Mechanisms of Vascular Disease

A Textbook for Vascular Specialists

Robert Fitridge
Editor

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Contents

Abbreviations

^{18}F-FDG	Fluorodeoxyglucose
^{18}F-FDG PET-CT	^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography
^{18}F-NaF	Sodium fluoride
3D-IF	3D-image fusion
5-HT	5-Hydroxytryptamine (also known as serotonin)
α-SMA	Alpha-smooth muscle actin
AAA	Abdominal aortic aneurysm
AAS	Acute aortic syndrome
AASV	Anterior accessory saphenous veins
AAV	ANCA-associated vasculitides
ABC	Automatic brightness control
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
ABI	Ankle-brachial index
AC	Abdominal compliance
ACE	Angiotensin-converting enzyme
ACS	Acute coronary syndrome
ACS	Abdominal compartment syndrome
ACT	Acceptance and commitment therapy
Acta2	Smooth muscle α-actin
ACTA2	Actin alpha 2 gene
ADA2	Adenosine deaminase-2
ADAMTS13	Disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13
ADAMTS3	Disintegrin and metalloprotease with thrombospondin motifs-3 protease
ADP	Adenosine diphosphate
AGE	Advanced glycated end-products
AK	Above-knee
AKI	Acute kidney injury

ALARA	As low as reasonably achievable
ALCS	Acute limb compartment syndrome
AMP	Adenosine monophosphate
AMPA	α-Amino-3 hydroxy-5-methylisoxazole receptors
ANA	Antinuclear antibodies
ANCA	Anti-neutrophil cytoplasmic antibody
Ang	Angiopoietin
AngII	Angiotensin II
Anti-GBM	Anti-glomerular basement membrane disease
AP	Antero-posterior
AP-1	Activating protein-1
APC	Activated protein C
APLAS	Antiphospholipid antibody syndrome
apoA-I	Apolipoprotein A-I
apoB	Apolipoprotein B
ApoE	Apolipoprotein E
APP	Abdominal perfusion pressure
ARCL1	Autosomal recessive cutis laxa type 1
ARDS	Acute respiratory distress syndrome
ASCVD	Atherosclerotic cardiovascular disease
AT	Antithrombin
ATP	Adenosine triphosphate
ATS	Arterial tortuosity syndrome
AVG	Arteriovenous grafts
AZA	Azathioprine
BAPN	3-Aminopropionitrile
BAV	Bicuspid aortic valve
BC	Boundary conditions
BC	Bacterial cellulose
BCL-2	B-cell lymphoma-2
BCRL	Breast cancer-related lymphoedema
BD	Behcet's disease
BEIR VII	Biological Effects of Ionizing Radiation VII Committee
BEVAR	Branched endovascular aneurysm repair
BGN	Biglycan gene
BK	Below-knee
BKCa	Large-conductance Ca^{2+}-activated K^+ channel
BMI	Body mass index
BMP	Bone morphogenetic protein
BNC	Bacterial nanocellulose
BTK	Bruton tyrosine kinase
BVS	Bioresorbable vascular scaffold
C-ANCA	Cytoplasmic anti-neutrophil cytoplasmic antibody
CAA	Coronary artery aneurysms
CAC	Coronary artery calcification

CAD	Coronary artery disease
CAD	Computer-aided design
CAK	Cumulative air kerma
CAM	Calmodulin
cAMP	Cyclic adenosine monophosphate
CBCT	Cone-beam CT
CBD	Cannabidiol
CCA	Clonal chromosomal abnormalities
CCK	Cholecystokinin
CCL5	C-C motif chemokine ligand 5
CCTA	Coronary computed topographic angiography
CD36	Cluster of differentiation-36
Cdc42	Cell division cycle 42
CDs	Clusters of differentiation
CE-MRA	Contrast-enhanced magnetic resonance angiography
CEA	Carotid endarterectomy
CEAP	Clinical, etiological, anatomical, pathophysiological
CEMRI	Contrast-enhanced magnetic resonance imaging
CETP	Cholesteryl ester transfer protein
CFD	Computational fluid dynamics
cGMP	Cyclic guanosine monophosphate
CGRP	Calcitonin gene-related peptide
CHCC	Chapel Hill Consensus Conference
CKD	Chronic kidney disease
CLI	Critical limb ischaemia
CMR	Cardiac magnetic resonance
CNC	Cardiac neural crest
CNCP	Chronic noncancer pain
cNOS	Constitutive nitric oxide synthase
CNS	Central nervous system
CO	Carbon monoxide
COX	Cyclo-oxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclo-oxygenase-2
CPIs	Checkpoint inhibitors
CRC	Colorectal cancer
CRM	Crew resource management (CRM)
CRP	C-Reactive protein
CRPS	Complex regional pain syndromes
CRRETAWAC	Cysteine-arginine-arginine-glutamic acid-threonine-alanine-tryptophan-cysteine peptide
CSE	Cystathionine γ-lase
CT	Computerised tomography
CTA	Coronary tomography angiography
CTLA4	Cytotoxic T-lymphocyte-associated protein 4

CV	Cryoglobulinaemic vasculitis
CVD	Cardiovascular diseases
CXCL1	Chemokine (C-X-C motif) ligand 1
CXCL16	C-X-C motif chemokine 16
CyA	Cyclosporin A
CYC	Cyclophosphamide
CYP	Cytochrome
DAG	Diacylglycerol
DAMPs	Danger (or damage)-associated molecular patterns
DAP	Dose area product
DAPT	Dual anti-platelet therapy
DEJ	Dermo-epidermal junction
DFI	Diabetic foot infection
DFO	Diabetic foot osteomyelitis
DFU	Diabetic foot ulcers
DICOM	Digital imaging and communications in medicine
DIT	Diffuse intimal thickening
DL	Decompression laparotomy
DLL4	Delta-like ligand 4
DLT	Decongestive lymphatic therapy
DM	Diabetes mellitus
DMARDs	Disease modifying antirheumatic drugs
DN	Diabetic neuropathy
DOAC	Direct oral anticoagulant
DOF	Degrees of freedom
DREZ	Dorsal root entry zone
DRG	Dorsal root ganglion
DRLs	Diagnostic reference levels
DSA	Digital subtraction angiography
DSCT	Dual-source CT
DTS	Dense tubular system
DUS	Duplex ultrasonography
DVT	Deep vein thrombosis
EC	Endothelial cell
ECM	Extracellular matrix
ECMO	Extracorporeal membrane oxygenation
ED	Effective dose
EDCF	Endothelium-derived contracting factor
EDH	Endothelium-dependent hyperpolarisation
EDHF	Endothelium-derived hyperpolarising factor
EDS	Ehlers-Danlos Syndrome
EDV	End-diastolic velocity
EETs	Epoxyeicosatrienoic acids
EFEMP2	Fibulin-4 gene
EFNS	European Federation of Neurological Societies

EGDT	Early goal-directed therapy
EGF	Epidermal growth factor
EGPA	Eosinophilic granulomatosis with polyangiitis
ELAM-1	Endothelial-leukocyte adhesion molecule
ELG	Endoluminal grafts
ELISA	Enzyme-linked immunosorbent assay
EMR	Electromagnetic radiation
eNOS	Endothelial nitric oxide synthase
EPC	Endothelial progenitor cell
EPCR	Endothelial protein C receptor
EPS	Extracellular polymer substances
ePTFE	Expanded polytetrafluoroethylene
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate
ET	Essential thrombocytosis
ET-1	Endothelin-1
EULAR	European League Against Rheumatism
EVAR	Endovascular aneurysm repair
FBLN4/EFEMP2	Fibrillin-4
FBN1	Fibrillin-1 gene
FDA	Food and Drug Administration
FDPs	Fibrin degradation products
FEA	Finite element analysis
FEVAR	Fenestrated endovascular aneurysm repair
FGF	Fibroblast growth factor
FLNA	Filamin A gene
FMD	Fibromuscular dysplasia
FOV	Field of views
FOXE3	Forkhead box E3 gene
FSI	Fluid–solid interaction
FSP	Fluorosurfactant polymer
FT	Fluoroscopy time
FTAAD	Familial thoracic aortic aneurysm dissection
FVL	Factor V Leiden
G-CSF	Granulocyte colony-stimulating factor
GABA	γ-Aminobutyric acid
GCA	Giant cell arteritis
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GLUT10	Glucose transporter 10
Gly	Glycine
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GP	Glycoprotein
GPA	Granulomatosis with polyangiitis
GPCR	G Protein-coupled receptor

GPELF	Global Programme to Eliminate Lymphatic Filariasis
GPI	Glycosylphosphatidylinositol
Gs-IVUS	Greyscale IVUS
GSM	Greyscale median
GSV	Great saphenous vein
GTPase	Guanosine triphosphatase
GWAS	Genome-wide association studies
Gy	Gray
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
HARM	Hyperintense acute reperfusion injury marker
HBOT	Hyperbaric oxygen therapy
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High-density lipoproteins
HDL-C	High-density lipoprotein cholesterol
HES	Hydroxyethyl starch
HETE	Hydroxyeicosatetraenoic acid
HIF	Hypoxia-inducible factor
HIT	Heparin-induced thrombocytopenia
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
HMGB1	High-mobility group box 1
HMW	High molecular weight
HMWK	High-molecular-weight kininogen
HO	Haem oxygenase
HPETE	Hydroperoxyeicosatetraenoic acid
hs-CRP	High-sensitivity C-reactive protein
HSPs	Heat shock proteins
HU	Hounsfield unit
HUV	Human umbilical vein
HUV	Hypocomplementaemic urticarial vasculitis
I/R	Ischaemia-reperfusion
IA	Intussusceptive angiogenesis
IAH	Intra-abdominal hypertension
IAP	Intra-abdominal pressure
IASP	International Association for the Study of Pain
IB-IVUS	Integrated backscatter IVUS
ICA	Internal carotid artery
ICAM-1	Intercellular adhesion molecule-1
ICD-11	International Classification of Diseases
ICG	Indocyanine green
ICRP	International Commission on Radiation Protection
ICU	Intensive care unit
ICV	Immune complex vasculitides

IDL	Intermediate-density lipoproteins
IFC-ACS	ACS due to intact fibrous caps
IFN	Interferon
IFN-γ	Interferon gamma
IFX	Infliximab
IgAV	Immunoglobulin-A vasculitis
IGF	Insulin-like growth factor
IgG4-RD	IgG4-related disease
IH	Intimal hyperplasia
IIF	Indirect immunofluorescence
IKCa	Intermediate-conductance Ca^{2+}-activated K+ channels
IL	Interleukin
IL-6	Interleukin-6
IL-6R	Interleukin-6 receptor
Ile	Isoleucine
IM	Internal membrane
iMAP-IVUS	iMAP-intravascular ultrasound
IMH	Intra-mural haematoma
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase
InsP3	Inositol 1,4,5-trisphosphate
IP	Prostanoid
IP3	Inositol (1,4,5) trisphosphate
IP3	Inositol trisphosphate
IPH	Intraplaque haemorrhage
IRAD	International Registry of Acute Aortic Dissection
irAEs	Immune-related adverse events
IRI	Ischaemia-reperfusion injury
IRP	Interventional reference point
ITPKC	Inositol-triphosphate 3-kinase
IVC	Inferior vena cava
IVIG	Intravenous immunoglobulin
IVUS	Intravascular ultrasound
IWGDF	International Working Group in Diabetic Foot
JAK	Janus kinases
KAP	Kerma area product
KATP	Adenosine triphosphate-sensitive K+
KD	Kawasaki disease
KLF	Krüppel-like factor
LAO	Left anterior oblique
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LDS	Loeys-Dietz syndrome
LECs	Lymphatic endothelial cells

LEF	Leflunomide
Leu	Leucine
LMWH	Low-molecular-weight heparin
LNP	Localised neuropathic pain
LOPS	Loss of protective sensation
LOX	Lysine 6 oxidase (lysyl oxidase)
LOX-1	Lectin-like low-density lipoprotein receptor-1
Lp-PLA2	Lipoprotein-associated phospholipase A2
LP(a)	Lipoprotein-(a)
LPS	Lipopolysaccharide
LR-NC	Lipid-rich necrotic core
LTA	Light transmittance aggregometry
LVA	Lymphatico-venous anastomosis
LVV	Large vessel vasculitis
M-CSF	Macrophage colony-stimulating factor
MAC	Medial arterial calcification
MAC-1	Macrophage-1 antigen
MACE	Major adverse cardiovascular events
MAGP2	Microfibril-associated glycoprotein 2
MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinases
MAT2α	Methionine adenosyltransferase IIα
MCP-1	Monocyte chemotactic protein-1
MCSV	Multiple cross-sectional views
MDCT	Multidetector-row CT
MEFV	Mediterranean fever gene
MEKK1	Mitogen-activated protein kinase 1
MFAP5	Microfibrillar-associated protein 5 gene
MFS	Marfan syndrome
MGP	Matrix gla-protein
MH1/2	Mad-homology 1 and 2
MHC	Major histocompatibility complex
MHC-II	Class II major histocompatibility complex
Mhy11	Smooth muscle myosin heavy chain
MI	Myocardial infarction
MIF	Macrophage inhibitory factor
MIP-1α	Macrophage inflammatory protein 1-alpha
MIP2	Macrophage inflammatory protein 2
miR	MicroRNAs
MLC20	Myosin light chain protein
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MLD	Manual lymphatic drainage
MLS	Meester-Loeys syndrome
MMF	Mycophenolate mofetil

mmHg	Millimetres of mercury
MMP	Matrix metalloproteinase
mmPb LE	Lead equivalence
MOD	Multi-organ dysfunction
MPa	Mega-Pascal
MPA	Microscopic polyangiitis
MPN	Myeloproliferative neoplasms
MPO	Myeloperoxidase
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MRL	Magnetic resonance lymphangiography
MRSA	Methicillin-resistant *S. aureus*
MRTA	Magnetic resonance tomographic angiography
MTHFR	Methylenetetrahydrofolate reductase
MTX	Methotrexate
MVV	Medium vessel vasculitis
MyD88	Myeloid differentiation primary response 88
MYH11	Smooth muscle myosin heavy chain
MYLK	Smooth muscle myosin light chain kinase
NADPH	Nicotinamide adenine dinucleotide phosphate
NaV1.7, NaV1.8 and NaV1.9	Voltage-gated sodium channels
NCP	Non-calcified plaques
NETs	Neutrophil extracellular traps
NeuPSIG	Neuropathic Pain Special Interest Group of the IASP
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B-cells
NGAL	Neutrophil gelatinase-associated lipocalin
NGF	Nerve growth factor
NIR	Near-infrared lymphangiography
NIRAF	Near-infrared autofluorescence
NIRF	Intravascular near-infrared fluorescence
NIRS	Near-infrared spectroscopy
NK-1	Neurokinin
NMB	Neuro-muscular blockade
NMDA	*N*-methyl D-aspartate
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOD	Nucleotide-binding and oligomerization domain
NOS	Nitric oxide synthase
NPWT	Negative pressure wound therapy
NSAIDs	Non-steroidal anti-inflammatory drugs
NSCLC	Non-small cell lung cancer
NSTEACs	Non-ST elevated acute coronary syndromes

NSTEMI	Non-ST segment elevation myocardial infarction
O_2^-	Superoxide anion
OA	Open abdomen
OCI	Operator-controlled imaging
OCT	Optical coherence topography
OH•	Hydroxyl radical
OM	Osteomyelitis
OPG	Osteoprotegerin
OPN	Osteopontin
OR	Odds ratio
OSL	Optically stimulated luminescence
OSR	Open surgical repair
OxLDL	Oxidised low-density lipoproteins
P-ANCA	Perinuclear anti-neutrophil cytoplasmic antibody
PAD	Peripheral artery disease
PAF	Platelet-activating factor
PAI-1	Plasminogen activator inhibitor-1
PAIs	Plasminogen activator inhibitors
PAMPs	Pathogen-associated molecular pathogens
PAN	Polyarteritis nodosa
PARs	Protease-activated receptors
PASV	Posterior accessory saphenous veins
PAU	Penetrating aortic ulcer
PBT	Probe to bone test
PC	Protein C
PCI	Percutaneous coronary intervention (angioplasty)
PCL	Polycaprolactone
PCSK9	Proprotein convertase subtilisin/kexin type 9
PCT	Procalcitonin
PD-1	Programmed cell death protein-1
PD-L1	Programmed cell death ligand-1
PDE-5	Phosphodiesterase type 5
PDGF	Platelet-derived growth factor
PDGFB	Platelet-derived growth factor B
PDGFRβ	Platelet-derived growth factor receptor β
PECAM-1	Platelet endothelial cell adhesion molecule-1
PEG	Polyethylene glycol
PET	Positron emitting topography
PGA	Polyglycolic acid
PGI2	Prostaglandin I2/Prostacyclin
PGM	Prothrombin gene mutation
PHD	Prolyl hydroxylase domain
PHN	Postherpetic neuralgia
PHZ	Para-anastomotic hyper-compliant zone
PI3K	Phosphatidylinositol 3-kinase

PIP2	Phosphatidylinositol 4,5-bisphosphate
PKC	Protein kinase C
PLA	Poly-lactic acid
PLC	Phospholipase C
PLGF	Placental growth factor
PLLA	Poly-L-lactic acid
PLP	Phantom limb pain
PMR	Plaque-to-myocardial signal intensity ratio
PMR	Polymyalgia rheumatica
PNH	Paroxysmal nocturnal haemoglobinuria
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PPARα	Peroxisome proliferator-activated receptor α
PPI	Proton-pump inhibitor (e.g. omeprazole)
PRKG1	Type I cGMP-dependent protein kinase gene
PRP	Primary Raynaud's phenomenon
PRTN3	Proteinase 3 gene
PRV	Polycythaemia rubra vera
PS	Protein S
PSC	Posterior subcapsular cataracts
PSD	Peak skin dose
PSGL	P-Selectin glycoprotein ligand-1
pSS	Sjögren's syndrome
PSV	Peak systolic velocity
PTA	Percutaneous transluminal angioplasty
PTFE	Polytetrafluoroethylene
PTS	Post thrombotic syndrome
PU	Polyurethane
PVNH	Periventricular nodular heterotopia
RAAA	Ruptured abdominal aortic aneurysm
Rac-1	Ras-related C3 botulinum toxin substrate 1
RAGE	Receptors for advanced glycated end products
RAK	Reference air kerma
RANTES	Regulated upon activation, normal T-cell expressed and secreted
RAO	Right anterior oblique
RCC	Renal cell carcinoma
RCTs	Randomised controlled trials
RFC-ACS	ACS due to ruptured fibrous caps
RGD	Arginine-glycine-aspartic acid
RGDS	Arginine-glycine-aspartic acid-serine
rHDL	Reconstituted high-density lipoprotein
RIAM	Rap1-GTP interacting adapter molecule
RLC	Integrin subunit alpha 9
RM	Regenerative medicine
ROS	Reactive oxygen species

RP	Raynaud's phenomenon
RPAK	Reference point air kerma
RSD	Reflex sympathetic dystrophy
RTX	Rituximab
SAA	Serum amyloid A
SAM	S-Adenosylmethionine
SAP	Stable angina plaques
SARA	SMAD anchor for receptor activation (involved in TGF-β signalling)
SBTE	Sheet-based tissue engineering
SC	Schwann cells
SCS	Spinal cord stimulation
SCVs	Small colony variants
SDF	Stromal cell-derived factor
SDF-1α	Stromal-derived growth factor-1α
SEP	Serum elastin peptide
SERPINA1	Alpha-1-antitrypsin gene
SFA	Superficial femoral artery
SGLT2	Sodium glucose cotransporter-2
SGS	Shprintzen-Goldberg syndrome
SIRS	Systemic inflammatory response syndrome
SKCa	Small-conductance Ca^{2+}-activated K+ channels
SLC2A10	Solute carrier family 2 member 10 gene
SLV	Single longitudinal view
SMAD2/3	Receptor-regulated mothers against decapentaplegic homologue 2 and 3 proteins
SMCs	Smooth muscle cells
SMP	Sympathetically maintained pain
SNPs	Single nucleotide polymorphisms
SNRIs	Serotonin/noradrenaline reuptake inhibitors
SOFA	Sequential [Sepsis-related] Organ Failure Assessment
SP	Substance P
SPP	Skin perfusion pressure
SR-A1	Scavenger receptor type 1
SR-A2	Scavenger receptor type 2
SR-PSOX	Scavenger receptor for phosphatidylserine and oxidised low-density lipoprotein
SRB1	Scavenger receptor B1
SRP	Secondary Raynaud's phenomenon
SSC	Surviving sepsis campaign
SSRIs	Selective serotonin reuptake inhibitors
SSV	Small saphenous vein
STEMI	ST-elevation myocardial infarction
Sv	Sievert
SvO2	Venous oxygen saturation

SVV	Small vessel vasculitis
T-regs	Regulatory T-cells
TA	Temporal artery
TAAD	Thoracic aortic aneurysm and dissection
TAAs	Thoracic aortic aneurysms
TAB	Temporal artery biopsy
TAC	Temporary abdominal closure
TAD	Thoracic aortic dissection
TAFI	Thrombin-activatable fibrinolysis inhibitor
TAK	Takayasu arteritis
TAO	Thromboangitis obliterans
TBAD	Type B aortic dissection
TBI	Toe:brachial pressure index
TCA	Tricyclic antidepressants
TCC	Total contact cast
TCFA	Thin-cap fibroatheroma
TcPO2	Transcutaneous oxygen tension
TCZ	Tocilizumab
TE	Tissue engineering
TEE	Transesophageal echocardiograpy
TENS	Transcutaneous electrical nerve stimulation
TESA	Tissue engineering by self-assembly
TEVAR	Thoracic endovascular aortic repair
TEVG	Tissue-engineered vascular graft
TF	Tissue factor
Tfh	T follicular helper cell
TFPI	Tissue factor pathway inhibitor
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor-beta
Th	T-helper cell
Th1	T-helper type 1 lymphocyte
THC	Tetrahydrocannabinol
TIA	Transient ischaemic attack
TIE-2	Tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domain-2
TIMP-3	Tissue inhibitors of matrix metalloproteinase-3
TIMPs	Tissue inhibitors of metalloproteinases
TIR	Toll/interleukin-1 receptor
TKIs	Tyrosine kinase inhibitors
TLD	Thermoluminescent dosimeter
TLRs	Toll-like receptors
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor-alpha
TNFi	Tumour necrosis factor inhibitors
TOE	Trans oesophageal echocardiography

TP	Thromboxane
tPA	Tissue plasminogen activator
TPs	Toe pressures
Treg	T regulatory
TRIF	Toll/interleukin-1 receptor domain-containing adaptor-inducing IFN-β
TrKa	Tropomyosin receptor kinase A
TRLs	Triglyceride-rich lipoproteins
TRP	Transient receptor potential ion channel
TRPV1	Transient receptor potential vanilloid type 1 ion channel
TTE	Transthoracic echocardiography
TXA2	Thromboxane A2
uPA	Urokinase-type plasminogen activator
VACM	Vacuum-assisted wound closure and mesh-mediated fascial traction
vasDCs	Vascular dendritic cells
VC	Vascular calcification
VCAM-1	Vascular cell adhesion molecule 1
VE-cadherin	Vascular endothelial cadherin
vEDS	Vascular EDS (EDS type IV)
VEGF	Vascular endothelial growth factor
VEGFA	Vascular endothelial growth factor A
VEGFR1	Vascular endothelial growth factor receptor 1
VH-IVUS	Virtual histology IVUS
VLA-4	Very late antigen-4
VLDL	Very low-density lipoproteins
VRT	Venous refilling time
VSMC	Vascular smooth muscle cell
VTE	Venous thromboembolism
vWF	von Willebrand factor
WBC	White blood cells
WHO	World Health Organisation
WIfI	Wound, Ischaemia and foot Infection classification system
WSS	Wall shear stress

Chapter 1
Vascular Endothelium in Health and Disease

Ran Wei, Paul M. Kerr, Stephen L. Gust, Raymond Tam, and Frances Plane

Key Learning Points

- All vascular endothelial cells have a common embryonic origin but show clear bed-specific heterogeneity in morphology, function, gene and protein expression, determined by both environmental stimuli and epigenetic features acquired during development.
- Endothelial heterogeneity is also demonstrated by regional differences in the release of vasoactive and inflammatory mediators in response to stimuli such as changes in shear stress and hypoxia, and in expression of pro- and anti-coagulant molecules.
- The first identified endothelium-derived relaxing factor (nitric oxide; NO) is a short-lived free radical synthesised from L-arginine by endothelial NO synthase (eNOS) and destroyed by reactive oxygen species (ROS).
- Carbon monoxide (CO) and hydrogen sulphide (H_2S) are two other gaseous mediators contributing to endothelium-dependent modulation of vascular tone.
- Endothelium-dependent hyperpolarisation (EDH) is an important regulator of blood flow and blood pressure and plays a predominant role in smaller vessels.
- Arachidonic acid is released from endothelial cell membrane phospholipids and metabolised into a number of vasoactive factors by cyclooxygenase (COX), lipoxygenase and cytochrome P450 monooxygenase enzymes.
- Endothelin-1 (ET-1) is a potent vasoconstrictor which is released from the vascular endothelium. NO strongly inhibits the release of ET-1 and ET-1 attenuates

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NO-mediated dilation. Therefore, ET-1 and NO are functionally interdependent and many of the cardiovascular complications associated with endothelial dysfunction may be due to an imbalance in this relationship.

