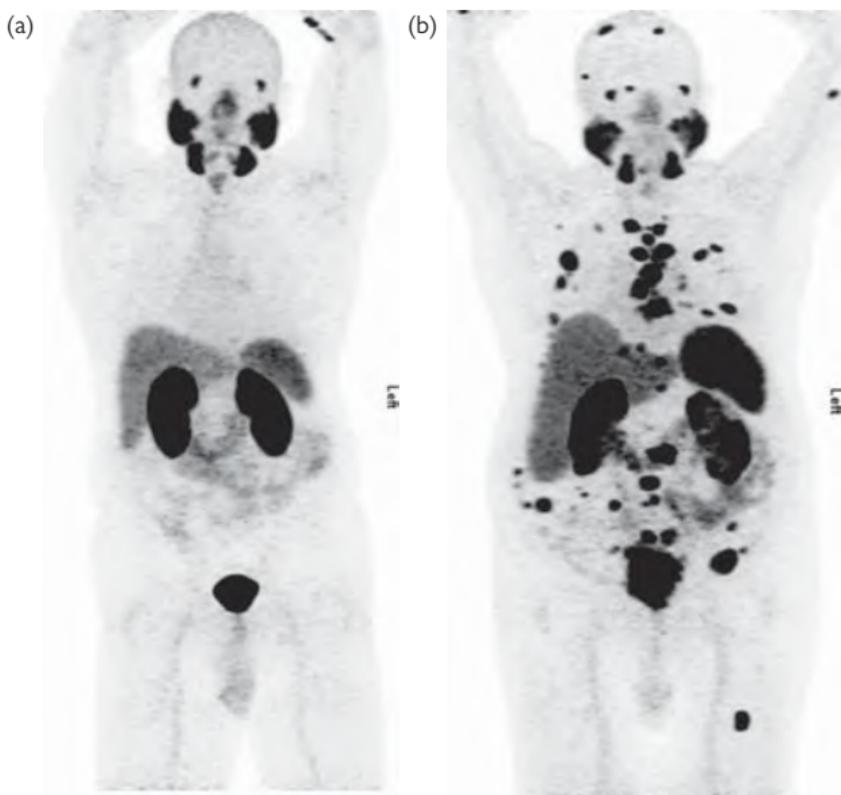


Fig. 14.21 (a) Normal ^{68}Ga -PSMA scan; (b) abnormal scan with multiple osseous and extra-osseous metastases.

¹¹C-choline/¹⁸F-fluorocholine PET/CT

Background

Following radical prostatectomy for treatment of prostate cancer, recurrent disease is suspected if there is rising PSA. ¹⁸F-choline PET/CT is often used in restaging of patients with prostate cancer (¹¹C-choline or ¹¹C-acetate PET can also be used where available).

Indications

Prostate cancer patients with rising PSA levels after radical prostatectomy and in patients with recurrent prostate cancer.

Patient preparation

Fasting for 6h recommended to minimize impact of dietary choline. Patient should be well hydrated.

Procedure

¹¹C-choline (half-life 20min) is injected IV and PET images acquired over 0–15min. ¹⁸F-choline (half-life 110min) is injected IV and scan is performed at 1h.

Results

Physiological uptake in the liver, pancreas, spleen, salivary and lacrimal glands, urinary tract, and less commonly in BM and intestines (see Fig.14.22a).

Interpretation

↑ tracer uptake in recurrent prostate cancer and metastases (see Fig.14.22b).

Advantages

High specificity in lymph node staging, high sensitivity in recurrent disease, and often helps in treatment planning.

Pitfalls

(a) Limited accuracy for the staging of the 1° tumour; (b) often unable to differentiate prostate cancer from benign prostate hyperplasia, chronic prostatitis, and high-grade intraepithelial neoplasia; and (c) high background signal frequently hampers lesion detection.

Further reading

Schillaci O, Calabria F, Tavolozza M, et al. ¹⁸F-choline PET/CT physiological distribution and pitfalls in image interpretation: experience in 80 patients with prostate cancer. *Nucl Med Commun* 2010; 31: 39–45.



Fig. 14.22 (a) Normal ¹⁸F-choline scan; (b) abnormal scan with pelvic nodal metastases (arrows).

¹⁸F-fluoride PET/CT

Background

¹⁸F-fluoride is a highly sensitive bone-seeking PET tracer used for detection of skeletal pathology. In general, the tracer uptake mechanism is similar to conventional SPECT tracer. The CT component of the PET/CT study allows localization, characterization, and differentiation of skeletal metastases from benign lesions.

Indications

Similar to ^{99m}Tc-MDP bone scan:

- Tumour staging—assess skeletal metastases.
- Bone pain.

Patient preparation

Patients should be (a) well hydrated and (b) instructed to empty their bladder immediately before imaging.

Procedure

¹⁸F-fluoride is injected IV. The adult activity is 185–370MBq. Paediatric activity should be weight-based (2.22MBq/kg).

Results

- Radiopharmaceutical uptake reflects osteoblastic activity (see Fig. 14.23a).
- Focal ↑ uptake in sclerotic metastases (see Fig. 14.23b), trauma, or infection.
- Diffuse ↑ uptake associated with advanced metastases, Paget's, and metabolic bone disease.

Interpretation

Highly sensitive, but non-specific. Interpretation relies on pattern recognition in the clinical setting. The CT component of PET/CT, even when performed primarily for attenuation correction and anatomic registration, also provides diagnostic information.

Advantages

Sensitive—detects early changes in bone physiology, often before abnormal plain radiographs, e.g. occult trauma, metastases, and sepsis.



Fig. 14.23 (a) Normal ¹⁸F-fluoride scan; (b) abnormal scan with multiple skeletal metastases in the spine.

Further reading

Segall G, Delbeke D, Stabin MG, et al. SNM practice guideline for sodium ¹⁸F-fluoride PET/CT bone scans 1.0. *J Nucl Med* 2010; 51: 1813–20.

Myocardial perfusion imaging

Background

MPI reflects regional blood flow during stress (\uparrow demand) and at rest, providing prognostically significant information that, both in isolation and in conjunction with coronary angiography, can be used to optimize patient management. Originally performed using ^{201}TI -thallous chloride, but now based on $^{99\text{m}}\text{Tc}$ -labelled tracers—sestamibi (methoxy-isobutylisonitrile) or tetrofosmin. Exercise or pharmacological stress are used to challenge the coronary artery reserve. Exercise is performed by treadmill or bicycle, whilst pharmacological stressors are either adenosine/dipyridamole infusion, which \uparrow coronary artery blood flow by vasodilatation, or dobutamine infusion with both inotropic and chronotropic activity. Can also be performed using PET/CT with ^{82}Rb -rubidium chloride or ^{13}N -ammonia as tracer.

Indications

Ischaemic heart disease.

Pre-angiography

- When conventional stress testing fails, e.g. bundle branch block.
- Left ventricular hypertrophy.
- Atypical chest pain.
- Recurrent chest pain post-intervention. Good prognostic indicator.

Post-angiography

- Assess functional significance of known stenoses.
- Identify critical vascular territory for intervention.

Patient preparation

- Stop β -blockers 24h prior to stress study.
- Sometimes helpful to withdraw all anti-anginal medication.
- Assess the optimal stress technique for individual patients, i.e. exercise or pharmacological.
- Attach 12-lead ECG.
- Insert IV cannula.
- Check baseline BP.

Procedure

Two-part investigation comparing myocardial perfusion during stress and at rest

Stress test

- Treadmill or bicycle exercise to $>85\%$ of maximum predicted heart rate or adenosine $140\mu\text{g}/\text{kg}/\text{min}$ IV infusion for 6min—sometimes with submaximal exercise or dobutamine $5\text{--}40\mu\text{g}/\text{kg}/\text{min}$ in $5\mu\text{g}/\text{kg}/\text{min}$ increments over 16min.
- Inject the radiopharmaceutical (^{201}TI -thallous chloride, $^{99\text{m}}\text{Tc}$ -sestamibi, or $^{99\text{m}}\text{Tc}$ -tetrofosmin) at peak stress.
- Tomographic imaging immediately (^{201}TI) or 15–60min post-injection ($^{99\text{m}}\text{Tc}$ compounds). Images generally acquired with ECG gating.

Rest study

- Second ^{99m}Tc radiopharmaceutical injection under resting conditions.
- Tomographic imaging as before.
- With ^{201}TI , second injection not necessary, since tracer redistributes into ischaemic areas over 4h, but top-up dose sometimes given.

Results

Myocardial uptake reflects radiopharmaceutical delivery and myocyte function (see Fig. 14.24).

Interpretation

Infarction causes matched perfusion defects during stress and rest. Inducible ischaemia creates a perfusion defect at stress, which reperfuses at rest = reversible ischaemia (see Fig. 14.25). The severity, extent, and number of reversible defects are prognostically significant. A normal MPI study implies risk of an adverse cardiac event of <0.5% per annum.

Advantages

Non-invasive; relatively inexpensive, compared with angiography.