- Angiogenesis is the growth of new blood vessels formed by endothelial cell tubes sprouting from existing vessels. This process involves the following sequence of events:
  - Activation of endothelial cells
  - Degradation of extracellular matrix by matrix metalloproteinases
  - Proliferation and directional migration of endothelial cells
  - Formation of endothelial tubes
  - Maturation of new vessels by recruitment of pericytes and smooth muscle cells to stabilise endothelial sprouts and secrete extracellular matrix molecules to form the vascular basement membrane
- Angiogenesis in response to ischaemia is largely controlled by hypoxia-inducible factor-1 (HIF-1). Recruitment and proliferation of bone marrow-derived endothelial progenitor cells to form new vessels (vasculogenesis) is a separate but complimentary process which occurs simultaneously in ischaemic and wound tissue to augment perfusion.
- The endothelium is one of the few surfaces that can maintain blood in a liquid state during prolonged contact. The endothelium also prevents thrombin formation by expressing tissue factor pathway inhibitor that binds to clotting factor Xa and thrombomoduli, which in turn bind to and inactivate thrombin, thus blocking its pro-coagulant activity. Several other haemostatic factors are also expressed by the endothelium, in particular von Willebrand factor (vWF) and plasminogen activator inhibitor-1 (PAI-1).
- Vascular endothelium has potent anti-platelet aggregation properties which are mediated by the synthesis of prostacyclin (PGI_2) and NO.
- The endothelial response to inflammation and infection involves the production of inflammatory cytokines (such as interleukin-8) and the expression of surface adhesion molecules (selectins), which facilitate leukocyte adhesion and migration to the site of inflammation/infection.

1.1 Introduction

The endothelium, first described over 100 years ago as an inert anatomical barrier between the blood and cells of the vessel wall, is now recognized as a dynamic organ with secretory, synthetic, metabolic, and immunologic functions. Endothelial cells play an obligatory role in modulating vascular tone and permeability, angiogenesis, and in mediating haemostatic, inflammatory and reparative responses to local injury. To fulfil these roles, the endothelium is continuously responding to spatial and temporal changes in mechanical and biochemical stimuli. Such responsiveness is affected through receptors for growth factors, lipoproteins, platelet

products, and circulating hormones, which regulate changes in RNA and protein expression, cell proliferation, and migration or the release of vasoactive and inflammatory mediators [1].

All vascular endothelial cells have a common embryonic origin but show clear bed-specific heterogeneity in morphology, function, and gene and protein expression, determined by both environmental stimuli and epigenetic features acquired during development. Thus, the endothelium should not be regarded as an homogenous tissue but rather a conglomerate of distinct populations of cells sharing many common functions but also adapted to meet regional demands [2]. In most blood vessels, continuous endothelium provides an uninterrupted barrier between the blood and tissues and ensures tight control of permeability at the blood-brain barrier. In regions of increased trans-endothelial transport such as capillaries of endocrine glands and the kidney, the presence of fenestrae, transcellular pores approximately 70 nm in diameter with a thin fenestral diaphragm across their opening, facilitate the selective permeability required for efficient absorption, secretion, and filtering. In hepatic sinuses, the presence of a discontinuous endothelium with large fenestrations (0.1–1 mm in diameter) lacking a fenestral diaphragm, provides a highly permeable and poorly selective sieve essential for transfer of lipoproteins from blood to hepatocytes [1, 3].

Beyond these structural variations, endothelial heterogeneity is also manifest in regional differences in the release of vasoactive and inflammatory mediators, in responsiveness to stimuli such as changes in shear stress and hypoxia, and in expression of pro- and anti-coagulant molecules. For example, the contribution of nitric oxide (NO) to endothelium-dependent vasodilation appears to be greater in large conduit arteries compared to small resistance vessels [4]. Expression of von Willebrand factor (vWF), a circulating glycoprotein that mediates platelet adhesion to the subendothelial surface of injured blood vessels, displays a mosaic pattern in the aorta and in selected capillary beds [5]. These regional differences between endothelial cells extend to their susceptibility to injury in the face of cardiovascular risk factors such as hypercholesterolemia, diabetes and smoking, and thus impact the function of the vasculature both in health and disease.

This chapter provides an overview of how the endothelium regulates four key aspects of cardiovascular homeostasis; vascular tone, angiogenesis, haemostasis and inflammation.

1.2 Endothelium-Dependent Regulation of Vascular Tone

Since the first report of endothelium-dependent modulation of the contractile state of smooth muscle cells in the artery wall [6], it has become apparent that endothelial cells release a plethora of vasoactive factors in response to a wide range of mechanical and chemical stimuli. Many of these factors also modulate processes such as inflammation, cell adhesion, and coagulation, highlighting the crucial physiological role of the endothelium, and why endothelial dysfunction is pivotal in the

development of cardiovascular diseases such as atherosclerosis and hypertension. Here we will focus on the four major pathways underlying endothelium-dependent modulation of vascular tone; the gaseous mediators NO, carbon monoxide (CO) and hydrogen sulfide (H_2S), endothelium-dependent hyperpolarisation (EDH), metabolites of arachidonic acid, and endothelin.

1.2.1 Gaseous Mediators

The first endothelium-derived relaxing factor described by Furchgott and Zawadski was subsequently identified as NO, a short-lived free radical synthesized from L-arginine by endothelial NO synthase (eNOS) and destroyed by reactive oxygen species (ROS). Once released from the endothelium, NO activates the haem-dependent enzyme soluble guanylyl cyclase in surrounding smooth muscle cells, leading to formation of cyclic guanosine monophosphate (cGMP). Subsequent activation of cGMP-dependent kinase leads to phosphorylation of a diverse range of target proteins such as large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, Rho kinase, myosin light chain phosphatase and phospholamban, that mediate smooth muscle cell relaxation and hence vasodilation [7]. This signalling pathway is terminated by phosphodiesterase enzymes that degrade cGMP. These enzymes are inhibited by the drug sildenafil used for treatment of erectile dysfunction [8]. NO can also act in a cGMP-independent manner (e.g. nitrosylation of proteins), which will not be discussed here but has been reviewed by Lima et al. [9].

eNOS is a bidomain enzyme; an N-terminal oxygenase domain with binding sites for haem, tetrahydrobiopterin, oxygen and the substrate L-arginine which supports catalytic activity, and also a C-terminal reductase domain which binds the co-factors nicotinamide adenine dinucleotide phosphate (NADPH), flavin mononucleotide and flavin adenine dinucleotide. Transfer of electrons from NADPH to flavins in the reductase domain and then to the haem in the oxygenase domain is required so that the haem iron can bind oxygen and catalyze the synthesis of NO from L-arginine. Binding of the ubiquitous Ca^{2+} regulatory protein calmodulin (CAM) facilitates transfer of electrons from the reductase to the oxygenase domain and is critical for activation of the enzyme [10].

eNOS is constitutively expressed in all endothelial cells but regulation of enzyme activity by physiological and pathophysiological stimuli occurs via a complex pattern of transcriptional and post-translational modifications. Both eNOS mRNA and protein levels are increased by fluid shear stress via activation of a pathway involving c-Src-tyrosine kinase and transcription factor nuclear factor κ-light-chain-enhancer of activated B cells (NFκB). At the post-translational level, eNOS activity is highly regulated by substrate and cofactor availability as well as by endogenous inhibitors, lipid modification, direct protein-protein interactions, phosphorylation, O-linked glycosylation, and S-nitrosylation.

Agonists at endothelial G-protein coupled receptors (GPCRs) such as bradykinin and acetylcholine elicit Ca^{2+}-CAM-dependent NO production via phospholipase

C-mediated generation of inositol 1,4,5-trisphosphate ($InsP_3$) and subsequent release of Ca^{2+} from intracellular stores. Activation of tyrosine kinase linked receptors such as the vascular endothelial growth factor (VEGF) receptor, and mechanical stimulation of the endothelium by shear stress, lead to phosphorylation of eNOS at Ser1177 to increase the Ca^{2+} sensitivity of the enzyme so that it can be activated at resting Ca^{2+} levels. Distinct kinase pathways can mediate eNOS phosphorylation; shear stress elicits phosphorylation of Ser1177 via protein kinase A, whereas insulin and VEGF cause phosphorylation of the same residue via the serine/threonine protein kinase Akt. Conversely, phosphorylation of the enzyme at Tyr657 within the flavin mononucleotide domain, or Thr495 within the CAM-binding domain, inhibits enzyme activity [11].

Within endothelial cells, eNOS is targeted to invaginations of the cell membrane called caveolae, membrane microdomains enriched in cholesterol and sphingolipids, and defined by the presence of the scaffolding protein caveolin-1. Caveolae sequester diverse receptors and signalling proteins including GPCRs, growth factor receptors, and Ca^{2+} regulatory proteins such as CAM. Thus, targeting of eNOS to this region facilitates communication with upstream and downstream pathways. Within caveolae, caveolin-1 tonically inhibits eNOS activity, thereby limiting the production of NO. The binding of Ca^{2+}-CAM leads to disruption of the caveolin-1/eNOS interaction and increases eNOS activity [12]. Other associated proteins such as platelet endothelial cell adhesion molecule-1 (PECAM-1) modulate eNOS activity by virtue of their function as scaffolds for the binding of signalling molecules such as tyrosine kinases and phosphatases [13].

The release of NO by stimuli such as shear stress, circulating hormones (e.g. catecholamines, vasopressin), plasma constituents (e.g. thrombin), platelet products (e.g. serotonin), and locally-produced chemical mediators (e.g. bradykinin) plays a critical role in mediating acute changes in local blood flow and tissue perfusion. Shear stress-stimulated NO production is central to exercise-induced increases in blood flow in skeletal muscle [14]. Production of NO in response to serotonin released from aggregating platelets, dilates coronary arteries to prevent clots from occluding vessels [15]. Mice lacking eNOS are hypertensive and infusion of L-arginine analogues, competitive inhibitors of eNOS, cause alterations in local blood flow and in systemic blood pressure, demonstrating the importance of endothelium-derived NO in long-term cardiovascular control in vivo [11]. In humans, elevated levels of an endogenous inhibitor of eNOS, asymmetric dimethylarginine, are associated with hypertension and increased cardiovascular risk [16].

In addition to its vasodilator actions, NO is now recognized as playing other protective roles in the vasculature as a regulator of inflammation and vessel repair. Loss of NO-mediated vasodilation, due to reduced expression or activity of eNOS and/or oxidative stress-mediated reductions in NO bioavailability, is a hallmark of endothelial dysfunction associated with cardiovascular risk factors such as hypercholesterolemia, smoking, diabetes and obesity. Loss of endothelium-derived NO tips the homeostatic balance in favour of vasoconstriction, proliferation, activation of platelets and blood clot formation, and inflammation.

Therefore loss of NO contributes to clinical manifestations such as high blood pressure, atherosclerosis, and thrombosis, which are associated with significant morbidity and mortality [17].

Although they have received much less attention than NO, two other gaseous mediators, CO and H_2S, also contribute to endothelium-dependent modulation of vascular tone. CO is generated within endothelial cells during catabolism of heme by the enzyme heme oxygenase (HO). Of the two known isoforms, HO1 is an inducible form associated with increased oxidative stress while HO2 is constitutively expressed in endothelial cells and, like eNOS, activated by Ca^{2+}-CAM. The strongest evidence for a physiological role of endothelium-derived CO in regulation of vascular tone has come from studies of the cerebral circulation in which GPCR agonists and hypoxia cause Ca^{2+}-CAM- and HO-dependent vasodilation. CO-mediated smooth muscle relaxation is due to activation of soluble guanylyl cyclase which increases cGMP levels, leading to cGMP-dependent kinase-mediated activation of BK_{Ca} channels. In addition, CO can bind directly to the heme moiety bound to BK_{Ca} channels to directly elevate Ca^{2+} sensitivity of the channels [18]. To date, little is known about the mechanisms regulating CO production or alterations in HO/CO signalling in disease states.

Release of endothelium-derived H_2S, synthesized from cysteine by cystathionine γ-lase (CSE), is stimulated by many factors including acetylcholine, increases in shear stress, oestrogens, and plant flavonoids. Evidence from mice lacking CSE suggests an important role for H_2S in the physiological maintenance of blood pressure [19]. Like eNOS, CSE activity is regulated by a complex integration of transcriptional, post-transcriptional, and post-translational mechanisms. The rapid metabolism of H_2S via an oxygen-dependent pathway within mitochondria suggests that it may play a greater role in hypoxic rather than normoxic tissues [20].

Several signalling mechanisms have been described for H_2S such as reaction with heme-containing proteins, protein S-sulfhydration, reaction with ROS, and reduction of protein disulfide bonds to thiols [21]. The vasodilator action of H_2S has largely been attributed to hyperpolarization of the smooth muscle membrane potential mediated by opening adenosine triphosphate-sensitive K^+ (K_{ATP}) channels; H_2S sulfhydrates K_{ATP} channels at Cys43 to facilitate the binding of phosphatidylinositol(4,5)bisphosphate, a physiological activator of these channels [22]. However, the sensitivity of H_2S-evoked vasodilation to blockers of small (SK_{Ca}) and intermediate (IK_{Ca}) conductance Ca^{2+}-activated K^+ channels has also raised the possibility that H_2S may evoke relaxation via EDH [23].

Interactions between NO and H_2S signalling pathways can occur at a number of points, making for a complex relationship between the two mediators; NO inhibits CSE activity via S-nitrosation but can increase CSE expression and cellular uptake of its substrate cystine, and conversely, H_2S potentiates cGMP accumulation via the inhibition of phosphodiesterase. Alterations in H_2S production and/or signalling have been observed in animal models of endothelial dysfunction associated with pathological conditions such as diabetes and obesity, leading to H_2S donors being considered as potential therapeutic agents for the treatment of these diseases [24].

1.2.2 *Endothelium-Dependent Hyperpolarisation*

Observations of agonist-induced endothelium-dependent vasorelaxation which persisted in the presence of inhibitors of COX and eNOS and was accompanied by hyperpolarisation of the vascular smooth muscle cell membrane potential led to identification of a third endothelium-derived relaxing factor, endothelium-derived hyperpolarising factor (EDHF). Hyperpolarisation of the smooth muscle cells reduces the probability of opening the voltage-dependent Ca^{2+} channels, thus reducing Ca^{2+} influx to cause relaxation. A range of agents have been proposed to account for the actions of EDHF including K^+, epoxyeicosatrienoic acids (EETs) and C-type natriuretic peptide. However, it is now widely accepted that, rather than a diffusible factor, endothelium-dependent hyperpolarisation (EDH) of vascular smooth muscle is mediated by direct electrical coupling between endothelial and smooth muscle cells via myoendothelial gap junctions [25].

The initiating step in EDH-mediated vasorelaxation is activation of endothelial SK_{Ca} and IK_{Ca} channels. These channels, activated by increases in intracellular Ca^{2+} via CAM which is constitutively associated with the channels, are voltage-independent and thus can operate at negative membrane potentials close to the K^+ equilibrium potential. Inhibition of endothelium-dependent relaxation by a combination of SK_{Ca}/IK_{Ca} channel blockers is now regarded as the hallmark of EDH-mediated vasodilation, but the relative contribution of the two channels varies between different stimuli. This ability of endothelial cells to generate stimulus-specific responses to diverse inputs is facilitated by organization of SK_{Ca} and IK_{Ca} channels into spatially distinct microdomains that allow for differential activation by localized increases in Ca^{2+}. SK_{Ca} channels are located within caveolae at inter-endothelial junctions on the luminal surface where together with eNOS, they can respond to local Ca^{2+} increases elicited by shear stress-induced activation of transient receptor potential vanilloid type 4 (TRPV4) channels. In contrast, IK_{Ca} channels are located on the abluminal surface close to myoendothelial contact points where they are activated by localized, $InsP_3$-mediated increases in Ca^{2+} evoked by GPCR agonists (Fig. 1.1) [26].

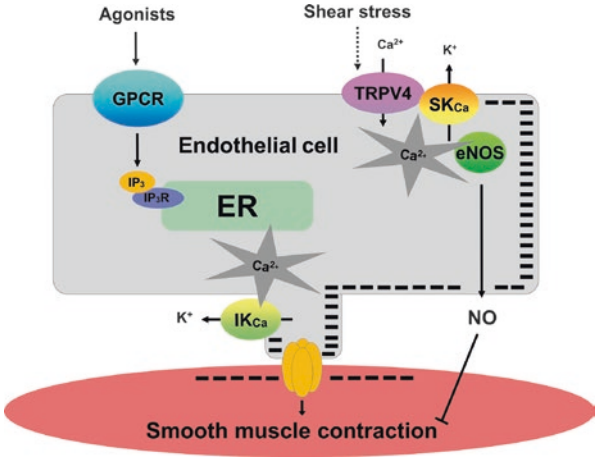

Fig. 1.1 Schematic showing differential localization and activation of endothelial SK_{Ca} and IK_{Ca} channels. SK_{Ca} channels are located on the luminal surface and respond to local Ca^{2+} increases elicited by shear stress-induced activation of TRPV4 channels. IK_{Ca} channels are located on the abluminal surface where they are activated by localized, $InsP_3$-mediated increases in Ca^{2+} evoked by GPCR agonists

The importance of EDH as a regulator of blood flow and blood pressure in vivo is demonstrated by enhanced resistance artery tone and elevated systemic blood pressure seen in mice lacking endothelial SK_{Ca} and/or IK_{Ca} channels. Loss of EDH, due to changes in expression or activity of SK_{Ca}/IK_{Ca} channels, contributes to experimental hypertension and diabetes-related erectile dysfunction. In contrast, resistance of the EDH pathway to the deleterious actions of ROS may allow EDH-mediated vasodilation to be maintained in the face of reduced bioavailability of NO in atherosclerosis and heart failure. Thus, selective activation of endothelial SK_{Ca} and IK_{Ca} channels is a potential therapeutic avenue for the future [27].

1.2.3 Metabolites of Arachidonic Acid

Within endothelial cells, arachidonic acid, released from cell membrane phospholipids by phospholipases, is metabolized by COX, lipoxygenase (LO), and cytochrome P450 monooxygenase (CYP) enzymes to yield an array of vasoactive factors.

COX enzymes metabolise arachidonic acid to endoperoxide intermediates which are then converted to a range of eicosanoids (e.g. prostacyclin (PGI_2), thromboxane A2 (TXA2)) through the actions of various synthases. Two isoforms of COX are found in the endothelium. The constitutively expressed COX-1 has long been regarded as vasculoprotective, the predominant product being PGI_2 which acts on prostanoid (IP) receptors to cause vasodilation and inhibition of platelet aggregation via activation of adenylyl cyclase to increase levels of cyclic-adenosine monophosphate (cAMP). PGI_2 also inhibits platelet and lymphocyte adhesion to endothelium, limits vascular smooth muscle cell proliferation and migration, and counteracts the production of pro-inflammatory growth factors [17]. However, evidence is now emerging that GPCR-mediated activation of endothelial COX-1 can generate other products such as TXA2 which activates thromboxane (TP) receptors on smooth muscle cells and so functions as an endothelium-derived contracting factor (EDCF). Stimulation of TP receptors elicits not only vasoconstriction but also proliferation of vascular smooth muscle cells, platelet adhesion and aggregation, and expression of adhesion molecules on endothelial cells. A shift from production of endothelium-derived relaxing factors to COX-dependent EDCFs is implicated in endothelial dysfunction associated with ageing, diabetes, and hypertension in both animal models and humans. Since activation of TP receptors is the common downstream effector, selective antagonists of this receptor may have therapeutic potential in the treatment of cardiovascular diseases [28].

COX-2 was first identified as an inducible form of the enzyme regulated at the level of gene expression and associated with inflammation. However, it is expressed in some blood vessels in the absence of overt signs of inflammation, and may be a major source of vasculoprotective PGI_2; hence the deleterious cardiovascular consequences seen in some patients treated with selective COX-2 inhibitors [29].