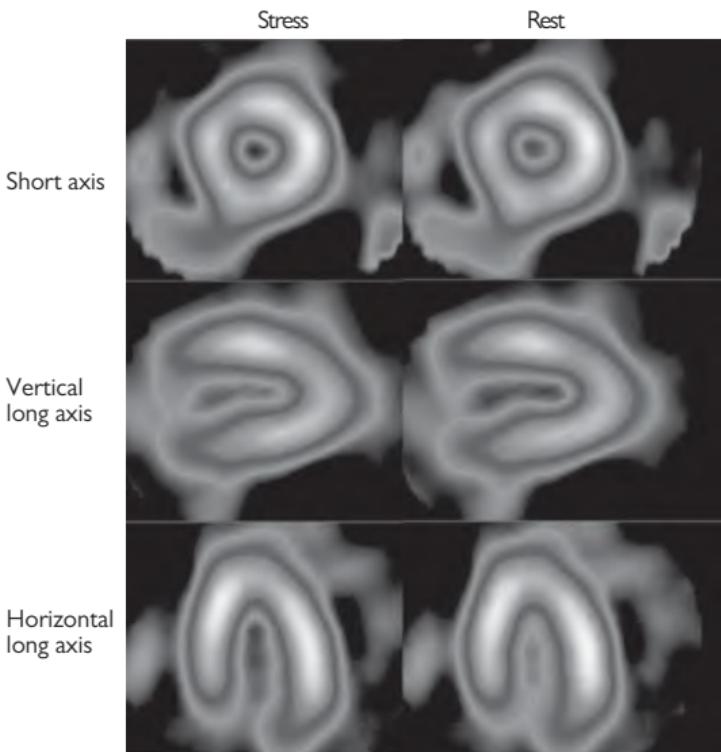


Fig. 14.24 Normal myocardial perfusion scan. (Colour plate 9.)

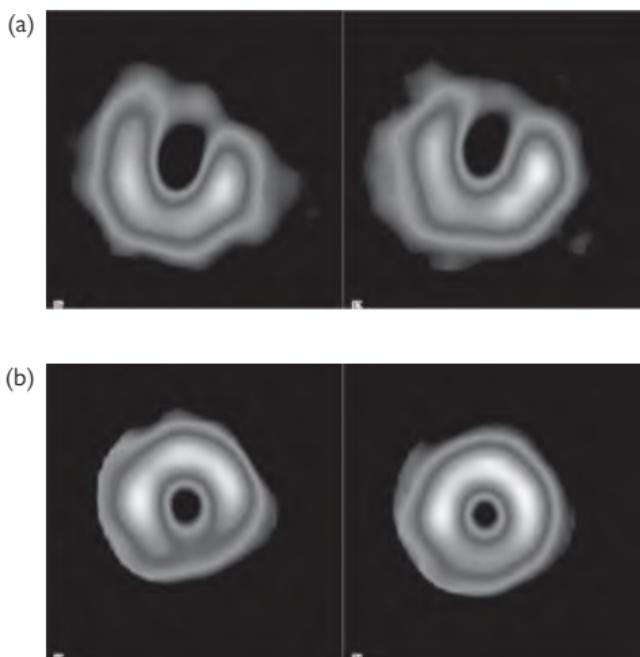


Fig. 14.25 Myocardial perfusion scan: (a) in fixed perfusion loss (anterolateral infarction) and (b) in inferior stress-induced (reversible) ischaemia. (Colour plate 10.)

Pitfalls

Less sensitive in multiple small-vessel coronary disease, e.g. DM. Sensitivity depends on stress test quality.

Further reading

Marcassa C, Bar JJ, Bengel F, et al. Clinical value, cost-effectiveness, and safety of myocardial perfusion scintigraphy: a position statement. *Eur Heart J* 2008; **29**: 557–63.

Verberne HJ, Acampa W, Anagnostopoulos C, et al. EANM procedural guidelines for radionuclide myocardial perfusion imaging with SPECT and SPECT/CT: 2015 revision. *Eur J Nucl Med Mol Imaging* 2015; **42**: 1929–40.

Radionuclide ventriculography: MUGA scan

Background

Increasingly replaced by TTE, TOE, and contrast ventriculography at time of cardiac catheterization. Multigated radionuclide angiography (MUGA) scans are less operator-dependent than either TTE or TOE, which is important in serial assessment. The cardiac blood pool is imaged dynamically after injection of radiolabelled RBCs. Wall motion, synchronicity of ventricular contraction, and EF are measured (see Fig. 14.26). The estimated EF unreliable in presence of dysrhythmias—acquisition requires a relatively regular heart rate.

Indications

- LVEF measurement, e.g. unechogenic patients (see Fig. 14.27).
- Monitor anthracycline cardiotoxicity.

Patient preparation

None.

Procedure

Radiolabel red cells (*in vivo* or *in vitro*) using ^{99m}Tc -pertechnetate. Image the patient supine in anterior and left anterior oblique projections. Camera acquisition gated to cardiac cycle. Imaging may be combined with low-impact exercise/pharmacological stress to assess cardiac reserve.

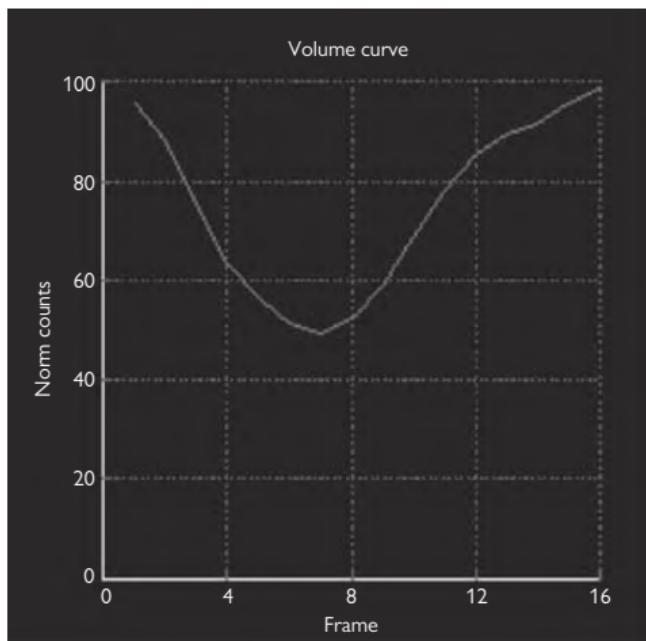


Fig. 14.26 Computer-generated EF of 40%.

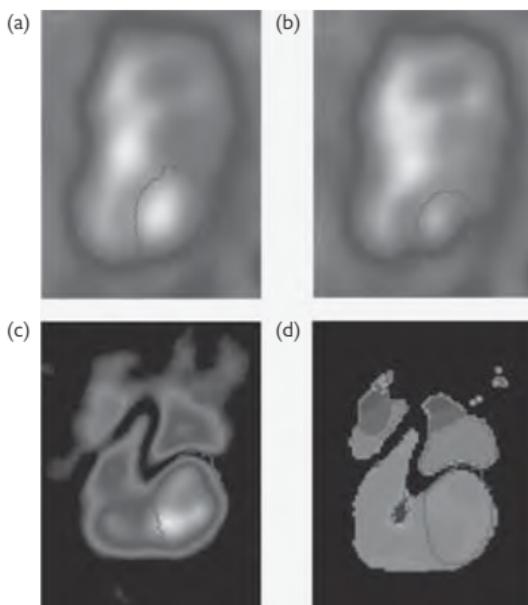


Fig. 14.27 MUGA scan showing (a) LV regions of interest at end-diastole and (b) end-systole for EF calculation; (c) amplitude image showing relative antero-septal hypokinesis, but (d) synchronous LV contraction. (Colour plate 11.)

Results

Visual image of 300–400 summated cardiac cycles. Computer-generated images used to assess regional wall motion and synchronous contraction (see Fig. 14.27). Computer-generated EF calculation. Normal EF 60–70%, ↓ with age.

Interpretation

To monitor treatment response in cardiac failure, cardiomyopathy.

Advantages

Good for serial measurements during anthracycline chemotherapy. Reliable in unechogenic subjects.

Disadvantage

Moderately high radiation dose: echocardiography preferable in most patients.

Pitfalls

Cardiac dysrhythmias interfere with gating, e.g. AF.

Further reading

Hesse B, Lindhardt TB, Acampa W, et al. EANM/ESC guidelines for radionuclide imaging of cardiac function. *Eur J Nucl Med Mol Imaging* 2008; 35: 851–85.

Radionuclide first-pass cardiac studies

Background

Nuclear medicine can assess simple shunts, e.g. left-to-right shunts (ventricular and atrial septal defects), but has no place in bidirectional shunting or multiple sources of pulmonary blood flow (e.g. patent ductus arteriosus). First-pass studies quantify shunt severity both pre- and post-surgical correction. Complementary to cardiac catheterization and Doppler colour flow echocardiography. Dynamic imaging of a small bolus of IV radioactivity, usually ^{99m}Tc -DTPA, outlines the cardiac venous return, pulmonary circulation, left heart, and systemic circulation. Largely superseded by angiography and MRI.

Indications

Measurement of simple left-to-right cardiac shunts.

Patient preparation

None.

Procedure

- ^{99m}Tc -DTPA administered IV as a bolus into a right antecubital vein with the shoulder abducted.
- Image immediately as a dynamic acquisition over 60s.

Results

Normal circulation will demonstrate sequential appearance of the right heart, pulmonary outflow tract, lungs, left heart, and aorta.

Interpretation

Variation in the normal sequence implies cardiac shunting. Interpretation must be performed in conjunction with definitive knowledge of the patient's anatomy, e.g. echocardiography with Doppler colour flow.

Advantages

Relatively non-invasive technique for serial follow-up of congenital heart disease. Mathematical analysis of the data allows quantification of shunt size and is important for consideration and timing of corrective surgery.

Lung scan: ventilation/perfusion imaging

Background

One of the most widely requested nuclear medicine studies. Sensitivity and specificity reduced in coexisting lung disease, when CTPA is more useful. All patients should have had a chest radiograph within 24h to aid V/Q interpretation and exclude other causes of pleuritic chest pain and hypoxia, e.g. pneumothorax.

Lung perfusion shown by injection of ^{99m}Tc particles which are trapped by the pulmonary capillary bed. Ventilation shown using radiolabelled gases or aerosols.

Indications

- Suspected pulmonary embolism (PE).
- Preoperative lung function assessment.