LO enzymes deoxygenate polyunsaturated fatty acids to hydroperoxyl metabolites. The three LO isoforms expressed in endothelial cells are 5-LO, 12-LO, and 15-LO, which correspond to the carbon position of arachidonic acid oxygenation. Each LO oxygenates arachidonic acid to form a stereospecific hydroperoxyeicosatetraenoic

acid (HPETE) which are unstable and rapidly reduced to the corresponding hydroxyeicosatetraenoic acid (HETE). 5-LO is the initial enzyme in the synthesis of pro-inflammatory leukotrienes but 5-LO products do not seem to be involved in regulation of vascular tone. In contrast, products from the 12-LO and 15-LO pathways are vasoactive but show species and vessel variation in the responses they elicit. 12-HETE causes relaxation of a number of peripheral arteries including human coronary arteries but causes vasoconstriction in dog renal arteries. In the same vessels, 15-HPETE and 15-HETE cause slight vasorelaxation at lower concentrations but contractions at higher concentrations mediated by activation of TP receptors.

Cytochrome P450 (CYP) enzymes add oxygen across the double bonds of arachidonic acid to produce four cisepoxides, 14,15-, 11,12-, 8,9-, and 5,6-epoxyeicosatrienoic acids (EETs). Two CYP enzymes have been cloned from human endothelium, CYP2C8/9 and CYP2J2, both of which produce mainly 14,15-EET with lesser amounts of 11,12-EET. The latter are also the major EETs released from endothelial cells in response to GPCR agonists (e.g. acetylcholine, bradykinin) and physical stimuli such as cyclic stretch and shear stress. EETs are rapidly metabolized by esterification into phospholipids or hydration to dihydroxyeicosatrienoic acids by soluble epoxide hydrolase. EETs can cause vasodilation via a number of different pathways. They can stimulate endothelial TRPV4 channels, which are non-selective cation channels that mediate Ca^{2+} influx, to activate eNOS or IK_{Ca}/SK_{Ca} channels to cause EDH. In contrast, endothelium-dependent flow-induced dilation is linked to release of 5,6-EET to activate smooth muscle TRPV4 channels which form a complex with BK_{Ca} channels, thus coupling local increases in Ca^{2+} to membrane hyperpolarisation and vasorelaxation [30]. Development of 14,15-EET analogues such as 14,15-epoxyeicosa-5Z-enoic acid revealed strict structural and stereoisomeric requirements for relaxations suggesting a specific binding site or receptor mediating EETs actions. G protein coupled receptor 40 (GPR40), a member of a family of GPCRs that have fatty acids as ligands, was recently detected in endothelial and smooth muscle cells, and suggested to be a low-affinity EET receptor. 11,12-EET stimulation of GPR40 increased expression of COX-2 and connexin 43, a key component of myoendothelial gap junctions, but a role for this receptor in EET-mediated changes in vascular tone remains to be established [31].

In some models of endothelial dysfunction reduced bioavailability of NO is counteracted by increased production of EETs which can maintain endothelium-dependent vasodilator responses. Thus, strategies aimed at enhancing production of endothelium-derived EETs or inhibiting their degradation, may represent a new therapeutic approach to endothelial dysfunction [32].

1.2.4 Endothelins

Endothelins are a family of 21 amino acid peptides of which there are three members (ET-1, ET-2, ET-3) with a high level of homology and similar structure [33]. Endothelial cells produce only ET-1; endothelin ET-2 is produced in the kidney and

intestine, while ET-3 has been detected in the brain, gastrointestinal tract, lung and kidney. ET-1 is a potent vasoconstrictor inducing long-lasting vasoconstriction at a half maximum effective concentration in the nano-molar range, at least one order of magnitude lower than values reported for other vasoconstrictor peptides such as angiotensin II [34].

ET-1 is not stored by endothelial cells. Production is regulated at the level of gene expression with the rate of transcription being responsive to stimulants and inhibitors to allow rapid changes in the amounts released. Pro-inflammatory factors such as transforming growth factor-β and tumour necrosis factor–α (TNFα), insulin, and angiotensin II up-regulate ET-1 mRNA whereas NO, PGI_2 and shear stress cause down-regulation. ET-1 is synthesized as a larger protein, the pre-proET-1 (203 amino acids) that is cleaved to pro-ET-1 (38 amino acids) and then to ET-1 (21 amino acids) by endothelin-converting enzymes. The half-life of ET-1 protein and mRNA is 4–7 min and 15–20 min, respectively, and most plasma ET-1 (90%) is cleared by the lungs during first passage.

The biological effects of ET-1 are mediated by two GPCR subtypes, ETA and ETB which have opposing effects on vascular tone. ETA receptors on vascular smooth muscle cells are responsible for the majority of ET-1 induced vasoconstriction; activation of phospholipase C increases formation of $InsP_3$ and diacylglycerol, and the resultant increase in intracellular Ca^{2+} and activation of protein kinase C cause vasoconstriction. ETB receptors are mainly present on endothelial cells and play an important role in clearing ET-1 from the plasma by internalising the receptor complex once ET-1 has bound. Activation of endothelial ETB receptors induces vasodilatation by stimulating the release of PGI_2 and NO. Inhibition of ETB increases circulating ET-1 levels and blood pressure in healthy subjects demonstrating that although ET-1 is regarded as primarily a vasoconstrictor, ETB-mediated vasodilation is also physiologically important [34].

ET-1 is not only a vasoactive factor. Acting via ETB receptors, ET-1 modulates the formation and degradation of extracellular matrix (ECM) and thus plays a role in vascular remodelling. Acting via ETA, ET-1 promotes smooth muscle proliferation contributing to neointima formation following vascular injury and to thickening of the arterial wall in pathological conditions such as pulmonary arterial hypertension, atherosclerosis and venous graft occlusion. As NO strongly inhibits the release of ET-1 from the endothelium, and ET-1 attenuates NO-mediated dilation, ET-1 and NO are functionally interdependent and many of the cardiovascular complications associated with endothelial dysfunction may be due to an imbalance in this relationship [35].

1.3 Angiogenesis

Angiogenesis is the growth of new blood vessels formed by endothelial cells sprouting from existing vessels. In adults it is a protective mechanism initiated in response to tissue hypoxia, ischemia or injury. It is also a key process in pathological

conditions such as proliferative diabetic retinopathy and neovascularization of tumours and as such, inhibitors of angiogenesis have received considerable interest as a potential therapeutic strategy. The angiogenic process depends on a complex transcriptional network coordinating production and release of numerous cytokines and growth factors [36].

Angiogenesis requires a sequence of individual processes:

1. Activation of endothelial cells,
2. Degradation of ECM by metalloproteinase enzymes,
3. Proliferation and directional migration of endothelial cells,
4. Formation of endothelial tubes,
5. Maturation of new vessels by recruitment of pericytes and smooth muscle cells to stabilize endothelial sprouts and secrete ECM molecules to form the vascular basement membrane (Fig. 1.2).

The endothelial cells that sprout from the parent vessel (tip cells) possess long and motile filopodia that extend towards the source of pro-angiogenic growth factors and respond to other guidance cues to enable directional vessel growth [37].

Endothelial cell migration requires the dynamic regulation of interactions between integrins and the surrounding ECM. Integrins are cell surface receptors which provide adhesive and signalling functions and link the actin cytoskeleton of the cell to the ECM at areas called focal adhesions. Phosphorylation of focal adhesion kinase, a cytoplasmic non-receptor tyrosine kinase, in response to pro-angiogenic signal molecules stimulates cell contraction thus allowing cell movement

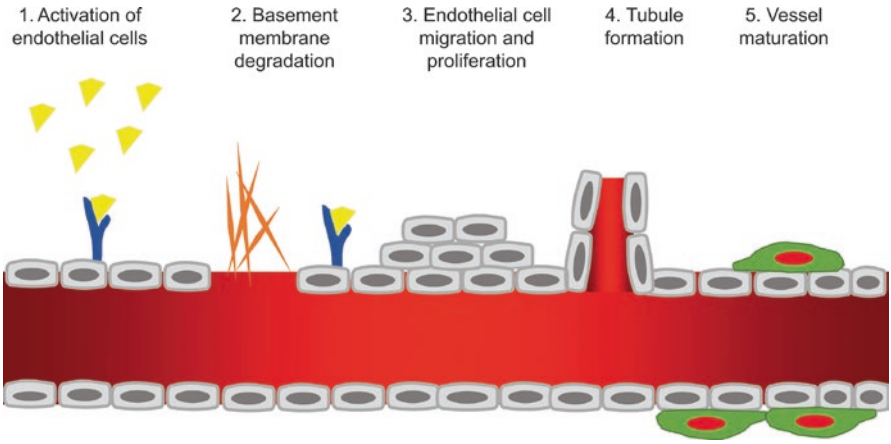

Fig. 1.2 Schematic of process of angiogenesis. Angiogenesis involves the following complex sequence of events: (1) Activation of endothelial cells. (2) Degradation of extracellular matrix by matrix metalloproteinases. (3) Proliferation and directional migration of endothelial cells. (4) Formation of endothelial tubes. (5) Maturation of new vessels by recruitment of pericytes and smooth muscle cells to stabilise endothelial sprouts and secrete extracellular matrix molecules to form the vascular basement membrane

on adhesive contacts. Subsequent integrin inactivation destroys the adhesive complex and allows detachment of the cell in its new location [38].

Cell-cell contacts between endothelial cells, essential for development of patent vessels, are mediated by cell surface receptors such as PECAM-1, a 130 kDa member of the immunoglobulin superfamily, which acts like a docking molecule to allow other proteins to provide further strength to vascular structures. Cadherins such as vascular endothelial cadherin are transmembrane proteins which provide weak adhesive cell-cell forces, further stabilized by catenins, intracellular proteins linking the cadherin cell surface molecule to the actin cytoskeleton.

Angiogenesis in response to hypoxia and ischaemia is largely controlled by the transcription factor hypoxia-inducible factor-1 [39]. HIF-1 has multiple subunits; HIF-1α which is produced continuously but rapidly degraded in the presence of oxygen, and HIF-1β which is constitutively expressed. Under hypoxic conditions, HIF-1α degradation is inhibited, and the stabilized protein translocates to the nucleus, where it dimerizes with HIF-1β. The dimer binds to hypoxia response elements on more than 60 HIF–responsive genes that function to enhance oxygen delivery and increase metabolism. Central angiogenic signals driven by increased HIF-1 activity include VEGF, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and angiopoietins [40].

FGF, VEGF and PDGF stimulate endothelial cell proliferation and migration. Their high affinity for heparan sulfate glycosaminoglycans on the endothelial cell surface facilitates binding to receptors and provides a reservoir of these factors in the ECM, which can be released during wounding or inflammation. FGF binds to the receptor tyrosine kinase FGFR-1 to increase endothelial migration and promote capillary formation. FGF also enhances PDGF expression via a VEGF-dependent mechanism illustrating the cross talk and synergism that occurs within these growth factor pathways. In addition, FGF-mediated proteolysis of ECM components and induction of the synthesis of collagen, fibronectin, and proteoglycans by endothelial cells contribute to ECM remodelling.

VEGF stimulates endothelial replication and migration and increases vessel permeability, facilitating extravasation of plasma proteins to form a provisional ECM to support cell migration. mRNA for VEGF and VEGF-receptors has been detected in the tips of invasive angiogenic sprouts, and antibody blockade of VEGF signalling significantly decreases microvessel outgrowth. PDGF produced by angiogenic endothelial cells is required for the recruitment, proliferation, and survival of pericytes for vessel stabilization and maturation. PDGF acts on two transmembrane receptor tyrosine kinases, PDGF-α and -β. PDGF-β expressed on pericytes is critical to their recruitment. Disruption of signalling at these kinases is associated with vascular abnormalities in physiological and pathological angiogenesis.

Angiopoietins are ligands of endothelial-specific Tie receptors that have multiple effects on the angiogenic process, particularly interactions between endothelial cells, pericytes, and the basement membrane. For example, angiopoietin-1 acts on Tie-2 to stimulate secretion of growth factors from endothelial cells, which in turn stimulate differentiation of surrounding pericytes into smooth muscle cells.

Conversely, angiopoietin-2 is an antagonist of the actions of angiopoietin-1 and so acts as a naturally occurring inhibitor of angiogenesis [40].

Recruitment and proliferation of bone marrow–derived endothelial progenitor cells (EPCs) to form new vessels (vasculogenesis) is a distinct but complimentary process which occurs simultaneously in ischaemic and wounded tissue to augment perfusion [41]. First described in 1997, classification of EPCs is still controversial, although the most well-accepted definition is that they are circulating endothelial cells expressing CD45, CD34 and CD133 surface antigens [42]. EPCs express proteins such as L-selectin and mucosal vascular cell adhesion molecule 1 (VCAM-1) which facilitate adhesion to mature endothelial cells. Incorporation of EPCs into the endothelial cell layer induces the release of proangiogenic factors such as VEGF, resulting in further recruitment of pro-angiogenic cells and enhanced angiogenesis. EPCs have been proposed as a potential cell-therapy to promote neovascularization in ischaemic tissues. This idea is supported by many studies demonstrating the role of EPCs in improved tissue perfusion in animal models of ischaemia, and clinical data showing that administration of EPCs to patients with myocardial infarction or chronic angina is associated with positive trends in perfusion [43].

1.4 Haemostasis

Endothelial cells play a pivotal role in regulating blood flow by exerting effects on the coagulation system, platelets, and fibrinolysis. Under normal physiological conditions, the endothelium provides one of the few surfaces which can maintain blood in a liquid state during prolonged contact [3]. A key factor in blood clot formation is activation of the serine protease thrombin which cleaves fibrinogen, producing fragments that polymerise to form strands of fibrin. Thrombin also activates factor XIII, a fibrinoligase, which strengthens fibrin-to-fibrin links, thereby stabilising the clot and stimulating platelet aggregation. Heparan sulfate proteoglycan molecules provide an anti-thrombotic endothelial cell surface by serving as co-factors for antithrombin III, causing a conformational change that allows this inhibitor to bind to, and inactivate, thrombin and other serine proteases involved in the clotting cascade. The endothelium also prevents thrombin formation by expressing tissue factor pathway inhibitor (TFPI) which binds to clotting factor Xa. TFPI and antithrombin III both contribute to physiological haemostasis, and both show impairment in acquired thrombotic states. A third endothelial anti-coagulation mechanism is expression of thrombomodulin. Binding of thrombin to cell surface thrombomodulin removes its pro-coagulant activity, and the thrombin-thrombomodulin complex activates protein C, a vitamin K-dependent anticoagulant. Activated protein C, helped by its cofactor protein S, inactivates clotting factors Va and VIIa [44].

The anti-platelet aggregation properties of the endothelium are largely mediated by release of PGI_2 and NO. As with smooth muscle relaxation, PGI_2 inhibits platelet aggregation through the activation of IP receptors and activation of adenylyl cyclase, whereas NO inhibits platelet adhesion, activation, secretion, and aggregation

through a cGMP-dependent mechanism. NO inhibits agonist-dependent increases in intra-platelet Ca^{2+} to suppress the Ca^{2+}-sensitive conformational change in the heterodimeric integrin glycoprotein IIb–IIIa required for fibrinogen binding. NO also promotes platelet disaggregation by impairing the activity of phosphoinositide 3-kinase, which normally supports conformational changes in glycoprotein IIb–IIIa, rendering its association with fibrinogen irreversible. Should a blood clot form, fibrinolysis depends primarily on the action of plasmin, an active protease formed from its precursor, plasminogen, upon stimulation by tissue-type plasminogen activator [44].

Under physiological conditions there is a haemostatic balance, and in addition to these anti-thrombotic mechanisms, the endothelium also synthesises several key haemostatic components, with vWF and plasminogen activator inhibitor-1 (PAI-1) being particularly important. PAI-1 is secreted in response to angiotensin IV, providing a link between the renin-angiotensin system and thrombosis. In addition to anti-coagulant activity, binding of thrombin to thrombomodulin accelerates its capacity to activate thrombin-activatable fibrinolysis inhibitor which cleaves fibrin and other proteins, resulting in the loss of plasminogen/plasmin and tissue plasminogen activator binding sites and thus retarding fibrinolysis. Perturbations such as those that may occur at sites of injury, inflammation, or high shear stress tip this haemostatic balance in favour of a pro-thrombotic and anti-fibrinolytic microenvironment. Critical steps include loss of cell surface heparan proteoglycan molecules and increased expression of the transmembrane glycoprotein tissue factor which initiates coagulation by stimulating the activation of clotting factors IX and X, and pro-thrombinase, with subsequent fibrin formation. Tissue factor accumulates in experimentally injured vessels and accumulation in some atherosclerotic plaques is likely to account for their high thrombogenicity [45].

1.5 Inflammation

The development of inflammatory reactions by the endothelium in response to injury or infection is critical for the maintenance and/or repair of the normal structure and function of the vessel wall. However, excessive inflammation can lead to severe tissue damage and contribute to the development of atherosclerosis. The interaction between endothelial cells and inflammatory cells such as leukocytes depends on the production of inflammatory cytokines (e.g. interleukin 8; IL-8) to attract leukocytes, and expression of adhesion molecules (e.g. selectins) to facilitate their adhesion and migration towards the site of infection. Loosely tethered leukocytes first roll over the endothelial surface, then arrest, spread, and finally migrate between endothelial cells to attach onto underlying ECM components [46] (Fig. 1.3).

Leukocyte rolling involves endothelial adhesion molecules which transiently bind to carbohydrate ligands on leukocytes to slow passage through the blood vessel. E- and P-selectin are expressed only on the surface of activated endothelial cells

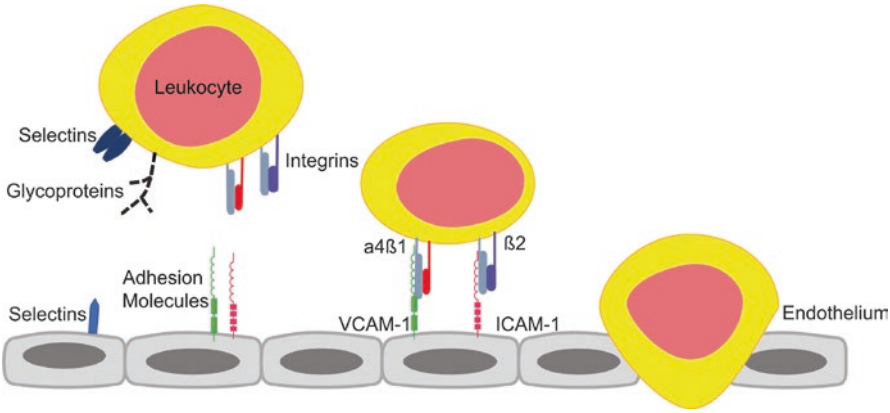

Fig. 1.3 Schematic of adhesion and migration of leukocytes. Inflammatory cytokines attract leukocytes and increase expression of adhesion molecules (e.g. selectins) to facilitate their adhesion and migration towards the site of infection. Loosely tethered leukocytes first roll over the endothelial surface, then arrest and migrate between endothelial cells to attach onto underlying ECM

whereas L-selectin is constitutively expressed on leukocytes and binds to ligands induced on the endothelium at sites of inflammation or on other leukocytes. The role of individual types of selectins in leukocyte rolling shows stimulus- and time-dependent variation. Immediate stimulation of leukocyte rolling induced by histamine or thrombin depends on rapid expression of P-selectin. Surface levels of this adhesion molecule decline after only 30 min. In contrast, TNFα stimulates delayed leukocyte rolling and adhesion to endothelial cells through the induction of E-selectin, surface levels of which peak after 12 h and decline after 24 h. Both E- and P-selectin are expressed on the surface of endothelial cells overlying atherosclerotic plaques, affirming the importance of these molecules in the development of atherosclerosis.

Firm adhesion of leukocytes is promoted by binding of cytokines to leukocyte GPCRs resulting in rapid activation of β1 and β2 integrins to increase their affinity for adhesion molecules of the immunoglobulin superfamily, intercellular adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM-1). ICAM-1 is constitutively expressed on endothelial cells, but levels are increased by stimuli such as TNFα peaking at 6 h and remaining elevated for 72 h. ICAM-1 mediates firm adhesion of blood cells by acting as a ligand for leucocyte β2 integrins. VCAM, a ligand for integrins α4β1 and α4β7, principally mediates the adhesion of monocytes, lymphocytes, eosinophils, and basophils to the endothelial surface. Expression of VCAM-1 is induced by cytokines, oxidized low-density lipoproteins, and ROS acting, as with induction of ICAM-1, primarily via NF-κB.

The migration of leukocytes through the endothelium requires the transient disassembly of endothelial cell junctions. Firm adhesion of leukocytes to the endothelium induces clustering of adhesion molecules like ICAM-1 and VCAM-1, triggering activation of intracellular signalling pathways which induce endothelial cell actin cytoskeleton and cell junction remodelling. The remodelling process

involves numerous pathways including Rho GTPase signalling, protein phosphorylation, and ROS generation, but a key event is reorganization of PECAM-1 dimers. PECAM-1 localizes to intercellular junctions of endothelial cells, forming homodimers linking two cells. Leukocytes also express PECAM-1 and the dissociation of PECAM-1 dimers between endothelial cells to form dimers between emigrating leukocytes and endothelial cells is critical for leukocyte migration [47].

1.6 Conclusions

The endothelium, once viewed as an inert physical barrier, is a dynamic secretory organ fulfilling numerous roles in maintenance of cardiovascular homeostasis. Endothelial cells from different parts of the vasculature show highly differentiated functions. Advances in defining endothelial functions at the molecular level may lead to targeted therapies to alleviate chronic endothelial dysfunction associated with the progression of cardiovascular disease.

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Chapter 2
Pathophysiology of Atherosclerosis

Sanuja Fernando, Christina A. Bursill, Stephen J. Nicholls, and Peter J. Psaltis

Key Learning Points

- Atherosclerotic plaques are formed by progressive accumulation of lipids and inflammatory cells, and extracellular matrix deposition in arterial intima.
- Atherosclerotic lesions progress through different stages from early fatty streaks to the formation of thin cap fibroatheromas that are vulnerable to plaque rupture and athero-thrombosis.
- Plaque erosion, which is distinct from rupture, is another increasingly recognised mechanism by which plaque thrombosis occurs to cause vessel occlusion.
- Atherosclerosis is mediated by different types of innate and adaptive immune cells as well as vascular endothelial and smooth muscle cells.
- The recruitment, activation, accumulation and cross-talk of these immune cells in response to modified lipoproteins, cholesterol crystals and other stimuli, creates an inflammatory cycle that aggravates plaque progression and instability.
- Plaque inflammation is countered by similarly complex healing mechanisms, involving production of anti-inflammatory cytokines, deposition of collagen and clearance of apoptotic cells.

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2.1 Introduction

In 1904 German pathologist Felix Marchand was the first to describe the term *"atherosclerosis"* from the Greek roots "*athere-"* meaning "gruel", and *"-sklerosis",* meaning "hardness". Atherosclerosis has become the leading cause of mortality and morbidity worldwide, causing clinical disease via vascular luminal narrowing which can ultimately lead to thrombus formation that obstructs blood flow to tissues, such as the heart (coronary heart disease), brain (ischaemic stroke) and lower extremities (peripheral vascular disease) [1]. It was formerly recognised as an ordinary lipid storage disease. However, major advances in basic, experimental and clinical sciences have illuminated the role of inflammation and the underlying complex cellular and molecular mechanisms that contribute to atherosclerosis [2]. It is now defined as a chronic inflammatory disease of the vasculature which is initiated early in life and develops over a number of decades. The intensity of the disease relies on many genetic, environmental and behavioural factors. The best-known risk factors include hypercholesterolaemia (specifically elevated low-density lipoprotein-cholesterol [LDL-C] levels), hypertension, diabetes mellitus, cigarette smoking, family history, obesity, male gender, age (male: >45 years and female: >55 years), sedentary lifestyle and diets with high saturated and trans-fatty acids.

Atherosclerosis is a multifocal disease that especially occurs at sites with low or oscillatory shear stress located near branch points in the arterial tree [1]. Thus, the most affected areas are the carotid bifurcations, coronary arteries, aortic and femoral artery bifurcations [3]. This lipid-driven inflammatory disease leads to subintimal plaque formation at specific sites due to maladaptive inflammation, fibrosis, necrosis and calcification.

2.2 Normal Vessel Wall

An understanding of the pathophysiology of atherosclerosis first requires knowledge of the normal artery wall structure (Fig. 2.1), its biology and function. Normal arteries have a trilaminar structure. The innermost layer, the **tunica intima** is the closest to the arterial lumen. This layer contains a monolayer of endothelial cells which resides on a basement membrane containing non-fibrillar collagen, such as type IV collagen, laminin, fibronectin, and other extracellular molecules. The middle layer, known as the **tunica media** is the thickest layer and lies under the intima separated by the internal elastic lamina. The media contains concentric layers of smooth muscle cells (SMCs), interleaved with layers of elastin-rich extracellular matrix and serves contractile and elastic functions of the vessel. The external elastic lamina bounds the tunica media and the outermost layer of the artery, the **tunica adventitia** which initially received little attention, although appreciation of its potential roles in arterial pathology has recently increased. The adventitia consists of a relatively loose array of collagen fibrils and contains blood vessels (*vasa vasorum*), nerve endings and lymphatics which nourish the cellular components of the arterial wall. It is also enriched

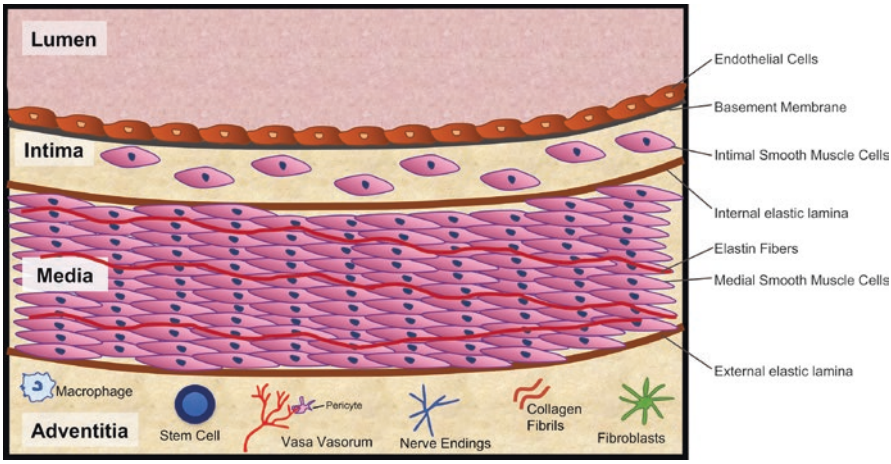

Fig. 2.1 Normal arterial wall. The normal arterial wall is composed of three layers: intima (monolayer of endothelial cells which reside on a basement membrane), media (concentric layers of smooth muscle cells interleaved with elastin fibres) and adventitia (fibroblasts, collagen fibres, nerve endings, vasa vasorum, stem cells, macrophages etc.). The internal elastic lamina separates the intima from the media and the external elastic lamina separates the media from the adventitia

with fibroblasts, different subsets of leukocytes (e.g. mast cells, macrophages, lymphocytes) and a diverse array of progenitor/stem cells [4, 5]. The adventitia provides a dynamic interface between the intima/media and the perivascular adipose and connective tissue, and is increasingly recognised as a complex biological processing centre which serves as an injury sensor for the rest of the vessel wall [6, 7].

Atherosclerosis is heralded by the build-up of lipid-rich plaques in the subintimal compartment of the vessel wall where maladaptive remodelling responses are involved in all three vessel wall layers discussed above.

2.3 Stages of Atherosclerosis

There are major histological and molecular changes which take place in the progression of atherosclerosis which have been elucidated over many years (Table 2.1). In this section, those changes will be briefly discussed in their order of occurrence, while in the following section, some of the cellular and molecular mechanisms of the atherosclerotic process will be elaborated.

2.3.1 Intimal Xanthomas or Fatty Streaks

Intimal xanthomas or fatty streaks are the earliest visible lesions in atherosclerosis which appear as areas of yellow discolouration in the inner surface of the artery. They reflect the adaptive intimal thickening which can exist as early as birth and

Table 2.1 Different stages in the development of atherosclerosis

Stage	Gross findings	Mechanisms
Intimal xanthomas/fatty streaks	Bright yellow minimally raised lesions that demonstrate abundant lipid when stained with oil red O	Endothelial dysfunction, lipoprotein entry and modification, leukocyte recruitment, foam cell formation
Fibroatheroma	Homogenous, white, raised and firmer areas	Migration of SMCs from arterial media to the subintima and proliferation of SMCs, SMC-rich plaque with proteoglycan matrix and focal accumulation of extracellular lipids, formation of the necrotic core
Thin-cap fibroatheroma	A thin, unstable fibrous cap (≤65 μm) with an underlying necrotic core	Type 1 collagen, very few SMCs (apoptosis), large necrotic core (>40% of plaque volume), adventitial inflammation, neovascularisation, spotty calcification, intraplaque haemorrhage
Plaque rupture	Ulcerated plaques showing surface thrombosis	Ruptured fibrous cap, presence of luminal thrombus, larger necrotic core, increased macrophage infiltration, increased expression of MMPs and MPOs
Fibrous calcified plaque	Eruptive nodular calcification with underlying fibrocalcific plaque	Formation of calcified sheets in the collagen-rich matrix
Endothelial erosion	Appear as gross plaques that are visible to the naked eye	Absent endothelium, abundant SMC and proteoglycans, scarcely calcified, less macrophages and less inflammation

Different stages in the development of atherosclerotic plaques are described briefly in their approximate order of occurrence, illustrating their gross histological findings and mechanisms involved [1, 4, 11, 12, 18, 31, 60, 62, 63, 95, 96]

grow, especially in areas of low shear stress. Fatty streaks are present in most children above the age of 3, where they increase rapidly in adolescence. Fatty streaks do not cause symptoms and in some locations in the vasculature can even regress over time. As time passes, areas of high oscillatory shear index tend to upregulate nuclear factor-kappa-β (NF-kβ), a fundamental transcription factor in the inflammatory cascade. Due to this inflammation, the endothelium becomes activated and permeable to leukocytes (neutrophils, monocytes) and toxic substances (for example, modified lipoprotein cholesterol particles) that are circulating in the blood. Monocytes which migrate to the arterial intima undergo differentiation into macrophages. The "fattiness" in this stage comes from the retention of modified lipoprotein particles in the subintimal compartment being taken up by macrophages to form foam cells.

2.3.2 Fibroatheroma

While endothelial cells and foam cells play key roles in the formation of the fatty streak, SMCs are critical to the development of fibroatheroma. SMCs which migrate to the subintima produce extracellular matrix that can trap lipoproteins and other

inflammatory cells which in turn accumulate and contribute to lesion growth. It is not clearly understood why only some fatty streaks progress to the fibroatheroma stage while others regress over time. Fibroatheromas consist of an acellular necrotic core, which is distinguished from lipid pool areas of pathological intimal thickening as it is made up of cellular debris. The necrotic core is covered by a fibrous cap consisting of SMCs in a proteoglycan-collagen matrix [8]. This fibrous cap is critical for the maintenance of the integrity of the lesion and is subjected to thinning, prior to rupture.

2.3.3 Thin-Cap Fibroatheroma

Thin-cap fibroatheroma (TCFA) is recognised by its morphological characteristics that depict plaques which are unstable or at risk of rupture [8, 9]. It is characterised by a thin fibrous cap made up mostly of type I collagen with an absence or paucity of SMCs. Fibrous cap thickness is an indicator of plaque vulnerability and is pathologically defined to be ≤65 μm in TCFAs [10]. A prevailing perception has been that rupture of fibrous caps typically occurs at the weakest points, often near shoulder regions of the plaque [11]. However, recent studies have found an equivalent number of ruptures occurring at the mid-portion of the fibrous cap which suggests the involvement of multiple catalysts to plaque rupture [12].

2.3.4 Plaque Rupture

Another hallmark of plaque instability is the formation of a necrotic core which results from apoptosis and death of foam cells and SMCs, and results in release of their intracellular contents and cholesterol crystal deposition [13]. This process, along with the increased expression of matrix metalloproteinases (MMPs) and myeloperoxidases (MPOs) produced by inflammatory cells (e.g. macrophages) [14], microcalcifications, and iron accumulation [15] collectively lead to weakening of the fibrous cap. In combination with oscillating shear stress within the arterial tree [16], this can eventually result in fibrous cap rupture. Once this occurs, the underlying necrotic core contents are exposed to circulating blood which activates a coagulation cascade involving platelets in response to the exposure of lipids and tissue factors which are present in the necrotic core [11].

2.3.5 Fibrous Calcified Plaque

Calcification tends to occur at a microscopic level in TCFAs and plaques that are vulnerable to rupture and is usually speckled or fragmented. This is often referred to as spotty calcification. When this fragmented calcification seen in

early lesions, spreads into the surrounding collagen-rich matrix, forming calcified sheets, the plaque transforms into a fibrous calcified plaque [17].

2.3.6 Plaque Erosion

Plaque erosion is a unique stage which is classified by the absence of endothelium at a site of erosion, with the exposed underlying plaque containing relatively low levels of inflammation and greater abundance of SMCs and proteoglycans [1]. Morphologically, eroded surfaces are less calcified and contain fewer macrophages and T lymphocytes. Plaque erosion is now recognised as the second commonest mechanism causing plaque rupture, by which coronary plaques become complicated by thrombosis and subsequent occlusion [18].

2.4 Pathophysiology of Atherosclerosis and Atherothrombosis

The molecular mechanisms involved in the progression of atherosclerosis are multifaceted and encompass endothelial dysfunction, lipoprotein entry and modification, leukocyte recruitment, foam cell formation, lymphocyte responses, SMC migration, fibrous cap formation, macrophage apoptosis, necrotic core formation, calcification, angiogenesis, intraplaque haemorrhage, fibrous cap degradation, plaque rupture, erosion, thrombosis and more. Between each of these processes, there are complex and variable interactions that occur in the development of any given plaque, which lead to unpredictable progression rates, heterogeneous morphologies, and variable clinical outcomes [1]. While most plaques remain asymptomatic (subclinical), some become obstructive (clinical ischaemia) while others elicit acute thrombosis leading to acute tissue infarction.

2.4.1 Endothelial Dysfunction

The endothelium was once thought of as the ‘cellophane wrapper’ of the vasculature with no specific function. However, major research advances in the last half century have led to a greater understanding of this dynamic organ. Sitting at the border between circulating blood and the SMCs in the vessel wall, the endothelium consists of a continuous cell layer with unique structural, metabolic and signalling functions that are mandatory to maintain homeostasis of the vessel wall. The normal functioning endothelium maintains vascular tone and permeability through vasodilation properties, anti-inflammatory, anti-thrombotic and anti-proliferative

functions. Endothelial dysfunction is characterised by a shift towards reduced vasodilation, pro-inflammatory, pro-thrombotic and increased proliferative properties. This process is initiated by perturbations to the endothelium which can occur due to various agents including chemical irritants and physical forces.

Nitric oxide (NO), primarily identified as endothelium-derived relaxing factor, is the major vasodilating substance released by the endothelium. NO is also an inhibitor of platelet aggregation and an anti-inflammatory substance. The presence of increased amounts of free radicals due to depletion of anti-oxidants can disrupt the balance of NO and damage the endothelium, leaving it overly permeable to toxic substances and inflammatory cells that should normally remain in the circulating blood.

Another important feature of the endothelium is its responsiveness to the physical forces caused by blood flow. It is the endothelium which determines which sections of the artery will first develop atherosclerosis. This phenomenon can be explained by hydrodynamic stress. In straight arteries, the steady laminar shear stress favours the endothelial production of NO leading to an athero-protective state. Conversely, disturbed flow which normally occurs at branching points of the arteries causes low shear stress, impairing these locally athero-protective endothelial functions [19, 20]. Consequently, arteries with fewer branches (e.g. the internal mammary artery) develop less plaque than arteries with bifurcated vessels (common carotid and coronary arteries).

When chemical irritants and physical forces interrupt normal endothelial homeostasis, an activated state results, manifested by impairment of the endothelium's role as a permeability barrier. This leads to uptake of lipoproteins and other toxic substances into the vessel wall, inflammatory cytokine secretions and increased expression of adhesion molecules, leading to leukocyte infiltration into the subintimal space. These mechanisms will be covered in the following sections.

2.4.2 Lipoprotein Entry and Modification

Due to increased endothelial permeability, certain toxic substances have the ability to transport through the endothelium into the subintimal space of the vessel wall. One such substance is lipids. This process is accentuated when there are increased levels of lipids in the blood stream and in particular, cholesterol. Cholesterol circulates in the blood as multiple distinct forms of lipoprotein particles. These particles consist of a lipid core surrounded by a hydrophilic phospholipid, free cholesterol and apolipoproteins. In order of increasing density, lipoproteins can be identified as chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (Fig. 2.2) [21]. In particular, LDL-associated cholesterol is known as a key driver of atherosclerosis. LDL is prominently present in the blood of patients with hypercholesterolaemia [22].

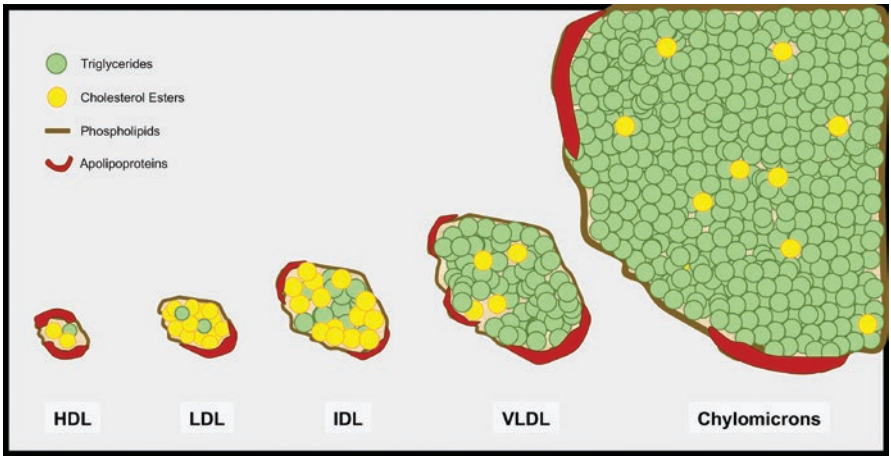

Fig. 2.2 Classification of lipoproteins. Cholesterol circulates in the blood in the form of lipoprotein particles. These lipoprotein particles consist of a lipid core surrounded by a hydrophilic phospholipid, free cholesterol and apolipoproteins. In order of increasing density, lipoproteins can be identified as chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL)

As these LDL particles enter the subintimal space of the arterial wall, they are retained by the proteoglycans in the extracellular matrix which leads to an increased resident time for the lipoproteins in the arterial wall. During this retention, LDLs are exposed to several chemical modifications. One such modification is oxidation, which occurs due to the interaction of lipoproteins with local reactive oxygen species (ROS), oxidative and lipid oxygenases, peroxidases and pro-oxidant enzymes. LDL may also undergo glycation, particularly in diabetic patients with sustained hyperglycaemia. Such modifications of LDL act quite early in the atherosclerotic process and contribute to the inflammatory mechanisms initiated by endothelial dysfunction and they tend to continue to promote inflammation throughout the life span of the plaque.

In addition to LDL, other lipoproteins particularly HDL can also undergo oxidation. Conventionally, HDL is known as the "good cholesterol" involved in reverse cholesterol transport and several other processes which interfere with the oxidation of LDL (although it is also readily oxidised). Oxidised HDL loses its "good cholesterol" functionality in reverse cholesterol transport [23].

2.4.3 Leukocyte Recruitment

An important stage in atherosclerosis is leukocyte recruitment which occurs early in lesion generation. The normal endothelium resists adhesive interactions with leukocytes. However, after the initiation of hypercholesterolaemia, leukocytes adhere to the endothelium, move between the endothelial cell junctions and penetrate through

endothelial cells (transcytosis) into the subintima. There are several types of leukocytes recruited in this manner including monocytes, neutrophils and T lymphocytes.

Monocyte recruitment is a key step in the progression of atherosclerosis. This recruitment relies on endothelial activation. When activated, the endothelium releases chemoattractant signals such as monocyte chemotactic protein-1 (MCP-1) and expresses several surface bound chemokines namely Chemokine (C-C motif) ligand 5 (CCL5) or RANTES (regulated on activation, normal T cell expressed and secreted), chemokine (C-X-C motif) ligand 1 (CXCL1) and interleukin-8 (IL-8) [24]. Following these chemotactic signals and enhanced chemokine expression, monocytes in blood roll and become tethered to the activated endothelial cells via selectin-dependent adhesion molecules, such as E-selectin and P-selectin. Following rolling, they form tighter adhesions with the endothelium via α4β1 integrin also known as very late antigen-4 (VLA-4) which binds to endothelial cell ligands including vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) [23, 25]. Finally monocytes are thought to reach the atherosclerotic lesion via trans-endothelial migration [23].

Once monocytes have been recruited into the subintima, they undergo differentiation in the presence of macrophage colony-stimulating factor (M-CSF), to form macrophages. These macrophages have the ability to express several different phenotypes and can exert multiple effects in lesion development (Table 2.2).

Table 2.2 Polarised macrophage phenotypes and their role in atherosclerosis

Macrophage type	Stimuli	Markers	Cytokines released	Role in atherosclerosis
M1	IFN-γ, LPS, TNF-α	CD86, MHCII, iNOS,	IL-1, TNF-α, IL-6, IL-12 high, IL-10 low, IL-23, RNI, ROI, CCL12, CCL18, CCL22, CCL24	Plaque progression, mediate Th1 responses and type I inflammation
M2a	IL-4, IL-13	MHCII, MR, CD206, SR-A1, Arg, CD23, CD163	IL-10, decoy-IL-1RII, IL-1ra, polyamine	Mediate Th2 responses and type II inflammation
M2b	Poly-IC + TLR/IL-R ligands	MHCII, CD86, CD80, Arg	TNF, IL-1, IL-6, IL-10 high, IL-12 low	Enriched in regressing plaques, Th2 activation, immunoregulation
M2c	IL-10	SLAM, CD206, MR, CD14, CD150, Arg	IL-10, TGF-β, IL-1ra, CCL16, CCL18, CXCL13, matrix vesicles	Immunoregulation, matrix deposition and tissue remodelling
M2d	IL-6, tumour derived factors	VEGF, Arg	IL-10, IL-12, TNF-α, TGF-β	Promote angiogenesis
Mox	oxLDL	VEGF	IL-10, IL-1β	Lesion development and plaque instability, redox and anti-oxidant activity, angiogenesis

(continued)

Table 2.2 (continued)

Macrophage type	Stimuli	Markers	Cytokines released	Role in atherosclerosis
Mhem	Heme	CD163, ATF1	LXR-β	Atheroprotective
M-Hb	Haemoglobin, haptoglobin	CD163, MR	ABCA1, ABCG1, LXR-α	Mediate cholesterol efflux, atheroprotective
M4	CXCL4	CD206, CD86, CD45, CD14	TNF-α, IL-6, IL-10, CCL18, CCL22	Minimal foam cell formation, potential pro-atherogenic roles

Monocytes which enter the vascular wall differentiate into macrophages. Different stimuli including growth factors, chemokines, lipoproteins or tissue factors induce specific types of macrophage polarisation. This table summarises some of these macrophage subtypes discovered to date [97–105]. *IFN* interferon, *IL* interleukin, *TNF* tumour necrosis factor, *CD* cluster of differentiation, *MHC* major histocompatibility complex, *iNOS* inducible nitric oxide synthase, *RNI* reactive nitrogen intermediates, *ROI* reactive oxygen intermediates, *CCL* CC chemokine ligand, *Th* T helper cell type, *MR* mineralocorticoid receptor, *SR* scavenger receptor, *Arg* arginase, *IL-1ra* interleukin 1 receptor antagonist, *Poly-IC* Polyinosinic-polycytidylic acid, *TLR* toll-like receptor, *SLAM* signalling lymphocytic activation molecule, *CXCL* chemokine (C-X-C motif) ligand, *VEGF* vascular endothelial growth factor, *TGF* transforming growth factor, *oxLDL* oxidised low density lipoprotein, *ATF* activating transcription factor, *ABCA1* ATP binding cassette transporter A1, *ABCG1* ATP binding cassette transporter G1, *LXR* liver X receptor

Some macrophages obtain pro-inflammatory properties, which has historically been referred to as an "M1-like" phenotype and they secrete pro-inflammatory cytokines, such as IL-1β and tumour necrosis factor-α (TNF-α), ROS and reactive nitrogen species, as well as many other mediators (plasminogen activators, cathepsins, matrix metalloproteinases) which are known to drive atherogenesis. Alternatively, other macrophages adopt an "M2-like" phenotype, which is traditionally believed to be more anti-inflammatory. These M2-like macrophages secrete factors such as transforming growth factor-β (TGF-β) and pro-resolving lipids which favour the resolution of inflammation. The differentiated macrophages also have the ability to take up modified lipoprotein particles to form foam cells (Fig. 2.3).

2.4.4 Foam Cell Formation

Foam cells are commonly known as the "hallmark of atherosclerosis" and their development in the arterial subintima heralds one of the early stages in plaque formation. Although macrophages are the main precursors of foam cells, SMCs and dendritic cells are also known to contribute to their existence owing to their ability to ingest and accumulate intracellular lipids. Notably, studies have found that foam cells which arise from SMCs display macrophage-like properties, including the expression of proteins that are known to be macrophage-specific [23].