Patient preparation

None. Relative contraindication in right-to-left intra-cardiac shunts; caution in severe pulmonary hypertension.

Procedure

- Lie the patient supine and inject ^{99m}Tc -macroaggregated albumin (MAA) IV.
- Obtain gamma camera perfusion images in four views.
- Ventilation images are obtained in same projections by continuous breathing of ^{81m}Kr gas or using ^{99m}Tc aerosol or $^{133}\text{xenon}$ gas.

Results

Homogeneous, matched V/Q patterns (see Fig. 14.28).

Interpretation

Four abnormal patterns recognized:

- Segmental perfusion loss with preserved ventilation: PE (see Fig. 14.29).
- Segmental matched perfusion and ventilation loss: pulmonary infarction/infection.
- Segmental/subsegmental ventilation loss with preserved perfusion: infection.
- Non-segmental, patchy, matched perfusion, and ventilation loss: COPD (see Fig. 14.29).

Advantages

Quick, non-invasive. Normal scan virtually excludes PE.

Pitfalls

Sensitivity reduced in underlying lung disease—COPD, asthma giving indeterminate results. False +ves with tumour, bullae, vasculitides, fibrotic lung disease, and old unresolved PE.

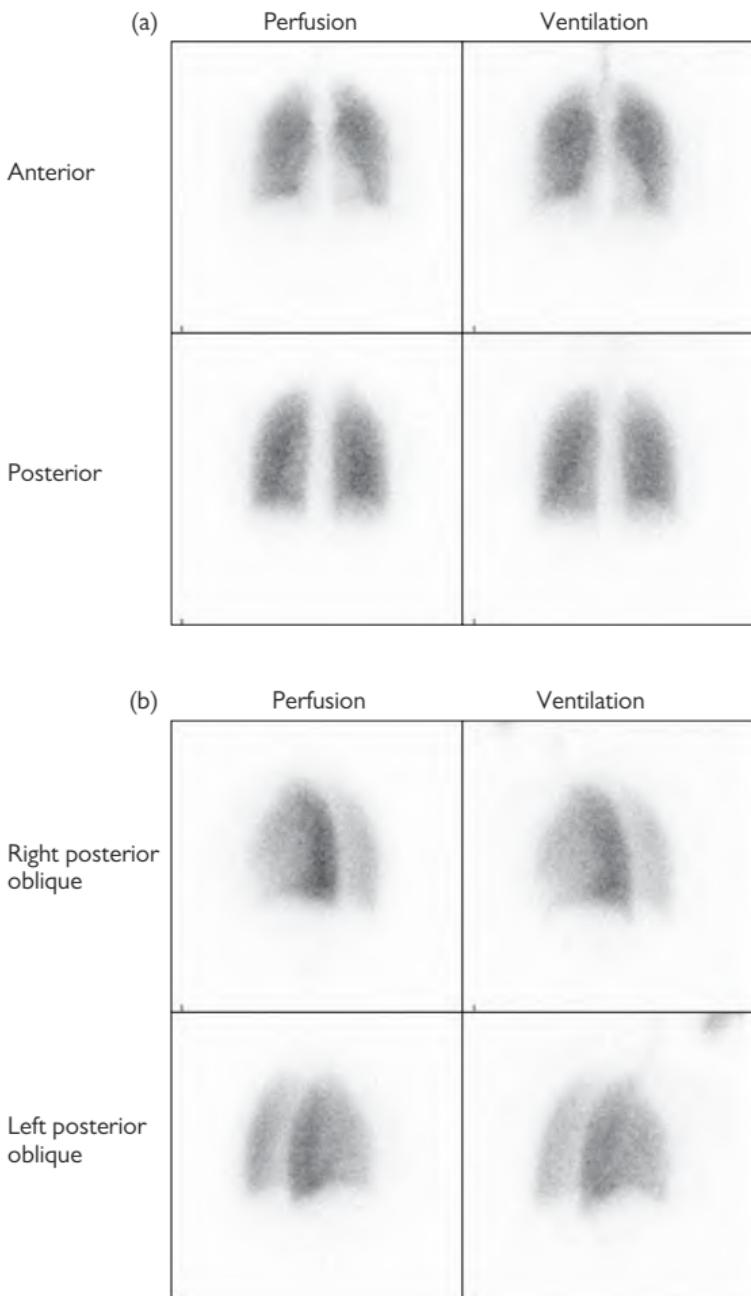


Fig. 14.28 Normal lung V/Q images: (a) anterior and posterior views; (b) oblique views.

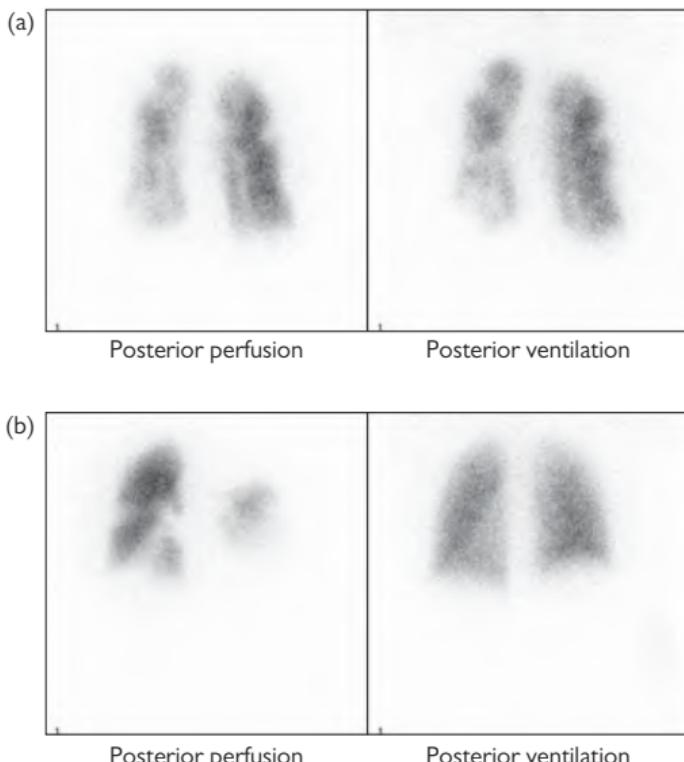


Fig. 14.29 Lung scans: (a) showing matched, non-segmental V/Q defects in COPD; and (b) showing segmental V/Q mismatch—extensive bilateral pulmonary thromboembolism and unmatched perfusion loss.

Further reading

Bajc M, Neilly JB, Miniati M, et al. EANM guidelines for ventilation/perfusion scintigraphy: Part 1. Pulmonary imaging with ventilation/perfusion single photon emission tomography. *Eur J Nucl Med Mol Imaging* 2009; 36: 1356–70.

Lung shunt studies

Background

Largely research procedure.

Indications

Suspected pulmonary AV shunting.

Patient preparation

None.

Procedure

Inject ^{99m}Tc -MAA IV; consider manoeuvres to reduce particle number. gamma camera lung, abdomen, and head imaging. Calculate relative uptake in lungs, kidneys, and brain. Express as fraction of cardiac output to quantify shunt fraction.

Results

Kidneys and brain not normally visible on lung perfusion imaging.

Interpretation

Abnormal extrapulmonary activity implies degree of shunting. Intensity of uptake rises with shunt severity (see Fig. 14.30).

Advantages

Non-invasive, quantitative. Can be used to monitor response to intervention.

Pitfalls

Injection extravasation invalidates shunt calculation.



Fig. 14.30 Lung shunt study showing extrapulmonary activity in the brain and kidneys.

Lung permeability studies

Background

Altered alveolar permeability affects gas exchange. Also shown by lung transfer factor measurement.

Indications

- *PCP infection*: rapid screening in high-risk patients with normal CXR.
- Monitor treatment response in CFA.

Patient preparation

None.

Procedure

Patient breathes ^{99m}Tc -DTPA aerosol. Gamma camera images of the thorax over 1h. Computer data analysis generates lung clearance curves, reflecting the integrity of the alveolar cell barrier.

Results

Clearance curves used to calculate the permeability index. Individual results, compared with centre-defined normal range.

Interpretation

Accelerated clearance in PCP, which \downarrow with successful treatment.

Advantages

Non-invasive. Allows rapid PCP diagnosis.

Pitfalls

Non-specific, e.g. accelerated clearance in smokers.

Lymphoscintigraphy

Background

Lymphoedema can be congenital or acquired. The lymphatic system normally drains subcutaneous tissues → local lymphatic channels and regional nodes. Lymphatic channels can be imaged using radiolabelled colloid particles (➡ Sentinel node imaging, p. 890).

Indication

Unexplained limb swelling, e.g. lymphatic hypoplasia.

Patient preparation

None.

Procedure

^{99m}Tc -nanocolloid injection SC into a finger or toe web space on the affected and contralateral limbs. Regional gamma camera imaging at 10min intervals over 1h.

Results

Normally rapid clearance via lymphatic channels to regional nodes (see Fig. 14.31a).

Interpretation

Slow clearance and failed regional node uptake in hypoplastic systems or metastatic regional node infiltration, dermal backflow (see Fig. 14.31b), depending on clinical context.

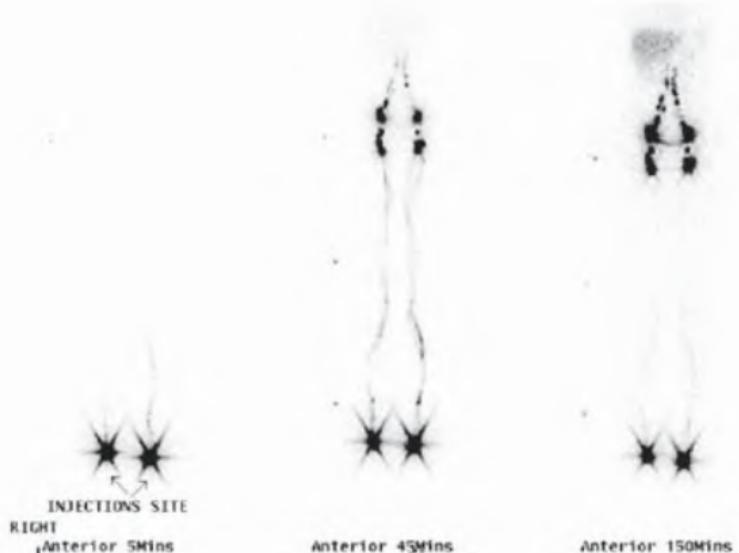
Advantages

Much easier than conventional (contrast) lymphography—avoids lymphatic channel cannulation.

Pitfalls

Lymphatic drainage may be disrupted by surgery or radiotherapy.

(a)



(b)

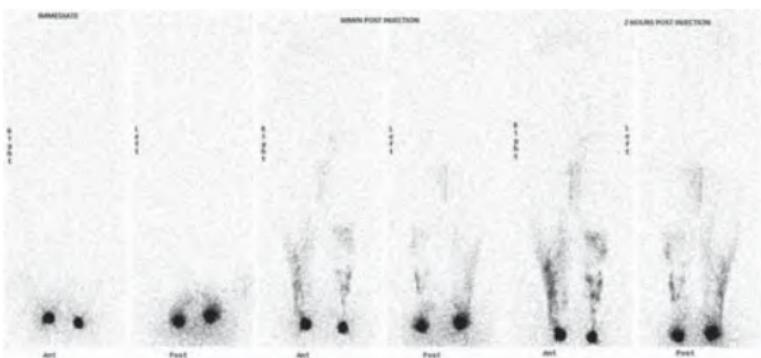


Fig. 14.31 (a) Normal lymphoscintograms. (b) Abnormal study: bilateral dermal backflow with non-visualization of lymphatic channels and lymph nodes.

Static cortical scintigraphy: dimercaptosuccinic acid imaging (DMSA)

Background

DMSA is concentrated by the proximal convoluted tubules in the renal cortex. ^{99m}Tc -DMSA provides good definition of functioning renal parenchyma. It should be used in conjunction with anatomical imaging, e.g. ultrasonography, to differentiate between scarring, cysts, or calculi.

Indications

- UTI: 'gold standard' for renal scarring.
- Measurement of relative renal function.
- Renal duplication assessment.
- Ectopic kidney localization.
- Renal trauma.
- Renal vein thrombosis.
- Pre-biopsy.

Patient preparation

None, but avoid dehydration.

Procedure

^{99m}Tc -DMSA injected IV. Static anterior, posterior, and posterior oblique images acquired 2–4h later.

Results

Visual image evaluation, assessing the integrity of cortical outlines for scarring (see Fig. 14.32a). Quantitative computer image analysis is used to measure relative renal function, i.e. the contribution of each kidney to overall GFR.

Interpretation

Cortical scars distort the renal outline (see Fig. 14.32b). Duplication may result in a non-functioning upper moiety, usually due to obstruction, or a scarred lower moiety, 2° to vesico-ureteric reflux. Relative renal function is usually $50:50 \pm 5\%$.

Advantages

Sensitive for renal scarring. Superior to US. Non-invasive.

Pitfalls

False +ves during or immediately after acute pyelonephritis. May give cortical defects that do not progress to scarring. Splenic impression at the left upper pole may be mistaken for scarring.

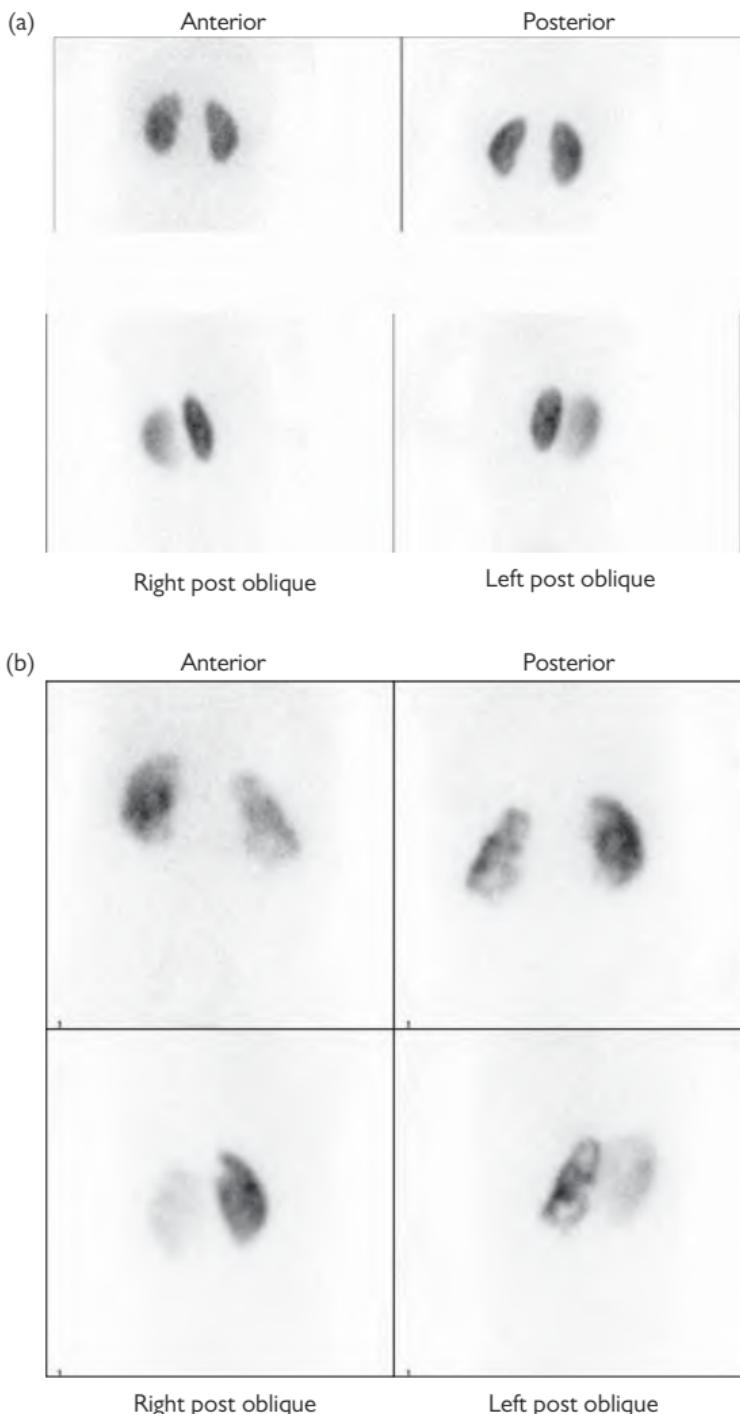


Fig. 14.32 DMSA static scan: (a) normal and (b) showing extensive left kidney cortical scarring.

Dynamic renography

Background

Nuclear medicine offers unique 'real-time' imaging of renal function, i.e. visualization of uptake, drainage, and bladder emptying. Available radiopharmaceuticals include:

- ^{99m}Tc -diethylenetriaminepentacetic acid (DTPA), cleared by glomerular filtration.
- ^{99m}Tc -mercaptoacetyltriglycine (MAG3), cleared by glomerular filtration and tubular secretion.
- ^{99m}Tc -ethylenedicycysteine (EC), cleared by glomerular filtration and tubular secretion.

Tubular agents preferred, particularly in the presence of renal impairment and in the immature kidney. ^{99m}Tc -DTPA reserved for assessment of ATN, post-transplant viability, etc.

Indications

- Assessment of renal drainage: discrimination between renal dilatation and outflow obstruction.
- Measurement of relative renal function.
- Loin pain.
- Post-pyeloplasty follow-up.
- RAS ( Captopril renography, p. 928).

Patient preparation

Good hydration essential. Empty the bladder immediately before undertaking the study.

Procedure

- Position the patient supine or seated erect, with the camera behind.
- Obtain good peripheral venous access. Bolus IV radiopharmaceutical injection ^{99m}Tc -MAG3 or ^{99m}Tc -DTPA, followed by 10–20mL of saline flush.
- Image immediately, acquiring real-time dynamic data for 20–30min.
- Diuretic administration is essential to distinguish dilatation from outflow obstruction.
- Post-voiding images are always required to assess the completeness of bladder emptying and may improve drainage of the upper renal tracts in high-pressure systems.

Results

Visual inspection of renal size, perfusion, function, and drainage (see Fig. 14.33a). Quantitative computer image analysis measures relative function and transit times, and generates drainage graphs.

Interpretation

Uptake and excretion of activity normally rapid. Dilated systems show progressive pooling in the renal pelvis that empties following diuretic challenge. Obstructed systems show progressive tracer accumulation with no diuretic response, often associated with reduced function on the affected side (see Fig. 14.33b).