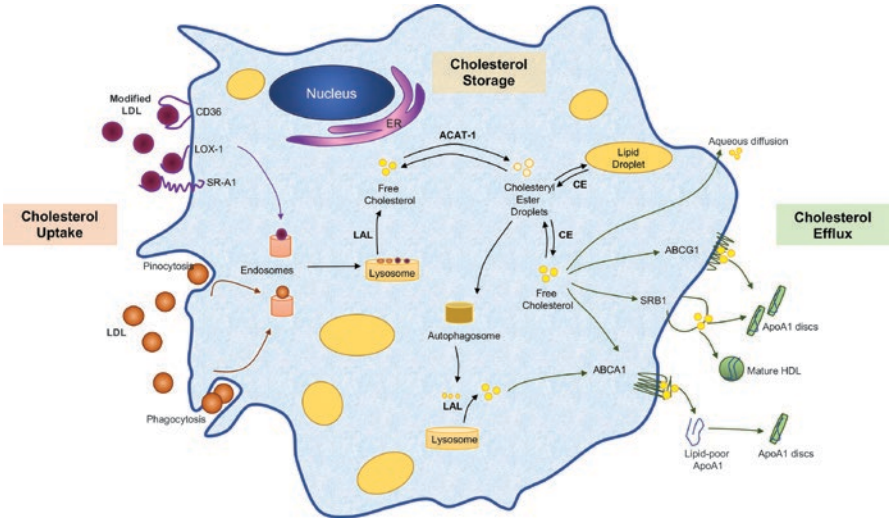

Fig. 2.3 Cholesterol handling in macrophages. Macrophages take up lipoprotein particles via phagocytosis or pinocytosis depending on the nature of the lipoproteins. Modified lipoprotein uptake primarily occurs via scavenger receptors (SRs), namely CD36, LOX-1 SR-A1. These lipoproteins are then carried to lysosomes in vesicles called endosomes. Subsequently lysosomal processing, esterification and storage of cytoplasmic lipid droplets occurs. Macrophages also have the ability to excrete out free cholesterol via a process called cholesterol efflux which uses the cell membrane transporters, ABCA1, ABCG1 and SRB1. HDL and ApoA1 particles capture the released cholesterol and circulate it in blood where it is taken to the liver for reverse cholesterol transport. *ACAT-1* Acetyl-CoA acetyltransferase, *CE* cholesterol esterase, *ER* endoplasmic reticulum, *LAL* lysosomal acid lipase

Macrophages have the ability to take up lipoproteins under certain conditions due to their phagocytic and endocytic nature. The uptake of these lipoproteins primarily occurs via a family of 'scavenger receptors' (SRs) that bind to and internalise these lipoproteins which then lead to lysosomal processing, esterification and finally storage of cytoplasmic lipid droplets (Fig. 2.3). Oxidised, acetylated and malondialdehyde modified LDLs are some of the modified lipoproteins that are taken up by SRs, which include cluster of differentiation-36 (CD36), lectin-like low density lipoprotein receptor-1 (LOX-1), scavenger receptors type 1 and 2 (SR-A1, SR-A2) and scavenger receptor for phosphatidylserine and oxidised low density lipoprotein (SR-PSOX), which is also known as C-X-C motif chemokine 16 (CXCL16). Studies have shown that CD36 and SR-A1 are responsible for 90% of the oxidised LDL loading by macrophages [26].

Macrophages also have the ability to excrete free cholesterol via a process called cholesterol efflux which uses cell membrane transporters, such as ATP-binding cassette transporter A1 (ABCA1), ATP-binding cassette transporter G1 (ABCG1) and scavenger receptor B1 (SRB1). HDL and apolipoprotein A1 (ApoA1) particles capture the released cholesterol and circulate it in blood where it is taken to the liver for reverse cholesterol transport [26].

Uptake of modified lipoprotein particles in the subintimal compartment is beneficial initially as it sequesters potentially damaging lipoprotein particles. However, when this increased lipoprotein uptake is paired together with an impaired efflux capacity, intracellular capacity of cholesterol surpasses the capacity of macrophages to handle it. As cholesterol starts accumulating, macrophages become enlarged with cholesterol and cholesterol esters residing within lipid droplets in the cytoplasm, resulting in the appearance of a foam cell. This negates the "protective role" of macrophages. Over time, the progressive accumulation of lipids inside foam cells causes endoplasmic reticulum stress leading to ROS production which triggers an apoptotic cascade. This leads to the release of proinflammatory cytokines which further promote atherosclerosis as more immune cells infiltrate the plaque as an attempt to rescue. This type of inflammatory amplification represents an *innate immune response* due to the non-dependence on antigenic stimulation.

Plaque growth and stability are influenced by accumulation and removal of macrophages and foam cells, and the production of pro- ("M1") and anti-inflammatory ("M2") cytokines, chemokines, ROS, and matrix degrading enzymes [27]. Murine modelling of atherosclerosis has suggested that macrophages accumulate primarily by monocyte recruitment in early plaque, and by local proliferation as plaque progresses [28].

It is also important to note that even though the role of macrophages in foam cell formation is reasonably established, the contribution of circulating monocytes is less well understood. Circulating monocytes also express the same SRs as macrophages (albeit at lower levels) and can potentially accumulate lipids prior to their migration to the arterial intima and differentiation into macrophages [25].

2.4.5 Lymphocyte Responses

The adaptive immune system which is usually activated in the presence of pathogens, also recognises modified LDL and other inflammatory cytokines released during plaque progression and directs immune cells to the site. While macrophages comprise the vast majority of immune cells in atherosclerotic lesions, T and B lymphocytes have also been found to migrate into the atherosclerotic aortic wall in an L-selectin-dependent manner similar to that of monocytes, discussed earlier (Fig. 2.4) [29, 30]. This migration initially occurs as an abortive attempt to remove or sequester abnormal lipid which is generally perceived as a danger signal.

Lymphocyte responses in plaque progression and the advances in the field of adaptive immunity in atherogenesis have been studied extensively. In brief, T lymphocytes participate in the formation of atherosclerotic lesions as early in the atherosclerotic process as monocytes. Several leukocyte adhesion molecules such as VCAM-1 and ICAM-1 also initiate T cell recruitment. T lymphocytes can exhibit functional diversity where T-helper type 1 (Th1) lymphocytes appear to accelerate atherosclerosis and regulatory T cells (T-regs) limit the disease process [31]. Th1 lymphocytes secrete inflammatory cytokines, such as interferon-γ (IFN-γ), TNF--α/β and IL-2 which activate macrophages, endothelial cells and SMCs leading to local inflammation [22, 30–32], whereas T-regs through the secretion of TGF-β and

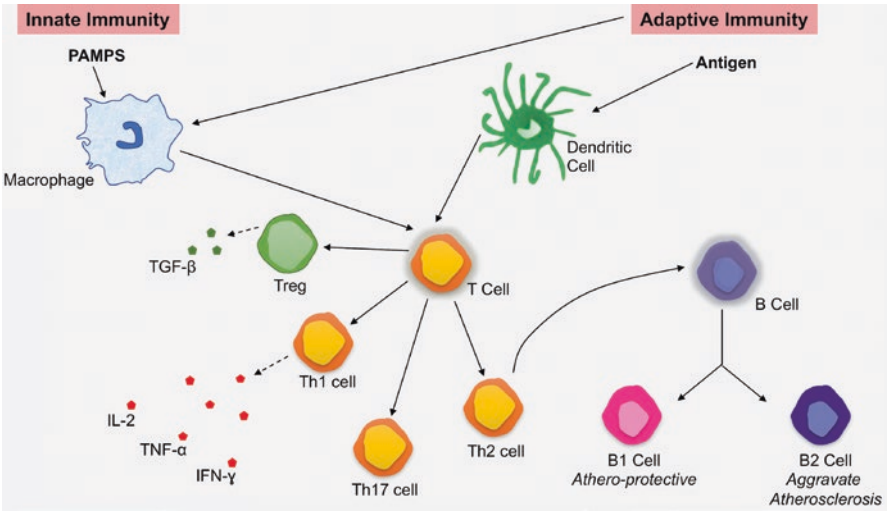

Fig. 2.4 Innate immunity and adaptive immunity. A simplified diagram of innate and adaptive immunity pathways operating during atherosclerosis. *PAMPS* pathogen-associated molecular patterns

IL-10 are believed to dampen this process [2, 33]. The balance between different T cell subsets is therefore an important determinant of plaque progression.

B cells also populate the lesion area, where they mostly assist with the mitigation of the disease [2, 34], as seen by B1 cells which produce natural antibody protection against atherosclerosis. However, several studies have reported that B2 cells might aggravate plaque progression [2].

2.4.6 Smooth Muscle Cell Migration and Fibrous Cap Formation

SMCs are largely responsible for the transition of atherosclerotic plaque from the fatty streak stage to the fibrous atheromatous plaque stage. In the normal arterial wall, SMCs express a differentiated phenotype and are surrounded by a basal lamina consisting of type IV collagen. They are normally contractile and do not divide or migrate. However, in atherosclerosis, lipoproteins, foam cells, activated endothelial cells and Th1 lymphocytes release inflammatory cytokines and proteolytic signals which degrade this supporting framework and initiate SMC migration into the arterial intima. This process starts with downregulation of genes responsible for SMC differentiation, such as smooth muscle α-actin *(Acta2)* and smooth muscle myosin heavy chain *(Myh11)*. At the same time the release of platelet-derived growth factor (PDGF) and TGF-β by macrophages and endothelial cells stimulates SMC migration across the internal elastic lamina into the subendothelial space, giving SMCs a 'synthetic' phenotype (Fig. 2.5) [35]. In the intima, foam cells release

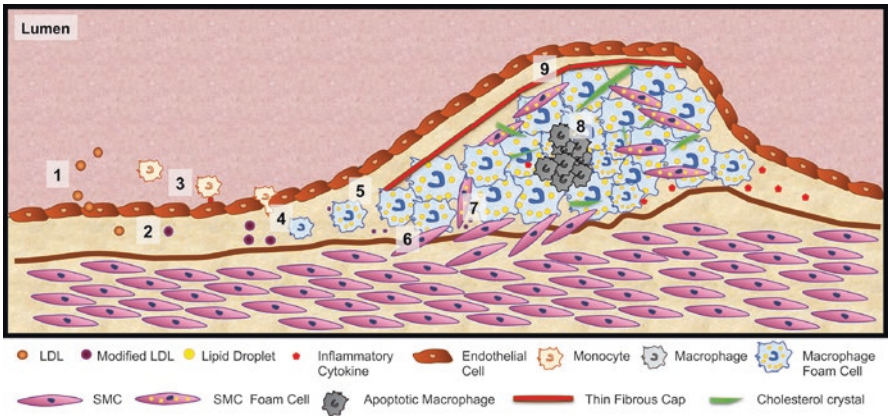

Fig. 2.5 Formation of atherosclerotic plaque. (1) LDL enters the subintima. (2) LDL becomes modified. (3) Monocytes which are circulating in blood become attached to the adhesion molecules expressed on the endothelial surface. (4) Monocytes differentiate into macrophages in the subintima. (5) Macrophages uptake the modified LDL particles and become foam cells. (6) SMCs migrate into the intima and proliferate. (7) SMCs also take up modified LDL particles and form foam cells. (8) Foam cells undergo apoptosis and start forming the necrotic core. (9) Thin fibrous cap is formed

an array of cytokines, namely TNF-α, IL-1, TGF-β and fibroblast growth factor (FGF) which stimulate SMC proliferation as well as the synthesis and secretion of new extracellular matrix proteins, proteoglycans and other proteins which are thought to be beneficial for arterial remodelling and plaque stabilisation [36–38]. These cytokines also provoke activation of other leukocytes and promote further cytokine release, thus reinforcing a positive feedback loop to maintain inflammation in the atherosclerotic lesion.

On the other hand, SMCs also play an important role in stabilising atherosclerotic plaques as they are responsible for the production of fibrillar collagens (type I and type III collagens), which leads to the formation of a fibrous cap on top of the plaque. This fibrous cap is vital in separating plaque's thrombogenic core from mediators of coagulation that circulate in the blood. The thickness and collagen content of the fibrous cap are important determinants of plaque stability. Plaques most at risk of rupture are defined as plaques with a fibrous cap less than 65 μm thick, also referred to as TCFA, as previously mentioned.

2.4.7 Macrophage Apoptosis and Necrotic Core Formation

Macrophage apoptosis (programmed cell death) occurs during all stages of plaque progression. The induction of this process likely involves chronic, cumulative stimuli over time rather than a single acute catastrophic stimulus. These take the form of; (1) oxidative stress; (2) high concentrations of pro-inflammatory cytokines, such as

TNF-α; (3) accumulation of unesterified cholesterol, oxysterols and modified LDL; (4) activation of Fas death pathway via Fas ligand and (5) endoplasmic reticulum stress.

In early lesions, the engorged foam cells become unstable over time and eventually undergo apoptosis. Studies in early atherosclerotic models have found an inverse relationship between macrophage apoptosis and lesion size, where increased macrophage apoptosis in early lesions was associated with decreased lesion size [39–41]. This is due to the rapid removal of apoptotic remnants by efferocytosis (see Glossary), leading to suppression of the pro-inflammatory responses. The overall effect is a reduction in lesion cellularity and size.

However, in advanced lesions the apoptotic macrophages are not cleared efficiently by efferocytosis. As a result, apoptotic macrophages accumulate and undergo secondary necrosis and their lipid-rich cargo is deposited in the tissue where it provokes further inflammation [42, 43]. Studies have reported observing apoptotic macrophages and SMCs in focal areas surrounding the necrotic core. The presence of free apoptotic remnants in advanced lesions that are not associated with phagocytic cells, indicates that impaired efferocytosis contributes to the growth of the necrotic core [44, 45].

The chemical composition of the necrotic core suggests that sources of lipids are also major contributors to its formation, which includes direct accumulation of cholesterol esters from LDL, free cholesterol and cholesterol crystal formation. It is not known why only some lesions develop necrotic cores while others do not. If there is no necrotic core present, there is typically no overlying fibrous cap to rupture. However, larger necrotic cores pose a greater risk of rupture than smaller ones.

2.4.8 Calcification

Vascular calcification occurs in the intima as well as the media of the vessel wall. These two processes occur independently of each other. Intimal calcification develops as a result of lipid accumulation and inflammation in atherosclerosis, whereas medial calcification arises mainly in patients with chronic kidney disease and type 2 diabetes mellitus [46]. Vascular intimal calcification is primarily the process of bio-mineralisation where insoluble calcium deposits in the form of calcium salts and is considered an active process where multiple mechanisms exist [47, 48]. It is believed that SMCs obtain osteogenic properties and calcify analogous to bone formation [17]. Extracellular vesicles are also thought to calcify when calcium phosphates appear inside them, and this leads to the formation of hydroxyapatite crystals, again akin to bone formation. Another popular theory for the mechanistic basis of intimal calcification is that apoptotic cells which arise from SMC and macrophage death also undergo calcification in the extracellular milieu where the same hydroxyapatite crystal formation occurs [49, 50]. It is also believed that these processes of calcification are stimulated by the loss of inhibitors of calcification which are usually present in the normal arterial wall such as Matrix Gla-Protein (MGP), osteopontin (OPN), fetuin and pyrophosphates [17].

Arterial calcification progresses with age and its extent generally correlates with plaque burden. The location and structure of calcification are the most important determinants of the hazards associated with it. Pathologically, largely calcified atheromatous lesions are much stiffer than more cellular lesions and are less likely to be associated with plaque rupture. It follows that gross calcification may in fact provide stability to plaques. Conversely, it has been reported that non-homogenous calcification, also known as spotty calcification or micro-calcification, is associated with high-risk, rupture-prone plaques due to the substantial stress it imposes on the overlying fibrous cap. Thus, depending on the degree and nature of calcification in atherosclerosis, it can either be a stabilising force for plaque or alternatively cause penetrating perturbations which can lead to a reduction in lesion stability [51, 52]. Statins have been shown to increase calcification and collagen production by vascular SMCs [53–55], and this is thought to increase the biomechanical stability of plaques which makes them less prone to rupture and thrombotic complications [55].

2.4.9 Neovascularisation and Intraplaque Haemorrhage

With progression of atherosclerotic lesions, the arterial wall thickness increases. It has been suggested that when this wall thickness exceeds 100 μm, oxygen supply to the plaque site becomes restricted. As a compensatory mechanism, hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF) and other angiogenic modulators are secreted. Together these factors promote neovascularisation (including angiogenesis) [56]. Neovessels originate from the adventitial *vasa vasorum* and grow into the base of the progressing atherosclerotic lesion. This provides an alternative route for oxygen and nutrients to enter the plaque site. These plaque neovessels are also leaky and express cellular adhesion molecules, favouring local extravasation of red blood cells, plasma proteins, circulating monocytes and other inflammatory cells which further contribute to the growth and destabilisation of the plaque.

Neovascularisation has been found to be present in 50% of coronary atherectomy samples from patients with unstable angina, compared to 10% from patients with stable angina, suggesting a possible role in plaque instability. This could be due to the neovascularisation within the plaque leading to extravasation of red blood cells and inflammatory mediators causing intraplaque haemorrhage. Once red blood cells are leaked into the plaque, cholesterol from their cell membrane becomes incorporated into the lipid core, increasing its volume [57].

2.4.10 Fibrous Cap Degradation and Plaque Rupture

The fibrous cap is located between the vascular lumen and the necrotic core. Plaques tend to rupture where the fibrous cap is at its thinnest and most infiltrated by macrophages and foam cells. In eccentric plaques, the weakest spot is usually

in the shoulder region or the cap margin, whereas the thinnest caps (≤65 μm) known as TCFAs are most prone to rupture [8, 18].

Thinning of the fibrous cap occurs due to its degradation. This fibrous cap degradation is hypothesised to occur due to two mechanisms [1]. The first mechanism involves the gradual loss of SMCs from the fibrous cap which are the main source of collagen synthesis. This results in thinning of the fibrous cap, thus contributing to plaque vulnerability. Secondly, infiltrating macrophages and foam cell formation, and subsequently their apoptosis lead to the continuous release of MMPs that degrade the collagen-rich matrix of the fibrous cap [58, 59]. The time taken for the fibrous cap to be degraded is not known. Further studies are needed to determine whether this evolves over decades or more acutely. Nevertheless, fibroatheromas have been commonly observed in patients as young as 30 years of age where acute coronary syndrome is very rare [60, 61]. This suggests that the thinning of the fibrous cap may take at least a few years to evolve [1].

Rupture of a thin cap exposes plaque components, such as the cap collagen, apoptotic microparticles and the lipid core to thrombogenic factors in blood, which can lead to subsequent thrombosis. The magnitude of thrombosis can be extremely variable and in the most severe cases leads to tissue- or even life-threatening luminal occlusion. The main determinants of thrombotic risk of plaque are explained as the Virchow triad; (1) thrombogenicity of the exposed plaque material, (2) local blood flow disturbances and (3) systematic thrombotic propensity [1, 10, 62].

2.4.11 Plaque Erosion

Plaque erosion is the second most common cause of atherothrombosis behind plaque rupture. It is best characterised in coronary atherosclerosis, where it can cause the full spectrum of clinical manifestations of acute coronary syndrome, including ST-segment elevation myocardial infarction (STEMI), non-ST segment elevation myocardial infarction (NSTEMI) and sudden cardiac death [63, 64]. Although the distinct morphological features of underlying plaque in cases of erosion have not yet been fully characterised, currently recognised hallmarks are an absent endothelium overlying a plaque which is typically scarcely calcified, with smaller lipid core, more abundant SMCs, but fewer macrophages and less inflammation than in ruptured plaques.

The mechanisms which lead to plaque erosion are still unclear. One current hypothesis is that toll-like receptor 2 (TLR2) expressed on endothelial cells interacts with gram-positive toxins and hyaluronan, triggering apoptosis and the release of ROS. This leads to endothelial dysfunction and local inflammation which attracts the infiltration of leukocytes, particularly neutrophils. Naturally, neutrophils act to eliminate pathogens by multiple means, both intracellular and extracellular by phagocytosis and degranulation [65], or by releasing neutrophil extracellular traps (NETs, see Glossary) [66]. NETs are composed of a core DNA element to which histones, proteins (for example, lactoferrin and cathepsins) and enzymes (for

example, myeloperoxidase and neutrophil elastase) are attached. NETs are thought to directly kill targets by means of antimicrobial histones and proteases in a process called NETosis [67]. Activated endothelial cells can induce NETosis. Neutrophils and NETs directly induce endothelial dysfunction and damage to endothelial cells, causing them to detach from the underlying internal elastic lamina basement membrane. This exposes them to thrombogenic factors in blood which leads to thrombosis [30, 68].

Compared to plaque rupture, plaque erosions are thought to convey a weaker thrombogenic stimulus. It has been observed that fatal thrombi resulting from plaque erosions seem to take longer to build up than those precipitated by plaque rupture [1, 69, 70].

2.5 The Role of Inflammation in Atherosclerosis

Inflammation underpins all stages of atherosclerosis and is a major driver of plaque complications, such as rupture or erosion, which ultimately results in thrombosis. Circulating leukocytes, which are the mediators of host defence and inflammation localise in the earliest lesions of atherosclerosis. The normal endothelium does not generally support the binding of these leukocytes. However, in early atherogenesis the endothelium becomes inflamed and has enforced expression of leukocyte adhesion molecules (VCAM-1, ICAM-1, P selectin and E selectin) due to the pro-inflammatory cytokines released by inflammatory cells. These proinflammatory cytokines (e.g. MCP-1) also provide a chemotactic stimulus for the adherent leukocytes directing their migration to the subintima. M-CSF which is responsible for the differentiation of monocytes into macrophages is also a major inflammatory mediator which augments the expression of scavenger receptors on macrophages to increase their uptake of modified lipoproteins to form foam cells.

Macrophages also produce the bulk of the enzymes that catabolise collagen, a key constituent of the plaque's fibrous cap. It has been reported that the overproduction of the interstitial collagenase members of the matrix metalloproteinase (MMP) family (MMP-1, MMP-8, and MMP-13) threatens the biomechanical stability of the plaque's protective fibrous cap, predisposing to plaque rupture [14, 71].

Macrophages comprise the vast majority of inflammatory cells in atherosclerotic plaques. Although in lower number, the cells of adaptive immunity, namely T lymphocytes and B lymphocytes also exist in atherosclerotic lesions [2]. Despite their minority status, lymphocytes, particularly T lymphocytes, appear to function decisively in the regulation of inflammation during atherogenesis by regulating the innate inflammatory response mediated by macrophages within plaques. The expression of class II histocompatibility antigens by neighbouring cells has provided evidence for the functional significance of these T cells. T lymphocytes produce IFN-γ, the inducer of class II major histocompatibility complex (MHC-II) antigens in SMCs and macrophages.

B cells also populate plaques. Humoral immunity appears to mitigate atherogenesis. Thus, B1 cells that give rise to natural antibody may protect against atherosclerosis. In contrast, B2 lymphocytes may aggravate atherogenesis [72]. Therefore, the net influence of B cell functions in atherosclerosis remains unsettled.