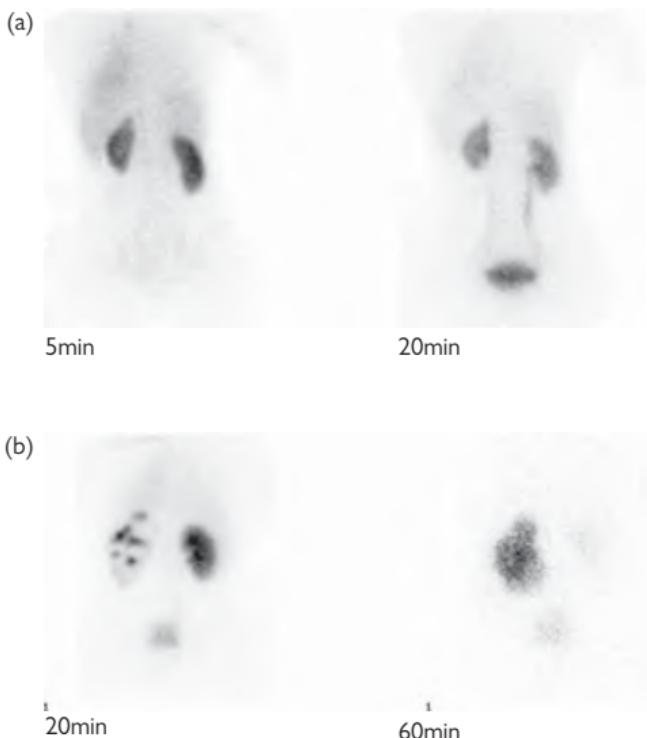


Fig. 14.33 Dynamic renogram posterior images: (a) normal, showing an early parenchymal image and later symmetrical excretion with bladder filling; (b) outflow obstruction: early image shows left hydronephrosis 2° to pelviureteric junction obstruction, with poor drainage at 60min.

Advantages

Sensitive, non-invasive, quantitative renal function assessment. Anatomical imaging, e.g. IVU, better for renal morphology, stones, etc.

Pitfalls

Movement artefact, chronic renal failure, and dehydration reduce data reliability. Renal drainage may be gravity-dependent—always complete the study with an erect image. Drainage curves invalidated by radiopharmaceutical extravasation.

Captopril renography

Background

RAS is a rare (<2%) cause of hypertension. Suspected in young adults presenting with hypertension, usually due to fibromuscular dysplasia. In patients >50 years, the commonest cause is atherosclerosis. Perfusion pressure is maintained by angiotensin II in RAS. Captopril is an ACE inhibitor, which blocks the conversion of angiotensin I to angiotensin II. Captopril reduces perfusion pressure, leading to a fall in the relative function and delayed tracer uptake on the affected side. Captopril administration is contraindicated in the presence of a solitary kidney.

Indications

Diagnosis of RAS (especially fibromuscular dysplasia) and prediction of response to revascularization.

Patient preparation

Well-hydrated. Baseline BP. IV access. Stop ACE inhibitors for 48h prior to the test.

Procedure

- Perform standard dynamic renogram using $^{99\text{m}}\text{Tc}$ -MAG3.
- Repeat renogram 1h after captopril 25mg single dose PO.
- Monitor BP—beware hypotension.

Results

Quantitative evaluation of R:L renal function and time to peak activity in each kidney.

Interpretation

RAS due to fibromuscular dysplasia—fall in relative renal function and delayed time to peak renal activity of >10min.

Advantages

Distinguishes generalized atherosclerosis (often poor BP outcome following angioplasty) from fibromuscular hyperplasia (good angioplasty response).

Pitfalls

- ↓ reliability in the presence of renal impairment.
- Severe hypotension.

Gastrointestinal bleeding: labelled red cell imaging

Background

The source of GI blood loss is usually identified by GI endoscopy but may be difficult to localize. Labelled red cell studies are useful when there is evidence of ongoing bleeding (typically falling Hb of 1g/L/day). The patient must be actively bleeding at the time of the study. This is a time-consuming investigation, with serial imaging beyond 24h often performed.

Indications

Localize source of active GI haemorrhage when other techniques (e.g. endoscopy or angiography) have failed.

Patient preparation

No recent contrast barium studies. Fasting during first 2h of imaging.

Procedure

Label red cells (*in vitro* or *in vivo*) using ^{99m}Tc -pertechnetate. Abdominal gamma camera blood pool imaging immediately and at intervals for up to 36h post-injection or until the bleeding source is identified.

Results

Activity normally restricted to vascular compartment.

Interpretation

Any activity in the gut lumen implies active haemorrhage. Serial images helpful (see Fig. 14.34).

Advantages

More sensitive and less invasive than angiography for intermittent bleeding.

Pitfalls

- Poor red cell label: degrades image quality, could lead to false +ve.
- Limits of detection: 0.5mL/min blood loss.

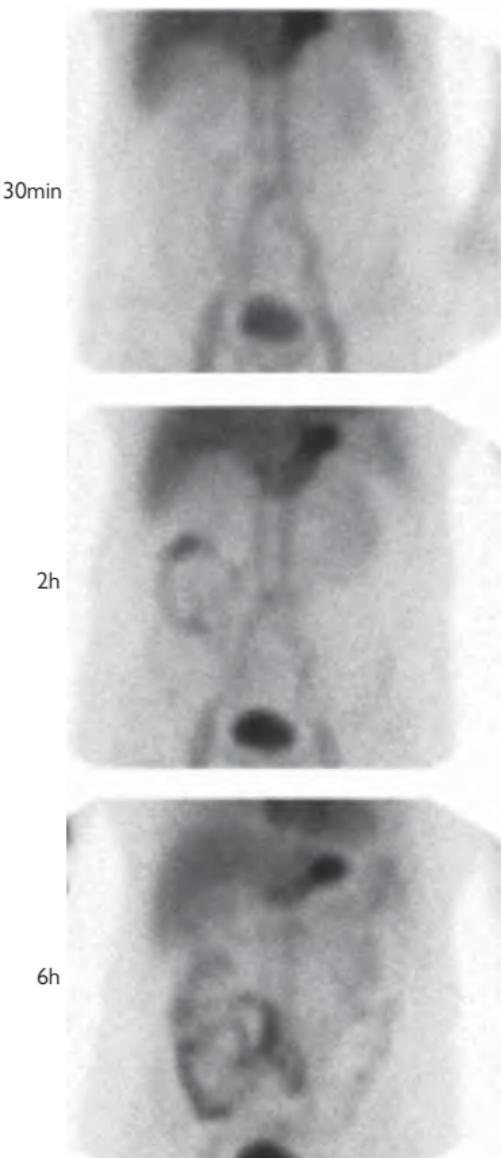


Fig. 14.34 Anterior abdominal images showing increasing red cell haemorrhage into the distal ileum.

Gastric emptying studies

Background

The diagnosis of dysfunctional gastric emptying can be difficult. In children, delayed gastric emptying may contribute to gastro-oesophageal reflux. In adults, both gastric stasis and 'dumping' syndromes occur, sometimes following previous surgery. Imaging following ingestion of radiolabelled solids or liquids demonstrates the timing and pattern of gastric emptying.

Indications

Altered GI motility—delayed or accelerated gastric emptying.

Patient preparation

Fast for 4h. Stop drugs likely to influence GI motility, e.g. domperidone, metoclopramide.

Procedure

Milk study

- Give radiolabelled (^{99m}Tc -DTPA) milk drink PO.
- Image the anterior abdomen immediately and at 10min intervals for 1h. Generate computer-derived clearance curves to calculate the emptying half-time.
- Delayed thoracic image helpful to exclude lung aspiration if clearance significantly delayed.

Dual isotope method

- Give ^{99m}Tc -labelled standard meal (e.g. porridge, egg) with ^{111}In -DTPA in water.
- Anterior abdomen gamma camera imaging as before using dual isotope settings.
- Generate solid and liquid phase clearance curves.

Results

Normal gastric emptying half-time (milk = 20min). Normal range for solids is centre-specific, depending on the standard meal composition (see Fig. 14.35a and b).

Interpretation

Visual image evaluation and half-time calculation.

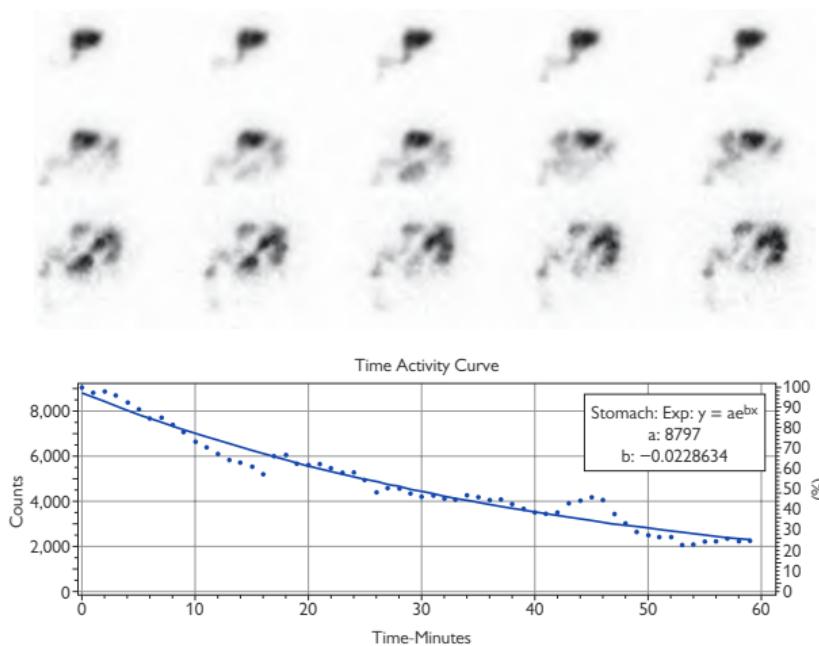
Advantages

Non-invasive and quantitative.

Pitfalls

Vomiting during study invalidates emptying time calculations.

(a)



(b)

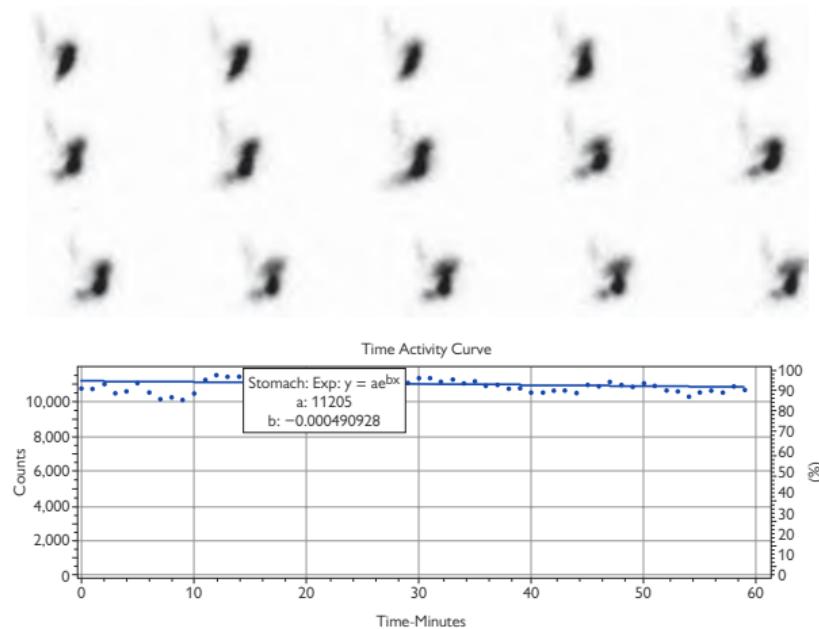


Fig. 14.35 (a) Normal gastric emptying study: anterior images showing clearance of ^{99m}Tc -labelled semi-solid meal into the proximal small intestine; (b) abnormal gastric emptying study: anterior images showing poor clearance of ^{99m}Tc -labelled semi-solid meal into the proximal small intestine.

SeHCAT studies

Background

The SeHCAT test is an important test for diagnosing bile acid malabsorption. SeHCAT is a taurine-conjugated bile acid analogue and it is incorporated with ^{75}Se (gamma-emitter) into the SeHCAT molecule (radiotracer) to assess *in vivo* the enterohepatic circulation of bile salts. The retention of radiotracer in the body is evaluated using a conventional gamma camera.

Indications

- Chronic diarrhoea.
- Diarrhoea-predominant IBS.
- Crohn's disease.
- Ileal resection, cholecystectomy, radiation-induced bowel damage, or ulcerative colitis.

Patient preparation

Colestyramine and colesevelam should be stopped for 3 days prior to scan.

Procedure

A capsule containing radiolabelled SeHCAT (370kBq) capsule is taken PO with water, and a scan is performed to measure SeHCAT activity at 1–3h. Patients will return on day 7 to undergo a second scan to measure the percentage of SeHCAT retention.

Results

The percentage of SeHCAT retention gives an indication as to whether the patient has bile acid malabsorption or not.

Interpretation

Retention values of <15% are considered abnormal and are suggestive of bile acid malabsorption (<5%, severe bile acid malabsorption; 5–10%, moderate; and 10–15%, mild). Retention values of >15% are normal.

Advantages

Easy to perform and well tolerated.

Further reading

- Boyd GS, Merrick MV, Monks R, Thomas IL. Se-75-labeled bile acid analogs, new radiopharmaceuticals for investigating the enterohepatic circulation. *J Nucl Med* 1981; **22**: 720–5.
Jazrawi RP, Ferraris R, Bridges C, Northfield TC. Kinetics for the synthetic bile acid ^{75}Se -selenohomocholic acid-taurine in humans: comparison with [^{14}C]taurocholate. *Gastroenterology* 1988; **95**: 164–9.



Meckel's scan: ectopic gastric mucosa localization

Background

Meckel's diverticulum is the commonest congenital anomaly of the GIT, occurring in ~2% of the population. Less than 10% contain ectopic gastric mucosa which may bleed, but diverticuli can also cause obstruction or become inflamed. Typically, childhood presentation. Nuclear medicine provides a straightforward imaging technique that targets gastric mucosal cells, which normally take up ^{99m}Tc -pertechnetate.

Indications

Unexplained abdominal pain or GI haemorrhage—after endoscopy/contrast radiology.

Patient preparation

- Fast for 4h.
- H₂ antagonist administration may improve specificity.
- No recent barium studies.

Procedure

Inject ^{99m}Tc -pertechnetate IV. Immediate and serial abdominal imaging over 1h.

Results

Normal uptake in gastric mucosa.

Interpretation

Focal abnormal uptake appearing at the same time as the stomach implies ectopic gastric mucosa (Meckel's diverticulum) (see Fig. 14.36), or occasionally a duplication cyst. Commonest site—right iliac fossa (RIF).

Advantages

Non-invasive.

Pitfalls

False +ves due to activity in the renal tract—lateral images usually help.



Fig. 14.36 Ectopic gastric mucosa in the right iliac fossa towards the midline (arrow).

Hepatobiliary scintigraphy

Background

Iminodiacetic acid (IDA) compounds are cleared from the circulation by hepatocytes and secreted into the bile in the same way as bilirubin. ^{99m}Tc -labelled IDA compounds show biliary excretion through the biliary tree and gall bladder → duodenum. Useful in acute/chronic acalculous cholecystitis and to diagnose biliary atresia.

Indications

- Acute cholecystitis.
- Trauma.
- Post-operative leak detection.
- Bile duct/stent patency.
- Gall bladder emptying.
- Bile reflux.
- Neonatal biliary atresia.

Patient preparation

- Adults: fast for 6h.
- Neonates: phenobarbital 5mg/kg/day PO for 3 days prior to study (enzyme induction).

Procedure

- Adults: IV injection of ^{99m}Tc -labelled IDA complex (mebrofenin). Gamma camera imaging over 1h.
- Neonates: IV injection of ^{99m}Tc -IDA. Immediate dynamic imaging for 5min, then serial static images for up to 24h or until activity reaches the small bowel lumen.

Results

Gall bladder and biliary tree normally shown with tracer excretion via the CBD into the duodenum by 30min post-injection. Cholecystokinin 0.5U/kg IV sometimes administered to stimulate gall bladder emptying (see Fig. 14.37a)

Interpretation

- Acute cholecystitis: absent gall bladder.
- Obstruction, leak, or reflux assessed visually (see Fig. 14.37b).
- Neonates: passage of activity into the gut lumen excludes biliary atresia.
- Quantification of T0 to T10 min images improves specificity for atresia diagnosis.

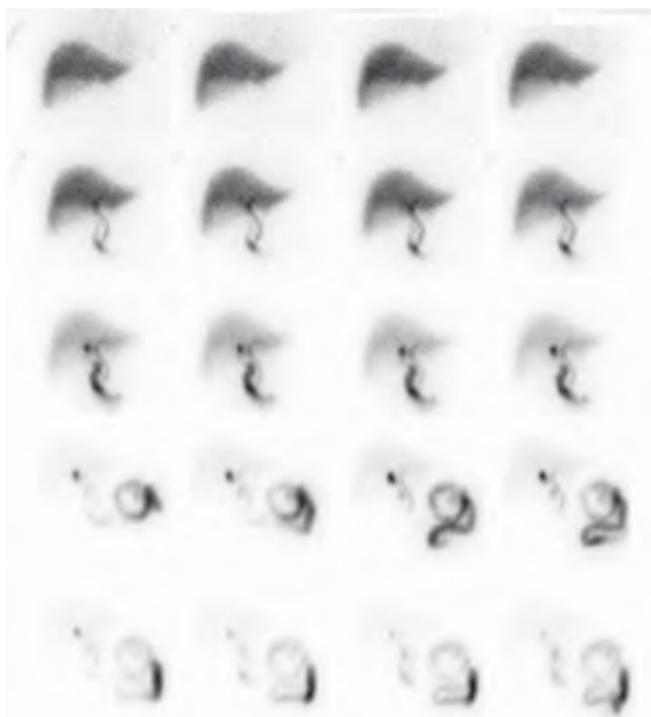
Advantages

Non-invasive. Straightforward pattern recognition.

Pitfalls

Delayed IDA excretion in severe jaundice: bilirubin >300 $\mu\text{mol/L}$.

(a)



(b)

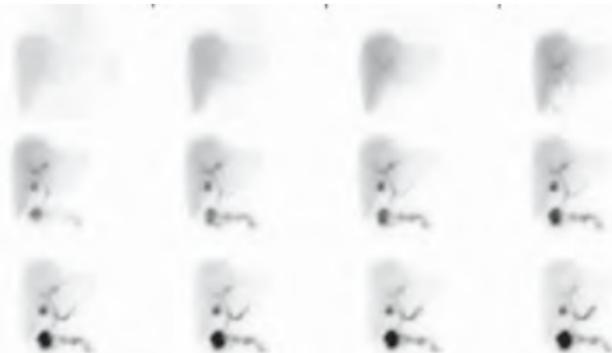


Fig. 14.37 (a) Normal hepatobiliary scan; (b) hepatobiliary scan showing leak post-laparoscopic cholecystectomy.

Splenunculus detection: heat-damaged red cell imaging

Background

Splenectomy may be indicated in haemolytic syndromes and in refractory haemorrhagic tendencies (e.g. ITP) if associated with hypersplenic thrombocytopenia. Remnant splenic tissue, or 'splenunculi', can give rise to recurrent thrombocytopenia—difficult to detect on anatomical imaging. As the spleen removes abnormal red cells from the circulating blood pool, radiolabelled heat-damaged red cells can be used to localize ectopic splenic tissue.

Indications

Recurrent thrombocytopenia post-splenectomy.

Patient preparation

None.

Procedure

- Obtain a venous blood sample.
- Radiolabel red cells *in vitro* using ^{99m}Tc -pertechnetate.
- Heat to 49.5°C for 20–30min.
- Cool and re-inject IV.
- Image the anterior abdomen 30min later.

Results and interpretation

Damaged red cells taken up by splenic remnants (see Fig. 14.38).

Advantages

Investigation of choice for splenunculus detection.