Even though the initial inflammatory response is inherently appropriate, it eventually becomes maladaptive due to defective inflammatory resolution with persistent recruitment of inflammatory cells to the lesion area creating a positive feedback loop as discussed above [21].

All these inflammatory mediators contribute to an essential link between arterial inflammation and thrombosis. Recognition of this link has led to efforts to predict future cardiovascular risk, for example through the detection of elevated levels of inflammatory biomarkers in the peripheral blood of apparently healthy men and women. Generally, the greater the systemic inflammatory response, the greater the vascular-associated inflammatory reaction will be [31].

Large scale population-based prospective studies have found increased cardiovascular risk associated with increased levels inflammatory cytokines, such as IL-6, IL-18 [73], MMP-9 [73] and TNF-α [74]; increased cell adhesion molecule expression (VCAM-1, ICAM-1, P selectin and E selection) [75–77]; elevated inflammatory lipoprotein subset lipoprotein(a) [78]; and elevated downstream acute phase reactants such as C-reactive Protein (CRP) [79–81], fibrinogen [78], homocysteine [78], and serum amyloid A.

2.6 Risk Factors

Detailed discussion of risk factors for atherosclerosis is beyond the scope of this chapter, but the following section will provide a concise overview of genetic and conventional risk factors and some of their notable mechanisms for mediating atherogenesis.

2.6.1 Genetic Risk Factors

Genetic predisposition remains a major risk factor of atherosclerosis. Population studies and *in vivo* animal models have confirmed several genetic variations that influence atherosclerotic plaque formation and progression. While there are notable exceptions, in most affected individuals, directly causative genes responsible for atherosclerosis remain elusive. A positive family history often reflects the complexities of several genes which add incremental risk to disease progression.

Familial hypercholesterolaemia is a genetic disease in which individuals display a two to three-fold increase in plasma cholesterol levels compared to the general population. This disease occurs due to the loss of the gene encoding for the low-density lipoprotein receptor (LDLR) or functional defects in the LDLR protein [82–84].

One in every 500 individuals are heterozygous for this condition and are prone to the development of premature atherosclerosis in early adulthood [85]. Individuals who are homozygous for the condition have a five to six-fold increase in their plasma cholesterol levels, resulting in even more accelerated disease progression and may die from myocardial infarction before the age of 20. Familial hypercholesterolaemia represents one of the few occasions where one gene is largely responsible for atherosclerosis. Several studies of another rare genetic disorder, primary dysbetalipoproteinaemia, also known as hyperlipoproteinaemia type III, have identified allelic variants of Apolipoprotein E (ApoE), which is involved in serum lipid metabolism and regulation. The most common allelic variations of ApoE known to occur in the population are E2 (60% frequency), E3 (30% frequency) and E4 (10% frequency), where E3 variants lead to an increase in plasma cholesterol levels whereas E2 variants lead to a reduction.

Recent genetic studies and numerous epidemiologic studies have identified lipoprotein-(a) [LP(a)] as a risk factor for atherosclerotic diseases. LPA is the gene encoding this lipoprotein which is believed to play a role in thrombosis. Lp(a) levels show tremendous variation due to genetic polymorphisms in the LPA gene. Increased levels of Lp(a) are associated with increased cardiovascular risk [86]. Variants in other apo-lipoprotein genes have also been associated with altered atherosclerotic risk, for example APOB and APOA5 [87], which are involved in the synthesis of different components that make up lipoprotein particles. These observations suggest that dysregulation of multiple aspects of lipid metabolism can increase atherosclerotic risk. Elevated levels of homocysteine in the blood are also known to be associated with an increased risk of atherosclerosis, likely due to the resulting endothelial dysfunction [88, 89].

Recent genome-wide association studies (GWAS) have identified a number of loci associated with atherosclerosis which further enhances our understanding of the spectrum of genetic variations and promises eventual developments in the identification, prevention and treatment of atherosclerosis [90].

2.6.2 Traditional Risk Factors

The most common traditional risk factor for atherosclerosis is known to be high levels of circulating cholesterol, known as hypercholesterolaemia. Observational studies have shown that societies with high consumption of saturated fat and prevalent hypercholesterolaemia have greater mortality from coronary disease than countries with traditionally low saturated fat intake [4, 91, 92]. Accordingly, data from the Framingham heart study and other cohorts have shown that the risk of ischaemic heart disease increases with higher total serum cholesterol levels. The coronary risk is approximately twice as high for a person with a total cholesterol level of 240 mg/dL (6.2 mmol/L) compared with a person whose cholesterol level is 200 mg/dL (5.2 mmol/L).

Elevated systemic blood pressure (either systolic or diastolic) also increases the risk of developing atherosclerosis. This risk is thought to increase gradually with

continuous high pressures. Mechanistically, hypertension can accelerate atherosclerosis in several ways. High pressures injure the vascular endothelium which leads to increased permeability. Cyclic circumferential strain due to hypertension stimulates SMC production of proteoglycans [93], which retain LDL particles leading to its modifications becoming more inflammatory. Angiotensin II which is released as a vasoconstrictor that mediates hypertension can also cause oxidative stress.

Cigarette smoking increases the progression of atherosclerosis by affecting numerous mechanisms which aggravate plaque development. Among these, smoking enhances endothelial dysfunction, increases oxidative modification of LDL, increases oxidative stress, decreases HDL levels and increases platelet adhesiveness which in turn increases the propensity for thrombosis [23, 32, 94].

Diabetes mellitus is another well-recognised risk factor for atherosclerosis. This predisposition is thought to be related to an increase in non-enzymatically glycated lipoproteins which leads to enhanced uptake of cholesterol by macrophages leading to foam cell formation. Diabetic individuals also have impaired endothelial function leading to the reduced bioavailability of NO which leads to increased leukocyte adhesion and migration to the intima.

2.7 Conclusion

The pathophysiology of atherosclerosis is a complex process which is initiated in the early days of childhood and develops over decades. It results in the accumulation of lipids in the vessel wall which narrow the arterial lumen, slowly leading to blood flow restriction and tissue ischaemia. On the other hand, atherosclerosis can also progress more rapidly resulting in acute atherothrombotic events that result in sudden tissue infarction. Despite ever increasing knowledge and advances in the field, vascular diseases caused by atherosclerosis remain among the leading causes of mortality and morbidity worldwide. It is undeniable that the improvements in cardiovascular care have increased patients' quality of life over the years. However, it is likely that the global burden of atherosclerosis will continue to rise as populations age and as developing countries continue to adopt the 'fast-food' diets and sedentary habitats of the western lifestyle. Ongoing research is tackling multiple lines of investigation into a vast number of molecular and cellular mechanisms involved in the pathogenesis of atherosclerosis. These findings will continue to spur the development of novel treatments for the disease.

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Chapter 3
Mechanisms of the Vulnerable Atherosclerotic Plaque and Imaging

Khizar Rana, Stephen J. Nicholls, and Johan W. Verjans

Key Learning Points

- Plaque rupture and thrombosis are responsible for approximately 2 out of 3 of acute ischaemic syndromes. Plaque erosion is distinct from plaque rupture, is less well understood, and accounts for the majority of remaining non-rupture events.
- Vulnerable plaque features that predispose to plaque rupture include thin-cap fibroatheromas, larger plaque volume, larger lipid-rich necrotic core, neovascularization, intraplaque haemorrhage and spotty microcalcification.
- Imaging modalities including ultrasound, CT and magnetic resonance imaging (MRI) have shown capability and value in detecting various vulnerable plaque features noninvasively.
- Positron emission tomography (PET) scans can be used to image the biology of plaque processes such as inflammation and calcification that are implicated in atherosclerosis and plaque rupture.
- Intravascular imaging, though invasive, offers superior spatial resolution to characterise plaques beyond that which can be achieved with non-invasive modalities.

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3.1 Introduction

Atherosclerotic disease is the leading cause of death worldwide [1]. Acute plaque thrombosis and subsequent occlusion of vessels is the mechanism underlying the acute-ischaemic syndromes. Acute plaque thrombosis can be secondary to (1) plaque rupture, (2) plaque erosions or (3) calcified nodules.

3.2 Plaque Rupture

Plaque rupture is defined as a disruption in the fibrous cap leading to a thrombosis that communicates with the necrotic core and is usually occlusive (Fig. 3.1). Plaque rupture is responsible for the vast majority (70–80%) of acute-ischaemic syndromes [2, 3]. Various plaque morphological features are known risk factors for a plaque rupture. These include thin-cap fibroatheromas, larger volume plaques, larger lipid-rich necrotic core, neovascularization, intraplaque haemorrhage and spotty microcalcification. These plaque morphological features will be discussed below.

3.2.1 Thin Cap Fibroatheromas

Atherosclerotic plaques with thin fibrous caps are more likely to rupture and expose the thrombogenic plaque core to the blood. An autopsy study of sudden cardiovascular deaths found that 95% of ruptured plaques had a fibrous cap thickness of less than 65 μm and this lead to the term thin-cap fibroatheromas (TCFAs) being introduced to describe such lesions [4, 5]. TCFAs are the likely precursors of the majority of fatal coronary plaque ruptures [6] (Fig. 3.1).

Fibrous cap thinning probably occurs due to two concurrent mechanisms; (1) decreased matrix production by smooth muscle cells (SMCs) and (2) increased matrix breakdown by infiltrating macrophages that secrete proteolytic enzymes such as matrix metalloproteinases (MMPs). Decreased production of collagen occurs due to the depletion of smooth muscle cells as a result of apoptosis [7, 8]. Additionally, in-vitro studies have shown that interferon-gamma released from activated T-cells can inhibit SMC collagen gene expression [9] and reduce the expression of an enzyme (lysyl oxidase) necessary for the crosslinking of collagen fibres [10]. Increased matrix breakdown is thought to result from the release of proteolytic enzymes such as matrix metalloproteinases, plasminogen activators and cathepsins by macrophage foam cells. The matrix metalloproteinases are released as latent zymogens which are subsequently activated by plasmin from macrophages, chymase from degranulating mast cells and trypsin [11]. Thus, thinning of the fibrous cap occurs probably due to the pro-inflammatory actions of infiltrating macrophages and activated T-cells.

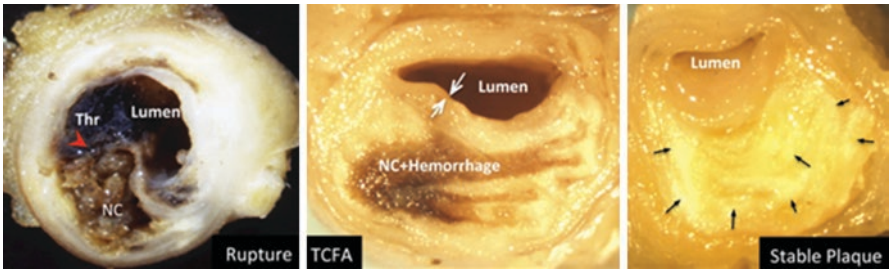

Fig. 3.1 Gross morphology of plaque rupture, thin-cap fibroatheroma and stable plaque. (Left) Plaque rupture, a disruption of the thin-fibrous cap (red arrow) can be seen with an overlying thrombus (Thr) forming. The thrombus is in direct contact with the necrotic core (NC). (Middle) Thin-cap fibroatheroma (TCFA). The gross features of a TCFA include a thin fibrous cap (white arrows indicating the thinnest point) overlying a large haemorrhagic necrotic core. (Right) Stable Plaque, The stable plaque is characterized by fibrous tissue with heavy calcifications (arrows). (Reprinted from Narula, Nakano [105])

3.2.2 Plaque Size and Positive Luminal Remodeling

Large plaques, which may not produce significant vessel stenosis, are responsible for the majority of acute plaque thromboses. This apparent anomaly can be explained by the fact that large, vulnerable plaques are associated with positive remodeling, whereby the degree of flow-limiting stenosis is attenuated by reactive changes in the underlying wall [12]. Metalloproteinases, the same enzymes that are believed to play a role in the thinning of fibrous caps, are believed to have an important role in the positive remodeling response [13, 14]. In vivo ultrasound studies have shown that large plaques and positive remodeling are associated with unstable angina whilst negative remodeling is associated with stable angina [12, 15]. Additionally, a larger luminal diameter, as seen in positive remodeling, is associated with a higher peak circumferential stress on the fibrous cap, potentially making it more prone to rupture.

3.2.3 Lipid-Rich Necrotic Core

In early atherogenesis, diffuse intimal thickening (DIT) consisting of SMCs, elastin and proteoglycans develops in the arterial wall. Lipoproteins then accumulate in the intimal wall and attract macrophages that engulf the lipoproteins and secrete proteolytic enzymes, leading to the development of a lipid-rich necrotic core.

Plaque rupture is more likely to occur where the fibrous cap is thinnest. For eccentric plaques, this is often at the junction of the fibrous cap and the adjacent wall, known as the shoulder region [16]. Plaques with a higher cross-sectional area of lipid-rich core have been associated with an increased risk of rupture and thrombosis in the aorta [17, 18]. A large, eccentric lipid core may cause redistribution of circumferential stress to the vulnerable shoulder region of the fibrous cap, and thus

increasing the likelihood of rupture or alternatively, an expansion of the lipid core may erode the fibrous cap from below. Additionally, the lipid-rich core has a high density of tissue factor making it highly thrombogenic when exposed to blood following plaque rupture [19].

3.2.4 Neovascularisation and Plaque Haemorrhage

Neovascularisation is a common feature of vulnerable plaques. In the coronary circulation, the intima lacks vasa vasorum, whereas the outer media and adventitia have a blood supply. Intimal thickening and inflammation in atherosclerosis increases the demand for oxygen above that which can be supplied by the vasa vasorum in the adventitia and hence causes hypoxia. Hypoxia and the concomitant inflammation induce the release of angiogenic factors (e.g. VEGF) that promote angiogenesis. The sprouting vessels extend from the adventitia, through the media and into the plaque [20]. However, these microvessels are thin-walled, lined with discontinuous endothelium and have a lack of supporting SMCs. Disruption of these fragile microvessels is believed to result in intraplaque haemorrhage and extravasation of proteins and inflammatory cells [21, 22].

Intraplaque haemorrhage is associated with an increase in the size of the necrotic core, and is more frequently seen in lesions prone to rupture [23]. Clinically, studies have shown a strong association between the presence of symptomatic carotid disease and the degree of plaque vascularity and quantity of intraplaque haemorrhage [24, 25]. Erythrocytes have membranes rich in cholesterol. In atherosclerotic plaques, the cholesterol in the erythrocyte membranes is liberated by an enzyme called sphingomyelinase, leading to increased cholesterol deposition in the plaque [26, 27]. Thus, neovascularization and intraplaque haemorrhage destabilise the plaque by increasing macrophage infiltration, cholesterol deposition and expanding the necrotic core.

3.2.5 Calcification

Coronary artery calcification (CAC) is an important marker of plaque burden that aids in cardiovascular risk stratification beyond that of the traditional risk factors [28, 29]. Different patterns of calcification can be observed. Spotty (micro) calcification is associated with acute coronary syndromes, whereas extensive calcification is regarded as a marker of plaque stability and is more prevalent in the stable angina cohort [30]. Spotty calcification is further associated with other features of a vulnerable plaque including a larger total atheroma volume, fibrofatty plaques and positive remodeling. Mechanistically, biomechanical studies suggest that the dense spotty calcification on the softer fibrous cap can create a larger stress concentration at the interface of the hard calcification and soft fibrous cap and this may lead to the sudden rupture of the fibrous cap [31]. Additionally, the size, shape, location, proximity to other calcifications and composition of the microcalcifications may also play an important role in determining the peak stress on the fibrous cap.

3.3 Plaque Erosion

Plaque erosions account for approximately one-third of lesions causing ACS [32, 33]. Plaque erosion is defined by the presence of a thrombus in direct contact with the intima, with absent endothelium and no identifiable fibrous cap rupture [34]. In contrast to plaque rupture, the underlying intima is rich in smooth muscle cells and proteoglycans and has minimal inflammatory cell infiltrate [5]. Erosions are also associated with a smaller plaque and necrotic core area, and lesser stenosis and plaque burden than ruptures [35].

Plaque erosions are more common in younger men and women (<50-years-old) and are associated with smoking, particularly in premenopausal women [5, 34]. Additionally, optical coherence tomography (OCT) studies have revealed that non-ST elevated acute coronary syndromes (NSTEACs) are more commonly associated with OCT-erosions and OCT-calcified nodules than ruptures [32].

The mechanism of plaque erosion has not been precisely elucidated. Proposed mechanisms involve a complex simultaneous interplay between processes causing endothelial cell loss, neutrophil recruitment, and thrombosis [36]. The underlying intima in plaque erosions is rich in smooth muscle cells and proteoglycans. Hyaluronan, a component of proteoglycans, has been shown to increase Toll-like receptor-2 (TLR-2) expression in vivo. Additionally, erosions causing locally disturbed blood flow can increase endothelial TLR-2 expression and apoptosis [37]. Increased TLR-2 expression may lead to the accumulation of neutrophils, endothelial cell apoptosis and thrombosis.

3.4 Calcified Nodules

A calcified nodule is the least common cause of thrombosis [34]. Calcified nodules can disrupt the overlying fibrous cap and cause thrombosis, however, it is not a common occurrence [5]. In an OCT study of 126 ACS patients, calcified nodules had an incidence of 7.9% [32]. They are more commonly seen in elderly individuals with heavily calcified arteries and most frequently found in the mid-right coronary artery [5]. In the PROSPECT study, calcified nodules identified with intravascular ultrasound (IVUS) resulted in very few coronary events at 3 years follow up [38].

3.5 Effect of Diabetes on Plaque Morphology

Diabetes in patients without clinical CAD is associated with an atherosclerotic burden equivalent to that of non-diabetics with clinical CAD, hence diabetes is thought to be a ‘CAD equivalent’ in terms of risk factors. Differences in plaque morphology have been shown between diabetic and non-diabetic patients. Diabetes has been associated with more widespread diffuse atherosclerosis, increased calcification [39], increased inflammatory infiltrate (macrophage,

T-cells), HLA-DR expression [40], CRP elevation, larger necrotic core, greater number of healed plaques [41], and increased presence of TCFAs [42, 43]. The underlying cellular mechanisms behind this are multifactorial. Autopsy studies have shown increased expression of receptors for advanced glycated end-products (RAGE) and RAGE-binding protein in plaques of diabetic patients. Hyperglycaemia can result in increased production of advanced glycated end-products (AGE) and AGE/RAGE signaling has been linked to increased calcification, oxidative stress and vascular inflammation, thereby contributing to accelerated atherosclerosis [41, 44].

3.6 Carotid Imaging

3.6.1 Carotid Ultrasound

Intraplaque haemorrhage and the lipid-rich necrotic core appear echolucent on ultrasound. Both of these plaque features cannot be distinguished on ultrasound. Echolucent plaques are associated with an increased rate of cerebrovascular events, independent of the degree of stenosis or cardiovascular risk factors [45, 46].

The grayscale median (GSM) score is a standardised way of characterising plaques. The more echogenic the plaque, the higher the GSM score. GSM measurements can be made from a single longitudinal view (SLV-GSM) or from multiple cross-sectional views (MCSV-GSM). Plaques with a lower GSM score (i.e. echolucent plaques) have been associated with an increased risk of stroke [47, 48]. In one study, the incidence of stroke was 9% for plaques with a GSM >50 and 40% in those with a GSM <50 ($p < 0.001$) [49]. Another study distinguished plaques causing amaurosis fugax from asymptomatic plaques by the GSM features of increased plaque heterogeneity (highest MCSV-GSM minus the lowest MCSV-GSM) and increased echolucency in plaques associated with amaurosis fugax [50].

Plaque calcification and the fibrous cap appear echogenic on ultrasonography. Ultrasonographic fibrous cap thickness measurement has shown to have excellent correlation with histological findings [51]. The Oxford plaque study showed that ruptured carotid plaques had a median representative cap thickness of 300 μm and a median minimum cap thickness of 150 μm, compared to 500 μm and 250 μm for non-ruptured plaques respectively [52]. This study proposed a representative cap thickness of less than 500 μm and minimum cap thickness of less than 200 μm to identify ruptured plaques on carotid ultrasonography.

Plaque ulceration is defined as an area of surface irregularity in the plaque-lumen border greater than 2 mm in depth and with a well-defined back wall at its base [53]. Standard and Doppler ultrasonography have a low sensitivity and specificity to detect plaque ulceration when compared with CT and MRI [54, 55], however contrast-enhanced ultrasonography has been shown to have improved

Table 3.1 The detection of vulnerable plaque features by ultrasound

Morphological features	Ultrasound findings
Intraplaque haemorrhage and large lipid-rich necrotic core (LR-NC)	Echolucent plaque
Thin fibrous cap	Echogenic fibrous cap with minimum cap thickness <200 μm
Plaque ulceration	Surface irregularity >2 mm depth, well-defined back wall on colour doppler
Neovascularisation	Enhancement with contrast

sensitivity and accuracy for the detection of plaque ulceration when compared to standard ultrasound [56].

Plaque neovascularisation and inflammation are active processes implicated in plaque rupture. However, these processes are unable to be evaluated by simple B-mode ultrasonography. Contrast-enhanced ultrasonography can however use an intravenous microbubble agent that remains within the vascular space to enhance areas of neovascularisation and inflammation in the plaque [57]. Contrast enhancement indicating neovascularisation was seen in more than 93% of soft, echolucent plaques [58]. These ultrasonography features may be used for the risk stratification of carotid plaques (Table 3.1).

Doppler imaging can be used to assess the haemodynamic significance of internal carotid artery (ICA) lesions. The ICA peak systolic velocity (PSV) and the visualisation of plaque on B-mode or Doppler are key determinants for the grading of lesions. The Society of Radiologists has published consensus guidelines on the grading of carotid artery lesions with ultrasonography [59]. The ICA/CCA ratio and end diastolic velocity (EDV) are additional measurements used to assist in grading lesions. The ICA is normal when PSV is <125 cm/s and there is no plaque visualised; there is <50% stenosis when PSV is <125 cm/s and there is plaque or intimal thickening visible; 50–69% stenosis is present when PSV is between 125 and 230 cm/s and plaque is visible; >70% stenosis when PSV is >230 cm/s and visible plaque and luminal narrowing is seen; near-occlusion is when there is "trickle" flow on colour Doppler and total occlusion is when there is no detectable lumen on B-mode ultrasound and no detectable flow on Doppler imaging [59]. It should be noted that there is not a linear association between PSV and the degree of stenosis—for a near-occlusion or complete occlusion, the PSV may be low or undetectable.

3.6.2 Carotid Computed Tomography

Multidetector-row CT (MDCT) and dual-source CT (DSCT) are the two main CT modalities used for carotid plaque imaging. Both techniques provide a high spatial and temporal resolution and can be used for plaque characterisation.