Pitfalls

Enlarged left lobe of the liver may obscure a small splenic remnant.

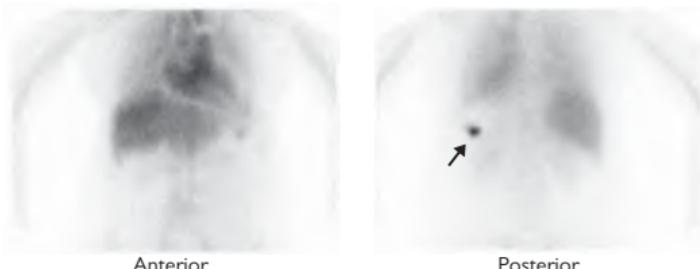


Fig. 14.38 Post-splenectomy. Intense uptake in a splenunculus lying in the splenic bed (arrow).



Hepatosplenic scintigraphy

Background

Largely superseded by ultrasonography and cross-sectional imaging. Maps reticuloendothelial tissue within the liver (Kupffer cells) and spleen, to identify SOLs and confirm the presence or absence of functioning splenic tissue.

Indications

Liver SOLs—now largely replaced by US, CT, or MRI.

Patient preparation

None.

Procedure

^{99m}Tc -colloid injected IV. Abdominal gamma camera images 30min post-injection.

Results

Normal, homogeneous liver and spleen uptake (see Fig. 14.39).

Interpretation

Focal ↓ uptake in SOLs. ↑ spleen and bone activity in portal hypertension. Focal ↑ uptake in the caudate lobe pathognomonic of Budd–Chiari syndrome.

Advantages

Cheap.

Pitfalls

Non-specific. Largely superseded by anatomical imaging.



Fig. 14.39 Normal hepatosplenic study.

Labelled leucocyte imaging

Background

Localization and assessment of acute or chronic infection/inflammation can be difficult. Nuclear medicine techniques show inflammation but do not differentiate infective from non-infective causes. Radiolabelled autologous leucocytes are injected and imaged. The normal distribution includes the liver and spleen, making peri-diaphragmatic collections difficult to identify. Delayed imaging useful in chronic low-grade infection, e.g. osteomyelitis, where cell migration to the site of inflammation is slow.

Indications

- Sepsis localization.
- IBD to determine disease activity, extent, severity.

Patient preparation

None. Avoid recent barium contrast radiology.

Procedure

- Obtain 40–60mL of blood sample.
- Separate the white cell layer, and radiolabel *in vitro* using ^{99m}Tc -exametazime (HMPAO) or ^{111}In -oxine.
- Re-inject labelled cells IV.
- Image 1 and 3h later (IBD), or 2, 4, and 24h for intra-abdominal sepsis/osteomyelitis.

Results

Physiological uptake in the RES. Variable GI and renal excretion, depending on the radiopharmaceutical used (see Fig. 14.40a).

Interpretation

Focal ↑ uptake indicates sepsis. Diffuse ↑ gut uptake reflects the extent and activity of IBD (see Fig. 14.40b).

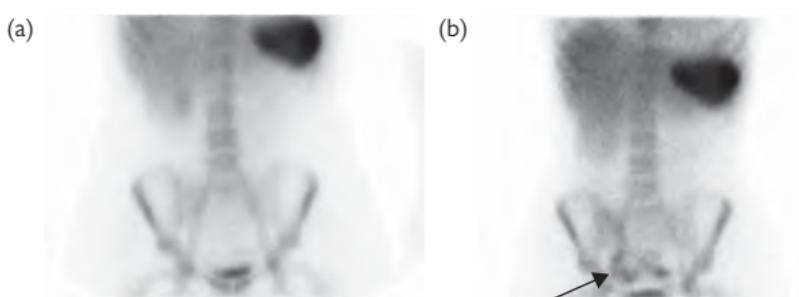


Fig. 14.40 Labelled leucocyte imaging: (a) normal, and (b) acute inflammatory bowel disease—intense uptake in small and large bowel loops (Crohn's disease).

Advantages

Very sensitive in IBD. Non-invasive, useful in sick patients, e.g. acute exacerbation of IBD.

Pitfalls

- *False -ves:* leucopenia and poor white cell label, perihepatic and perisplenic collections obscured by normal liver and spleen uptake.
- *False +ves:* physiological gut and renal activity.
- Damaged white cells during labelling causing lung sequestration.
- ^{99m}Tc -exametazime (HMPAO) preferred for routine imaging and IBD—lower radiation dose and earlier result than ^{111}In -oxine label.
- Reserve ^{111}In -oxine for low-grade bone sepsis localization.
- Requires aseptic facilities and trained personnel.
- Risk to staff (blood handling) and patient (contamination, re-injection into wrong patient).

^{67}Ga scintigraphy

Background

Previously used to diagnose and monitor sarcoid and lymphoma, but increasingly superseded by ^{18}F -FDG PET ( Labelled leucocyte imaging, pp. 944–5) and cross-sectional imaging. Sometimes useful in ‘pyrexia of unknown origin’ (PUO), e.g. immunocompromised patient where there is a suspicion of *Pneumocystis jiroveci* infection (cf. lung permeability studies).

Indications

- PUO and infection localization, especially in AIDS.
- Lymphoma follow-up.
- Sarcoidosis follow-up.

Patient preparation

None.

Procedure

- Inject ^{67}Ga -citrate IV. Gamma camera imaging at 48–96h with tomography.
- Non-specific gut retention reduced by laxative administration.

Results

Normal uptake in lacrimal glands, nasal mucosa, blood pool, liver, spleen, testes, ♀ perineum, breast (see Fig. 14.41a).

Interpretation

- Focal lymph node uptake in lymphoma and sarcoid distinguishes active disease from post-therapy scarring/fibrosis (see Fig. 14.41b).
- In AIDS, ↑ lung uptake indicates infection—PCP, CMV, mycobacterium—chest radiograph correlation essential.
- ↑ activity in IBD and focal sepsis; largely superseded by WBC imaging.

Advantages

Excellent, non-invasive marker of disease activity in lymphoma—but likely to be superseded by ^{18}F -FDG.

Pitfalls

Poor specificity. High radiation dose often difficult to justify when alternative techniques available. Prolonged test (48–96h).

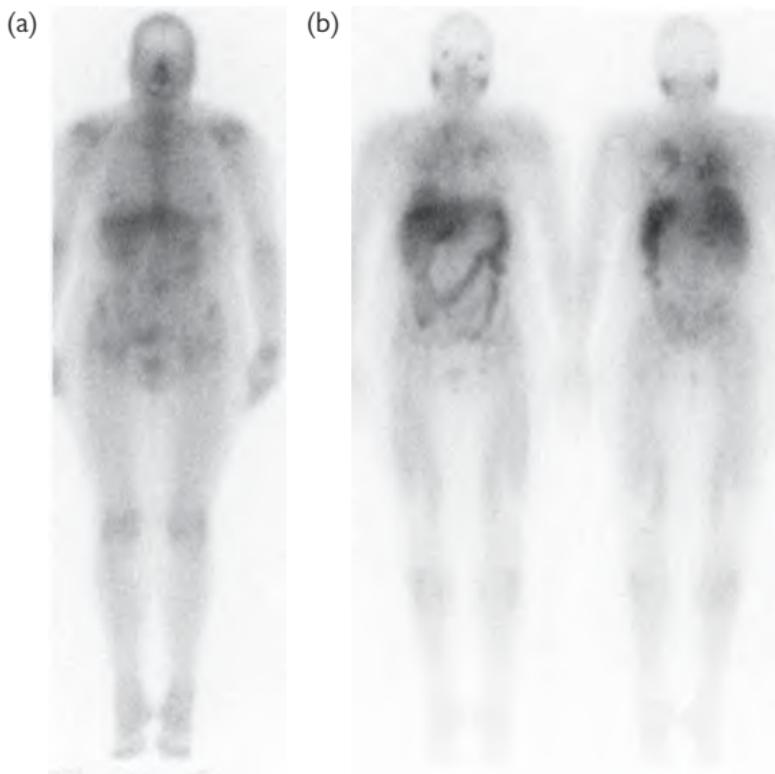


Fig. 14.41 (a) Normal ⁶⁷Ga scan; (b) abnormal tracer uptake in the lacrimal glands, parotid glands, mediastinum, and lungs, in keeping with known sarcoidosis.

Dacrocintigraphy

Background

Epiphora may arise from excessive tear production or inadequate drainage due to lower lid ectropion or nasolacrimal obstruction, i.e. nasal puncti, lacrimal sac, nasolacrimal ducts. Straightforward technique to assess function of the nasolacrimal apparatus.

Indications

Epiphora.

Patient preparation

None.

Procedure

One to two drops of ^{99m}Tc -labelled DTPA or pertechnetate instilled into the outer canthus of each eye. Immediate dynamic gamma camera imaging for 20min, with delayed static scans as required.

Results

Normal rapid radiopharmaceutical clearance through the nasolacrimal apparatus.

Interpretation

Delayed clearance implies obstruction—level of dysfunction usually identified, i.e. punctum, lacrimal sac, nasolacrimal duct (see Fig. 14.42a and b).

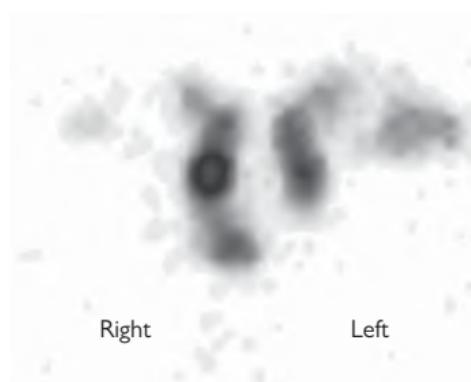


Fig. 14.42 Dacrocintigram (lacrimal drainage) showing normal lacrimal drainage on the right, and on the left obstructed drainage at the proximal nasolacrimal duct.