CT angiography (CTA) of the carotid arteries has a higher spatial resolution and quicker acquisition time than magnetic resonance angiography (MRA) [60]. CTA can provide information on the degree of luminal narrowing, as well as plaque morphological features including plaque calcification, fibrous cap thickness, intraplaque haemorrhage and lipid-rich necrotic core [60]. Comparison of carotid CTAs with histological examination of endarterectomy specimens has allowed for the correlation of different plaque features with CT Hounsfield densities [61]. Calcifications are readily identified due to their high density (HU >250). There is a significant degree of overlap between the CT Hounsfield densities of lipid-rich necrotic core (LR-NC) and connective tissue, as well as some overlap between connective tissue and haemorrhage. However, as a general rule, LR-NC has a low density (HU < 30) whilst connective tissue and haemorrhage have an intermediate density (between 30 and 150HU) (Fig. 3.2).

CTA does, however, show good reliability when considering large (>5-pixels) LR-NC and haemorrhages. CTA is also reliable in detecting ulcerations as small as 1 mm and measuring the fibrous cap thickness [61]. Fibrous cap thickness measurement on CTA was found to have excellent correlation with histological examination

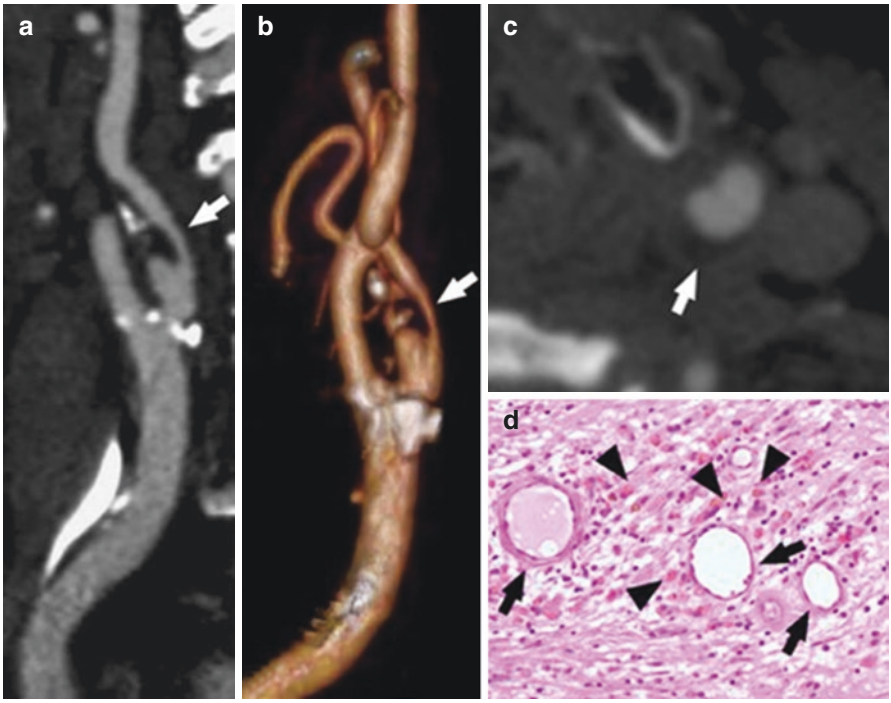

Fig. 3.2 Computed tomography (CT) imaging of Carotid Plaque. CT reconstruction (**a**) and volume rendering (**b**) showing a wide ulcerated carotid plaque causing significant stenosis. CTA axial image (**c**) shows a hypodense plaque (white arrow). Histological section (**d**) reveals the presence of neovessels (black arrows) and macrophages (black arrowheads). (Reprinted from Saba, Anzidei [106])

($P < 0.001$) [61]. Additionally, carotid plaque enhancement with contrast agents has shown good correlation with intraplaque neovascularisation [62].

Plaque features derived from CTA have shown to be correlated with cerebrovascular events including strokes and TIAs. A higher risk of stroke was found with the following features on CTA in one study: an increased wall volume, a thinner fibrous cap, higher number of lipid clusters, lipid clusters closer to the lumen, and fewer calcium clusters [63]. Other studies have also shown a correlation between non-calcified plaques or plaques with calcification at their base [64], carotid plaque enhancement [62] and fissured fibrous caps [65] with cerebrovascular events. Plaque morphological features derived from CTA can assist in determining the risk of future cerebrovascular events. Interventions may be directed at those deemed to be at high risk, however further studies are needed in this area.

3.6.3 Carotid Magnetic Resonance Imaging

Carotid MRI enables accurate visualisation of vulnerable carotid plaque features including thin or ruptured fibrous caps, large lipid-rich necrotic core, intraplaque haemorrhage, and carotid wall thickness. These features are associated with an increased risk of future cerebrovascular events [66].

Histological correlation of endarterectomy specimens with carotid MRI features has led to the development of a tissue type classification system linking MRI features to the histological findings (Table 3.2, Fig. 3.3). The lipid-rich necrotic core is seen centrally and forms the bulk of the plaque. A lipid-rich necrotic core may be seen with or without intraplaque haemorrhage (IPH). The intensities of IPH vary depending on whether it is acute (<1 week), recent (1–6 weeks), or old (>6 weeks) [67].

Table 3.2 Correspondence of plaque features with in vivo MRI findings

Plaque features	T1-weighted sequence	T2-weighted sequence	Proton density-weighted sequence	Time of flight
LR/NC with no IPH	Iso/hyperintense	Hypo/isointense	Iso/hyperintense	Isointense
LR/NC with IPH—Fresh	Hyperintense	Hypointense/ isointense	Hypointense/ isointense	Hyperintense
LR/NC with IPH—Recent	Hyperintense	Hyperintense	Hyperintense	Hyperintense
LR/NC with IPH—Old	Hypointense	Hypointense	Hypointense	Hypointense
Calcification	Hypointense	Hypointense	Hypointense	Hypointense
Dense fibrous tissue	Isointense	Isointense	Isointense	Hypointense
Loose matrix	Hypo/isointense	Hyperintense	Hyperintense	Hypointense

LR/NC lipid rich necrotic core, *IPH* intraplaque haemorrhage
Chu, Kampschulte [67], Saam, Ferguson [112]

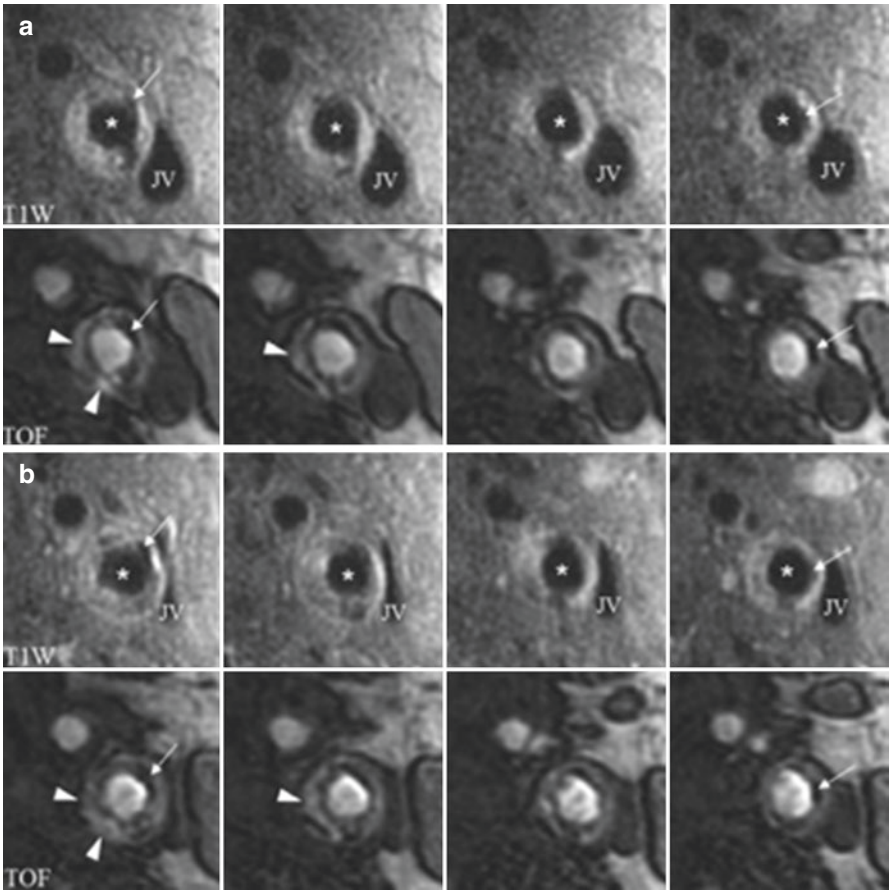

Fig. 3.3 T1-weighted (T1W) and time of flight (TOF) section through a left internal carotid artery lesion. Baseline imaging (**a**) and follow-up imaging at 18 months (**b**). The lesion contains intraplaque haemorrhage (arrow heads) and calcifications (arrows). A reduction in lumen area and increased wall thickness can be seen from (**a**–**b**). *JV* jugular vein. ∗Lumen of the internal carotid. (Reprinted from Underhill, Yuan [107])

Gadolinium-based contrast agents can be administered intravenously in carotid MRIs. The gadolinium enhances areas with increased vascularity. Thus, contrast-enhanced magnetic resonance imaging (CEMRI) can be used to help differentiate the fibrous cap which becomes enhanced, from the poorly vascularised LR-NC which does not enhance. CEMRI allows for quantification of the plaque volume, fibrous cap thickness and LR-NC—important determinants of plaque vulnerability [68]. Additionally, the rate of contrast enhancement is significantly correlated with neovascularisation and macrophage content. Thus, carotid MRIs can provide detailed information on the features of a vulnerable plaque. But how do these imaging technologies correlate with the risk of future clinical events?

MRI allows for the visualisation of high-risk plaque features that are not detectable on standard carotid ultrasonography. High-risk plaque features derived from MRI including IPH, large LR-NC and thin/ruptured fibrous caps have been associated with an increased risk of future ipsilateral ischaemic events [66, 69]. Detailed information on plaque morphology may allow clinicians to monitor high-risk plaque features and their response to medical therapy, as well as providing new indications for medical and/or surgical therapy directed at high-risk plaques that may not be symptomatic or significantly obstructive. Conversely, moderate stenoses (50–70%) on Doppler ultrasonography which do not have high-risk plaque features on MRI are reassuring markers of plaque stability and hence aid in clinical decision making.

3.7 Molecular Imaging

Positron emission tomography (PET) scans can be used to image functional processes implicated in atherosclerosis and plaque rupture. PET scans involve the administration of positron-emitting isotopes which decay resulting in the production of two photons that travel in opposite directions. The photons are detected and used to produce an image reflecting the location and density of the isotopes.

3.7.1 Imaging Inflammation: Fluorodeoxyglucose (^{18}F-FDG)

Fluorodeoxyglucose (^{18}F-FDG) is a radioactive glucose analogue that accumulates in cells in direct proportion to their rate of glycolysis and hence is a marker of glucose metabolism. In atherosclerosis, ^{18}F-FDG can be exploited as a marker of macrophage activity (Fig. 3.4) [70, 71]. Histological studies have shown that the intensity of ^{18}F-FDG uptake correlates well with the macrophage content of plaque endarterectomy specimens [71, 72]. Increased macrophage activity predominantly reflects the degree of inflammation in the plaque (Fig. 3.5) [70, 71]. However, there are also other factors that contribute to an increased signal. Hypoxia in atherosclerotic plaques can stimulate neoangiogenesis, glucose uptake by macrophages, and inflammation causing an increase in glycolysis and accumulation of ^{18}F-FDG [73, 74]. The ^{18}F-FDG signal may also be impacted by delivery. Increased microvascular permeability and microvessel density may increase delivery of ^{18}F-FDG and thus the signal may not entirely reflect the degree of inflammation [75].

Clinically, the utility of ^{18}F-FDG has not been well defined, however studies have shown promise. ^{18}F-FDG scans have been shown to help in predicting early stroke recurrence [76], cardiovascular events in a cohort with neoplastic disease [77], and can help predict the risk of cardiovascular disease beyond the Framingham risk score in some populations [78]. However, ^{18}F-FDG is a non-specific marker of metabolism and can be taken up by the myocardium, making it difficult to clearly assess the signals from the coronary arteries [70].

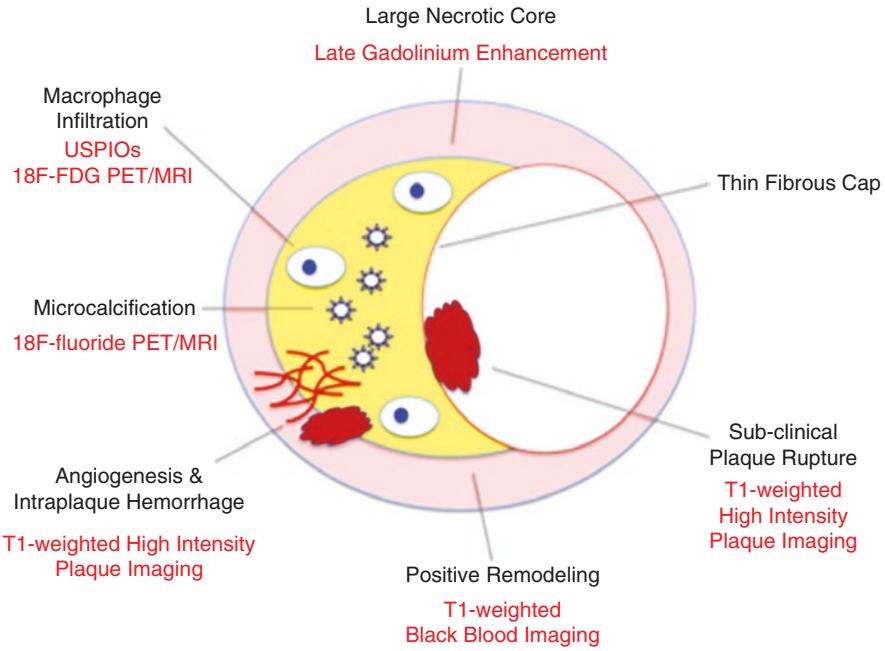

Fig. 3.4 Vulnerable plaque features on MRI. Vulnerable plaque features that can be detected with magnetic resonance imaging. (Reprinted from Dweck, Puntman [108])

^{18}F-FDG imaging has also been shown to play a role in assessing the clinical utility of drugs to reduce inflammation in plaques. A FDG-PET study provided evidence for dose-dependent reductions in carotid plaque ^{18}F-FDG uptake with statin therapy, independent of changes in blood lipid profiles [71]. This study showed that patients randomized to atorvastatin 80 mg had significantly reduced inflammation in the index vessel as compared to patients randomized to atorvastatin 10 mg at 12-weeks [71]. Additionally, ^{18}F-FDG imaging can be used as an endpoint in clinical trials to test the efficacy of various anti-atherosclerotic or anti-inflammatory drugs for atherosclerosis.

3.7.2 Imaging Microcalcification: Sodium Fluoride (^{18}F-NaF)

Spotty (micro) calcification is a feature of vulnerable plaques, whereas dense macrocalcification is associated with stable plaques [30]. Microcalcification cannot be detected with plain CT-scans whilst macrocalcification can be detected on CT. Sodium fluoride (^{18}F-NaF) is a promising isotope that can help image calcifications that were previously not detectable on plain CT. NaF is incorporated into hydroxyapatite at sites of active calcification [79]. It has traditionally been used to image bone diseases and cancers, however its application is now being extended

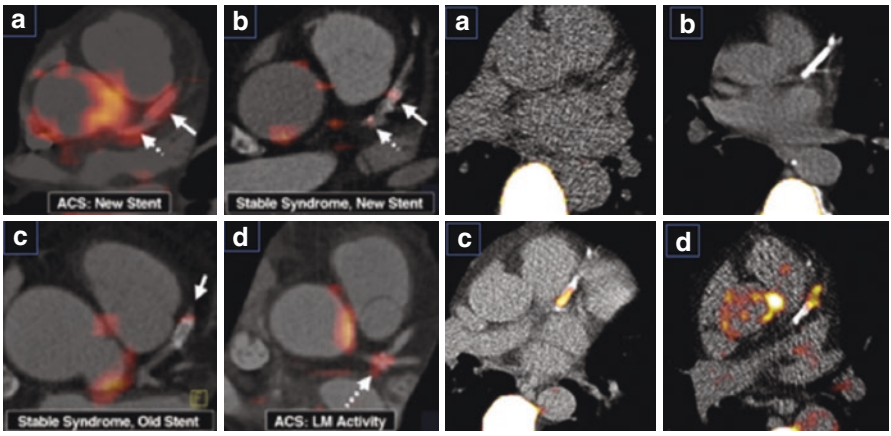

Fig. 3.5 Molecular imaging of vulnerable plaque with Fluorodeoxyglucose (left panels) and Sodium Fluoride (NaF) (right panels). (Left panels) Fluorodeoxyglucose (FDG) uptake as a marker of plaque inflammation. ^{18}F-FDG PET/CT preliminary imaging of coronary artery inflammation in subjects with Coronary Artery Disease (CAD). F-FDG imaging in a patient with recent ACS and a new stent (**a**) indicates a higher degree of tracer uptake than those with a stable syndrome and a new or old stent (**b**, **c**). (**d**) shows ^{18}F-FDG uptake at the LMCA trifurcation in an Acute Coronary Syndrome (ACS) patient. Reprinted from Rogers, Nasir [109]. (Right panels) Sodium Fluoride (NaF) as a marker of active plaque calcification. ^{18}F-NaF PET/CT imaging of plaque osteogenic activity in CAD patients. (**a**) Control subject with no calcification or NaF uptake. (**b**) Subject had extensive calcification in the left anterior descending artery as demonstrated by the hyperdense lesions however had no NaF uptake, indicating no current active calcification processes. (**c**) Shows increased NaF uptake on top of existing calcification. (**d**) Shows increased NaF uptake in the LAD adjacent to existing calcification. (Reprinted from Dweck, Chow [80])

into the vascular arena. ^{18}F-NaF uptake is a useful marker of active calcification and does not represent the pre-existing calcium burden of a vessel (Fig. 3.5).

Increased NaF uptake has been correlated with higher rates of prior cardiovascular events, angina and Framingham risk scores [80]. Increased ^{18}F-NaF uptake has been associated with histological evidence of active calcification, macrophage infiltration, apoptosis and necrosis [81]. It is further associated with other high-risk plaque features on intravascular ultrasound (IVUS) including positive remodeling, microcalcification, and necrotic core [81]. ^{18}F-NaF PET-CT is a non-invasive method of imaging high-risk plaques, however its role in altering management needs further investigation.

3.8 Intravascular Imaging

3.8.1 Intravascular Ultrasound (IVUS)

Intravascular ultrasound (IVUS) involves the same principle as conventional ultrasound but is able to take higher resolution images of atherosclerotic plaque. A transducer produces sound waves at 20–40 MHz, and the amplitude of the reflections are

digitised to produce gray-scale images [82]. Gray-scale IVUS has limited use in defining plaque morphology. In GS-IVUS, lipid-rich plaques have low-echogeneity, calcified plaques have high echogenicity with acoustic shadowing, fibrous plaques have intermediate echogeneity between the soft plaques and the echogenic calcifications and mixed plaques have a combination of the above. However, many of the findings in GS-IVUS are non-specific. For example, an area of echolucency may be attributable to one of several plaque features including lipids, necrotic zone, intramural haemorrhage, or thrombus [83].

To counter these issues, post-processing modules including virtual histology IVUS (VH-IVUS), iMAP-IVUS and integrated backscatter IVUS (IB-IVUS) have been developed. VH-IVUS uses the amplitude and frequency of the reflected waves to generate images. VH-IVUS has been shown to have reasonable accuracy (>90%) in identifying fibrous tissue, fibro-fatty regions, necrotic core and calcium-dense regions in plaques [84, 85]. VH-IVUS can also quantify plaque burden, lumen area, positive remodelling, and identify VH thin-cap fibroatheromas (Fig. 3.6). VH-TCFAs have been defined as plaques with a necrotic core ≥10 % in contact with the lumen without overlying fibrous tissue and percent atheroma volume≥40% [86]. VH-IVUS characterised features including plaque burden >70%, minimal lumen area <4 mm^2, VC-TCFAs, necrotic core area, dense calcium area and remodelling index have been associated with

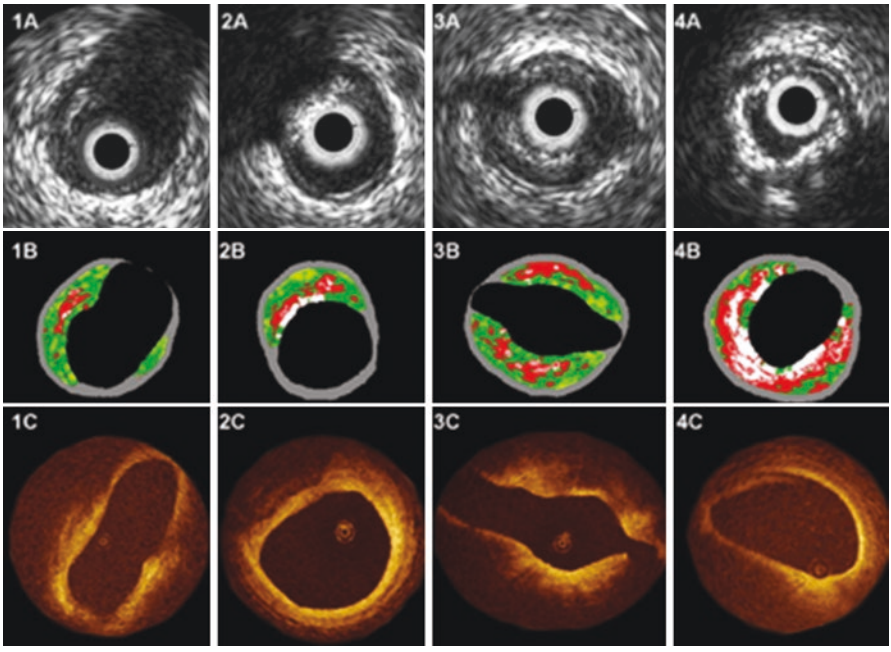

Fig. 3.6 Intravascular ultrasound, virtual histology and OCT to highlight plaque morphology. (**A**) Grayscale IVUS, (**B**) Virtual Histology, (**C**) OCT imaging for four different types of plaque: (1) fibroatheroma, (2) calcified fibroatheroma, (3) thin-cap fibroatheroma, (4) calcified thin-cap fibroatheroma. (Reprinted from Gonzalo, Garcia-Garcia [110])

future major adverse cardiovascular events (MACE) [87, 88]. However, IVUS is not able to detect thin-fibrous caps (<65 μm) due to its inferior spatial resolution [89].