Advantages

Non-invasive. Avoids nasolacrimal duct cannulation (cf. dacrocystography).

Pitfalls

Obstructed systems result in excess radiolabelled tears on the cheek, altering drainage times.

Salivary gland scintigraphy

Background

^{99m}Tc -pertechnetate uptake in the salivary glands reflects intact parenchyma. Salivary gland scintigraphy demonstrates both parenchymal function and excretory function. Salivary gland scintigraphy is a safe and sensitive technique to assess the function and morphology of salivary glands.

Indications

- Sjögren's syndrome.
- Chronic sialadenitis.
- Post-multiple radioiodine therapies.
- Irradiation of the head and neck.

Patient preparation

None.

Procedure

After IV injection of ^{99m}Tc -pertechnetate, dynamic scintigraphy is performed for 15–20min, and a sialogogue (e.g. lemon juice) stimulation is delivered and imaging is continued for further 15–20min to assess excretory function. Time–activity curves for the four major salivary glands are generated.

Results

Normal uptake of radiopharmaceutical in parotids and submandibular glands, with spontaneous excretion following lemon juice stimulation.

Interpretation

Delayed or reduced tracer uptake or accumulation and would be compatible with salivary gland dysfunction usually identified (see Fig. 14.43).

Advantages

Easy to perform, reproducible, and well tolerated.

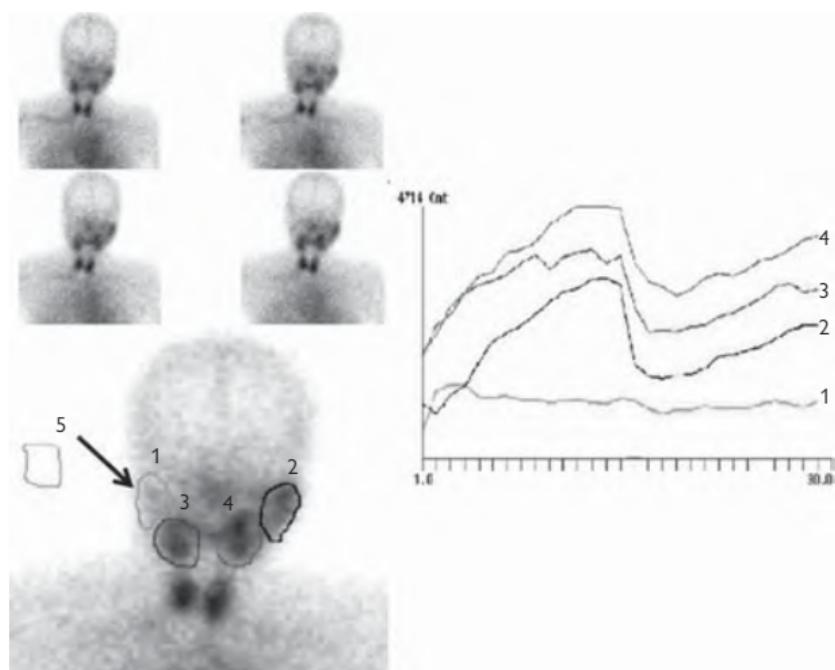


Fig. 14.43 The left parotid and submandibular glands show prompt tracer uptake and excretion. The right parotid gland shows poor uptake and function (arrow).

Glomerular filtration rate measurement

Background

In many instances, GFR estimation from CrC is adequate, but accurate measurement is essential in renal impairment or to monitor nephrotoxic drug therapy. Glomerular compensation prevents early renal damage detection by CrC measurements—60% of filtration activity can be lost before CrC falls.

Indications

Accurate GFR to monitor renal failure, cytotoxic chemotherapy, immunosuppression, e.g. ciclosporin.

Patient preparation

Well hydrated.

Procedure

- IV injection of ^{51}Cr -EDTA or $^{99\text{m}}\text{Tc}$ -DTPA.
- Venous sampling 2 and 4h later.
- Count plasma sample radioactivity and known standards in gamma counter. Correct for height and weight.

Results

Normal GFR = 125mL/min (age-dependent).

Interpretation

↓ values in chronic renal failure.

Advantages

More reliable and reproducible than CrC—avoids need for urine collection.

Pitfalls

Accuracy depends on accurate measurement of dose and good injection technique—avoid any extravasation. Unreliable results in severe peripheral oedema.

Urea breath test

Background

Helicobacter pylori infection is associated with duodenal ulceration. Eradication therapy reduces ulcer recurrence. *H. pylori* produces urease which converts labelled urea → labelled CO₂, detected in breath samples.

Indications

H. pylori detection—diagnosis and confirmation of eradication.

Patient preparation

Stop antibiotics, H₂ antagonists, proton pump inhibitors for 2–4 weeks.

Procedure

- Patient swallows urea drink labelled with ¹³C (stable isotope) or ¹⁴C (radioactive isotope).
- Breath samples (CO₂) collected over next 30min.
- Labelled CO₂ measured by mass spectroscopy (¹³C) or liquid scintillation counting (¹⁴C).

Results

Normal range varies according to local protocol.

Interpretation

↑ exhaled CO₂ levels imply abnormal urea breakdown by urease-producing bacteria in the stomach, e.g. *H. pylori*.

Advantages

- Very sensitive marker of active *H. pylori* infection (cf. serology).
- Non-invasive (cf. endoscopy) and avoids sampling errors.
- Good for non-invasive monitoring of recurrent symptoms.

Pitfalls

Occasional false +ves in oral *H. pylori* infection.

Red cell survival studies

Background

Although infrequently performed, provides evidence of abnormal red cell survival and localizes sites of red cell destruction. The investigation is prolonged, with initial *in vitro* red cell labelling and daily activity measurements over target organs—spleen, liver, and heart—for 14 days.

Indications

- Haemolytic anaemia (to confirm ↓ RBC survival, i.e. active haemolysis).
- Localize abnormal red cell sequestration.
- Predict response to splenectomy.

Patient preparation

None. Avoid blood transfusion during study.

Procedure

- Obtain a venous blood sample, and label the patient's red cells with ^{51}Cr -chromate.
- Re-inject cells and measure blood activity over 14 days using gamma counter.
- Measure activity over the liver, spleen, and heart using gamma probe daily for 14 days.

Results

- Normal red cell half-life >24 days.
- Equal fall in the heart, liver, and spleen counts with time.

Interpretation

Short red cell life confirms abnormal destruction. Ratio of counts in liver:spleen indicates the site of red cell destruction.

Advantages

Only available technique.

Pitfalls

Lengthy and labour-intensive. Sensitivity reduced by blood transfusion during 14 days' measurement period. Consistent probe positioning essential for accurate organ sequestration curves.

Red cell volume/plasma volume measurement

Background

Polycythaemia is suspected when Hb and Hct are raised. Routine laboratory screening does not differentiate true polycythaemia, i.e. elevated red cell mass, from apparent, stress, or pseudo-polycythaemia, i.e. reduced plasma volume. Radiolabelled red cells can be used to measure red cell mass. Plasma volume can be calculated from the Hct or measured independently using radiolabelled human serum albumin.

Indications

Polycythaemia, to distinguish between true polycythaemia (\uparrow RBC mass) from apparent polycythaemia (\downarrow plasma volume).

Patient preparation

Avoid recent therapeutic venesection. Less sensitive in patients already receiving myelosuppressive therapy.

Procedure

- Obtain 10 mL of venous blood.
- Radiolabel red cells using ^{99m}Tc or ^{51}Cr .
- Re-inject radiolabelled blood and, if required, ^{125}I -albumin.
- Obtain venous samples at 15 and 30min.
- Count activity in blood samples, compared with known standards, using gamma counter to establish plasma and red cell volumes.

Results

Compare measured red cell mass and plasma volume with predicted values for height and weight.

Interpretation

Distinguish relative polycythaemia (due to \downarrow plasma volume) from genuine elevation of red cell mass.

Advantages

Only technique available.

Pitfalls

Recent venesection or myelosuppressive therapy reduces test reliability. Plasma volume measurement unreliable in severe peripheral oedema.

Bile salt deconjugation studies

Background

Bile salts are synthesized and stored in the liver and are essential for adequate GI absorption. They are excreted into the gut as conjugated, water-soluble bile salts and recycled by the enterohepatic circulation with terminal ileal reabsorption. Bile salt deconjugation products are insoluble and cannot enter the enterohepatic circulation, leading to bile salt malabsorption. Bacterial overgrowth after small bowel surgery or terminal ileal disease can ↑ deconjugation.

The ^{14}C -labelled synthetic bile acid glycocholine is administered PO. Rapid transit into the large bowel will result in breakdown of the label by the normal large bowel flora and a rise in detected exhaled $^{14}\text{CO}_2$. Small bowel bacterial overgrowth, e.g. due to a 'blind loop', will also result in ↑ liberation of $^{14}\text{CO}_2$.

Indications

- Bacterial overgrowth.
- Bile salt malabsorption.

Patient preparation

- Starve overnight.
- Avoid antibiotics for 1 month before study.

Procedure

- Give oral ^{14}C -labelled glycocholic acid in water.
- Count $^{14}\text{CO}_2$ activity in breath samples over 6h using β liquid scintillation counter.

Results

Glycocholate is deconjugated into ^{14}C glycine and cholic acid by small intestine bacteria, releasing expired $^{14}\text{CO}_2$. Correct result for age-related variations in endogenous $^{14}\text{CO}_2$ production.

Interpretation

↑ $^{14}\text{CO}_2$ levels imply bacterial colonization or bile salt malabsorption.

Advantages

Accurate. Only available test.

Pitfalls

False –ves (unusual).

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