3.8.2 *Optical Coherence Tomography (OCT)*

Optical coherence tomography (OCT) uses near-infrared light to produce images with a superior spatial resolution of 10–30 μm. This allows OCT to measure the fibrous-cap thickness [90], macrophage density [91], collagen and smooth muscle cells content [92], characterise plaques as fibrous, fibrocalcific, and lipid-rich plaques [93], and identify neovessels, cholesterol crystals, ruptures and thrombi [94, 95]. The correspondence between plaque and OCT features can be seen in Table 3.3 and Fig. 3.7. However, as OCT has a penetration of approximately 1.5 mm, it is not able to image the deeper plaque and estimate the size of the necrotic core or identify positive remodeling. Additionally, the OCT rays can be attenuated by blood and so OCT requires a blood-free field unlike IVUS. It can also be difficult to differentiate calcified plaques from necrotic core as they both appear as signal poor areas with the only difference being the delineation of their borders (sharply delineated borders in calcified plaque, poorly delineated in necrotic core) [96].

Plaque features derived from OCT including OCT-TCFA, and thinner fibrous cap thickness have been associated with lesions causing acute MI and NSTEMI/Unstable angina, as opposed to stable angina lesions [97]. OCT derived features including lipid-rich plaque, thin fibrous cap, TCFA and fibrous cap macrophage density have been associated with positive remodeling [98].

Table 3.3 Correspondence of plaque features with optical coherence tomography (OCT)

Plaque features on optical coherence tomography (OCT)	
Plaque features	Appearance on OCT
Fibrous plaque	Homogenous signal, highly backscattering signal
Calcified plaque	Signal poor area with sharply delineated borders
Fibrocalcific plaque	Fibrous tissue + calcification (both as described above)
Fibrous cap	Signal-rich area overlying a signal poor area
Necrotic core	Signal-poor area with poorly delineated borders covered by a fibrous cap
OCT-TCFA	Necrotic core with overlying fibrous cap with thickness <65 μm
Macrophages	Signal-rich punctate regions at border of fibrous cap and necrotic zone
Cholesterol crystals	Linear regions of high signal intensity
Red thrombus	Lower signal (than white thrombus), <250 μm half-width (distance from max signal intensity to half-signal intensity)
White thrombus	Higher signal, >250 μm half-width
Intimal vasculature	Signal-poor, sharply delineated in multiple contiguous frames

Tearney, Regar [95], Kume, Akasaka [90]

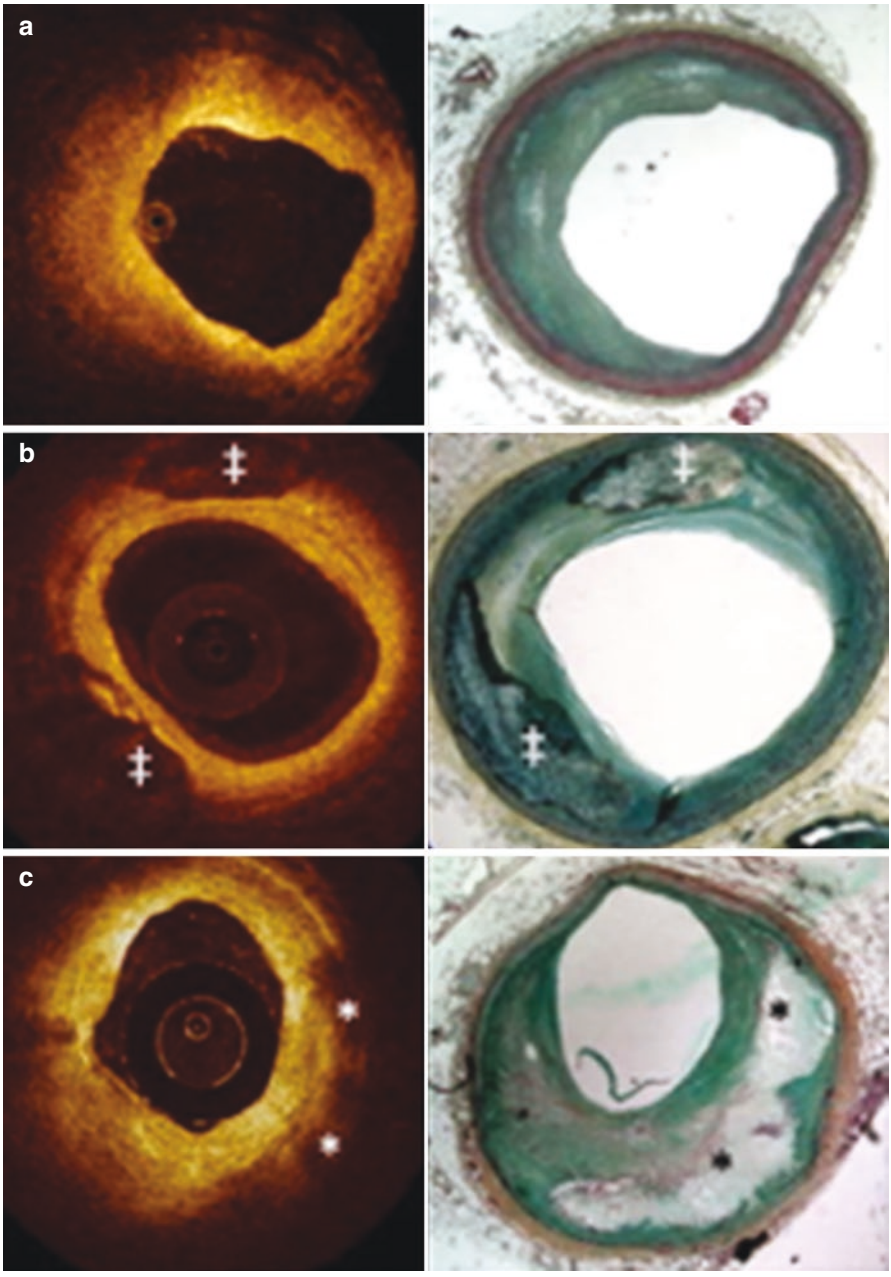

Fig. 3.7 Optical coherence tomography correlation with histology. (**a**) Fibrotic plaque shows a high signal and low attenuation. (**b**) A calcified plaque shows calcified regions (‡) that have a poor signal with sharply delineated borders. (**c**) The lipid rich plaque has a lipid core (∗) that has a diffuse border and high light attenuation. The thickness of the overlying fibrotic cap can be measured; in this case a thick cap (>200 μm) is present. (Reprinted from Bezerra, Costa [111])

Table 3.4 The strengths and limitations of intravascular ultrasound and optical coherence tomography to image vulnerable plaque features

Intravascular ultrasound and optical coherence tomography for imaging of vulnerable plaque features				
	Resolution	Penetration	Strengths	Limitations
Intravascular ultrasound	70–200 μm axial 200–400 μm lateral	5–10 mm	• Fibrous tissue, fibro-fatty, calcium dense, necrotic core • Plaque burden (PAV) • Lumen area • Positive remodelling • VH-TCFA	• Fibrous cap thickness (<65 μm)
Optical coherence tomography	10–30 μm	1.5 mm	• Fibrous cap thickness • Collagen content • Macrophages • Neovessels • Plaque rupture • Thrombus • Can detect plaque erosion	• Flush required as blood attenuates light • Limited penetration depth to image deeper plaque, estimate necrotic core size, positive remodelling • Poor discrimination between calcified areas and lipid core

Due to its spatial resolution of 10–30 μm, OCT is unable to image individual cells and subcellular processes implicated in atherosclerosis and coronary events (Table 3.3). μOCT is a further development from the standard OCT with a significantly superior resolution of 1-μm. μOCT has shown potential to visualize processes including leukocyte adhesion and diapedesis, clot morphologies, cholesterol crystals, microcalcifications, fibrin strand formation, ECM production, and quantify macrophage distribution [94, 99, 100]. Visualising these subcellular processes in vivo may help provide a new level of insight to characterize and identify vulnerable plaques (Table 3.4).

3.8.3 Intravascular Molecular Imaging

Intravascular near-infrared fluorescence (NIRF) involves the use of targeted molecular contrast agents to highlight via fluorescence certain processes implicated in plaque rupture. The advantage of near-infrared light includes lower attenuation of light rays through blood and lower autofluorescence [101]. Studies in this arena have included using indocyanine green to highlight endothelial abnormalities [101], and mapping arterial inflammation with the use of a contrast agent that highlights the inflammation regulated cysteine protease [102]. However, many of the contrast agents used in NIRF have not been approved for use in humans. Alternatively, near-infrared autofluorescence (NIRAF) can detect fluorescence from naturally

occurring molecules, negating the need for contrast agents. NIRAF has been safely used in humans. Promisingly, a significantly higher maximum NIRAF signal was associated with vulnerable plaques including OCT-delineated TCFA and plaque rupture cases [103]. However, further studies are needed to understand the molecular sources of NIRAF and its clinical significance.

3.9 Conclusion: Vulnerable Plaques and Vulnerable Patients

Post-mortem studies and subsequent imaging studies have clearly demonstrated the association of acute cardiovascular events with certain forms of plaque disruption [33, 95]. In approximately two thirds of all cases, plaque characteristics were associated with thin-capped atheroma along with a large necrotic core, suggesting the preceding destabilization. Plaque erosion accounts for the most of the of remaining events, but is less well understood, but can be imaged using OCT. Many attempts so far have been made to diagnose and predict events based on plaque characteristics that could be associated with a high-risk plaque. Nevertheless, this has been less successful than hoped, illustrated by the relatively disappointing results of the PROSPECT trial [104].

It seems evident that at least another decade of research is needed to develop better tools to assess high-risk plaques in coronary, carotid and peripheral arterial disease. In the meanwhile, another important realisation is that many chronic diseases, including atherosclerotic disease, are helped by taking a more holistic systems-based approach. This involves detecting the *patient* at high risk of cardiovascular events by taking into account variables based on blood biomarkers and myocardial vulnerability, in addition to the vulnerable plaque [33].

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Chapter 4
Current and Emerging Therapies for Atherosclerosis

Adam J. Nelson and Stephen J. Nicholls

Key Learning Points

- Lifestyle intervention, although difficult to sustain, remains the foundation of atherosclerosis treatment. Cardiac rehabilitation may be an underappreciated catalyst.
- A number of emerging agents are in varying stages of development and are targeting the full spectrum of atherosclerotic biology from lipids through to inflammation and thrombosis.
- Studies of emerging agents have re-emphasized the presence of a number of high risk groups including those with peripheral arterial disease and those who have sustained recent events.
- Risk assessment is likely to aid in determining how to use emerging agents: highest risk patients are likely to derive greatest overall benefit. Current risk assessment tools, however, are limited and may not represent key subgroups

4.1 Introduction

Atherosclerotic cardiovascular disease (ASCVD) continues to be a major global public health challenge. Despite therapeutic advances and their associated reduction in morbid and mortal cardiovascular events, ASCVD remains a leading cause of death and drain on health care expenditure worldwide. Many patients continue to experience clinical events, despite the use of evidenced-based therapies, supporting the need to identify additional strategies to achieve greater reductions in

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cardiovascular risk. Given the systemic nature of atherosclerosis, increasing evidence has demonstrated that the clinical benefits primarily targeted to reduce coronary events extend to other vascular territories. This has major implications for the development of guidelines for prevention of coronary, cerebrovascular and peripheral vascular disease.

4.2 Risk Stratification

Optimal approaches to cardiovascular prevention will require careful definition of the risk that an individual has of experiencing a clinical event. With an ageing population, lifelong risk of cardiovascular disease will ultimately increase. However, making decisions to intensify risk reduction strategies can be challenging. Early commencement of multiple medical therapies to reduce the risk of a clinical event many decades in the future is unlikely to be cost effective and unnecessarily exposes a large number of individuals to medications associated with side effects. Considerable work has been undertaken to more effectively determine the absolute risk that an individual has of experiencing a cardiovascular event, with those at the highest level of risk receiving more intensive therapy, as they are likely to derive the greatest absolute benefit from their use.

The patient with clinically manifest atherosclerotic disease, regardless of vascular territory involved, has the greatest absolute risk of a subsequent cardiovascular event. These are the patients typically enrolled in most clinical trials and receive the greatest clinical benefit from established therapies. Accordingly, the presence of symptomatic atherosclerotic cardiovascular disease identifies a patient who should receive intensive risk factor modification in prevention guidelines.

An increase in the number of evidence-based therapies is likely to have important consequences for both the health economy (payers) and for patients; greater pill burden, medication interactions and adverse drug reactions, inconvenience and potential out of pocket cost. These factors are likely to influence how these agents are best applied to derive net clinical benefit in the context of aggressive risk modification.

The association of concomitant risk factors, recurrent clinical events and presence of polyvascular disease (atherosclerotic disease in multiple arterial beds) are increasingly being integrated into clinical risk algorithms to determine how to more optimally risk stratify patients in secondary prevention [1].

Primary prevention of atherosclerotic cardiovascular disease presents a more challenging scenario. While lifelong risk of cardiovascular events across the population is considerable, the ability to predict risk in a more proximate (5–10 years) period has proven to be more difficult. Clinical risk calculators have been developed, which incorporate a range of factors, including age, gender, cholesterol, additional lipid parameters (triglycerides, high-density lipoprotein cholesterol [HDL-C], lipoprotein(a) [Lp(a)], blood pressure, diabetes, smoking, obesity and family history of premature atherosclerotic cardiovascular disease [2]. In general, where these

calculators determine a 10-year risk, they guide use of increasingly intensive therapy: lifestyle measures for those whose risk is less than 10%, intensive risk factor modification where risk greater than 20% and more tailored therapy for those between 10 and 20% [3].

However, the use of conventional risk prediction strategies in asymptomatic individuals has a number of challenges. They are derived from examination of large populations and their ability to accurately predict in specific individuals is limited. Some individuals determined to be low risk will experience clinical events, suggesting that other factors may underscore their atherosclerotic disease. Specific groups associated with increased cardiovascular risk, including patients with genetic dyslipidaemia, systemic inflammatory disease, human immunodeficiency virus (HIV) infection, end stage renal disease and indigenous populations [4–8], are not well represented by these calculators and often require early use of intensive risk factor modification. Patients with diabetes, for example, are considered a coronary risk equivalent [9], and are triaged to intensive medical therapy in prevention guidelines. These calculators estimate absolute risk yet provide no information with regard to modifiability of risk and cost effectiveness.

Development of a number of blood based and vascular imaging biomarkers have been reported to provide incremental risk prediction in asymptomatic individuals [10, 11], compared with conventional algorithms. While these have been incorporated into recent guidelines as adjunctive measures to predict risk [1], there have been limited clinical trials performed to determine how to use these investigations to optimise preventive therapies. The SCOT-HEART study has demonstrated that use of computed tomography coronary angiography to detect plaque triages appropriate use of statin therapy and reduces cardiovascular events on long term follow up [12]. In parallel, imaging studies may also play an important role in promotion of adherence with medical therapies [13]. Ultimately, the use of risk prediction strategies to tailor specific therapies to individual patients requires investigation in order to develop precision medicine approaches to cardiovascular prevention.

4.3 Lifestyle Measures

All approaches to the prevention of atherosclerotic cardiovascular disease should involve a foundation of lifestyle measures. Despite reductions in age-related mortality with use of evidence-based therapies in developed nations, the global spread of cardiovascular disease has resulted from an increase in obesity and associated metabolic risk factors, including early onset type 2 diabetes mellitus [14]. Accordingly, increasing efforts are required to promote lifestyle measures for prevention of incident cardiovascular disease along the life course of the disease process [15]. Similarly, in patients with established disease, use of intensive risk factor modification agents does not justify a lack of attention to lifestyle measures.

Smoking is a highly preventable cause of cardiovascular disease. Mechanistic studies have demonstrated a range of adverse effects on endothelial function,

thrombosis, inflammation and oxidative stress [16], which can be reversed with smoking cessation [17, 18]. While public health measures have led to a reduction in smoking rates in Western countries, there remains a considerable challenge worldwide [19]. A range of counselling and pharmacotherapy approaches have been employed in smoking cessation strategies for individuals. The adverse cardiovascular effects of smoking have been extended to the setting of secondary exposure [20]. As a result, this needs to be considered in developing individual prevention plans.

The increase in abdominal adiposity highlights the need to address diet and exercise in all approaches for cardiovascular disease prevention. In addition to caloric restriction, efforts to tailor dietary balance are of topical interest. Increasing consumption of highly processed foods, rich in carbohydrates, saturated fat and salt, associate with risk factors and premature cardiovascular disease [21–23]. Evidence that specific dietary interventions reduce cardiovascular events in large clinical trials in the contemporary era are lacking. Prevention guidelines, accordingly, emphasise a reduction in consumption of these dietary factors. In addition, 30–60 min of daily exercise have been demonstrated to have benefits on risk factor control and atherosclerotic plaque [24, 25], with no data to suggest incremental benefit from use of more intense or prolonged exercise regimens [26]. The potential for disease reversibility with diet and exercise interventions appears to be greatest in adolescents, suggesting more modifiable disease [27].

4.4 Anti-platelet and Anti-thrombotic Therapies

The seminal event underlying most ischemic events involves the formation of thrombus within the arterial lumen, secondary to either rupture or erosion of an atherosclerotic plaque. Accordingly, major advances in the treatment of acute and chronic ischaemic syndromes have been produced by the use of agents targeting platelet function, the coagulation cascade or dissolution of established clot. Early randomised controlled trials established that prompt administration of fibrinolytic agents in the setting of acute arterial occlusion led to rapid reperfusion of vascular territories and became standard of care for management of ST segment elevation myocardial infarction [28]. In many centres with rapid access to cardiac catheterisation laboratories, primary percutaneous coronary intervention has subsequently become the preferred treatment strategy for these patients [29]. Use of adjunctive antiplatelet therapy, primarily with aspirin, has proven to reduce both early and long term recurrent ischaemic events in patients with clinically manifest atherosclerotic disease. More recent studies have demonstrated incremental clinical benefit from use of dual antiplatelet therapy, typically in the form of aspirin combined with clopidogrel, prasugrel or ticagrelor, in high risk patients for at least 12 months after an acute event [30–32]. Increasing evidence suggests ongoing benefit with dual antiplatelet therapy beyond 12 months and in more stable patients with high risk features, such as the presence of peripheral arterial disease [33, 34]. The use of antiplatelet therapy in the primary prevention setting, however, appears to be less

clear. Meta-analyses and the results of recent clinical trials have failed to demonstrate clear cardiovascular benefit from widespread use of aspirin in patients without symptomatic disease [35, 36]. At this point in time, it would seem that use of aspirin for primary prevention is best reserved for specific high risk patients.

The use of anticoagulant therapy has evolved in the setting of atherosclerotic disease [37]. While early administration of heparin either in its unfractionated or low molecular weight formulations has been demonstrated to be efficacious as adjunctive therapy in patients with acute ischaemic syndromes [38], longer term use of warfarin has failed to produce consistent benefit in clinical trials [39]. The development of more novel anticoagulant agents, which primarily target factor X, have been increasingly used in combination with antiplatelet therapy in patients with concomitant atrial fibrillation and atherosclerotic disease, by virtue of greater ease of use compared with warfarin. Clinical trials have demonstrated these combinations to be highly effective and while associated with an increased risk of bleeding, provide a useful therapeutic approach in the early setting following an acute ischemic event [40–42]. Recent studies have also demonstrated that administration of low dose rivaroxaban reduced clinical events in patients with more stable, atherosclerotic disease [43], a benefit which seemed to be greater in higher risk patients such as those with manifest peripheral arterial disease [44]. While the mechanism underlying this benefit remains uncertain, the findings do suggest that patients may benefit long term from use of anticoagulant therapy in addition to antiplatelet agents to further reduce their risk of recurrent cardiovascular events.

4.5 Blood Pressure Therapies

Hypertension is a highly prevalent and modifiable risk factor, with evidence from large population studies that the curvilinear increase in risk is observed at systolic blood pressure levels within the range considered by many to be normal (i.e. 115 mmHg) [45]. Randomised controlled trials performed over a number of decades have confirmed that use of blood pressure lowering agents in patients with established hypertension, with systolic blood pressure levels greater than 160 mmHg, reduce cardiovascular event rates [46, 47]. This is further confirmed by meta-analyses which have demonstrated a linear relationship between both blood pressure lowering and achieved systolic blood pressure levels and cardiovascular benefit [48]. Accordingly, treatment guidelines for prevention of cardiovascular disease recommend blood pressure lowering with the target largely determined by the overall level of cardiovascular risk of the patient. More recent updates to treatment guidelines have advocated more intensive blood pressure lowering, aiming for a level below 130/80 mmHg in many higher risk patients [49]. To achieve such levels, many patients will require use of multiple blood pressure lowering agents. Conventional therapy often begins with use of either a calcium channel antagonist or pharmacological inhibitor of the renin-angiotensin-aldosterone system, with addition of the other class, beta-blockers or diuretics in combination. Patients with

very high levels of blood pressure may also require use of more centrally acting agents, although such patients are typically managed in specialist blood pressure clinics. Particular attention in the setting of such high and refractory levels of hypertension should be paid to be possibility of underlying secondary causes (e.g. renal artery stenosis, hyperaldosteronism, phaeochromocytoma, hyperthyroidism, kidney disease, Cushing's syndrome), which may require alternative interventions.

Additional approaches to management of refractory hypertension include the potential to disrupt renal sympathetic nerve supply by use of radiofrequency ablation therapy [50]. While early studies of this catheter based approach to treatment of patients with unacceptably high blood pressure levels, despite use of up to 4–5 agents, appeared promising with reports of effective blood pressure lowering when measured in the clinic, this did not prove to be the case in either 24 h blood pressure monitoring or in a large, sham controlled clinical trial [51]. Ongoing efforts are in progress to continue to advance effective approaches in this space in clinical studies.

There has also been considerable interest in the use of pharmacological agents to delay or prevent the development of hypertension. This is based on observations that cardiovascular risk begins to increase at levels not typically considered high enough to warrant therapy and due to the natural history, in which blood pressure tends to increase with age. While trials have demonstrated that early intervention does reduce the progression to hypertension in this setting, the overall clinical effect, both in terms of cardiovascular events and safety with the potential for symptomatic hypotension, remains uncertain. As a result, there is an ongoing need for clinical trials to determine optimal blood pressure levels to commence therapy in higher risk patients.

4.6 LDL Cholesterol Lowering Therapies

Considerable evidence has implicated low-density lipoprotein cholesterol (LDL-C) as a causal factor in atherosclerotic disease. Population studies demonstrate a curvilinear relationship between both LDL-C and apolipoprotein B (apoB) levels and cardiovascular risk. Genetic studies have established that polymorphisms producing lower LDL-C levels associate with less cardiovascular risk, the relationship being proportional to the difference in apoB levels [52]. Monogenic states of hypercholesterolemia are well characterized by a greater incidence of premature cardiovascular disease. Numerous clinical trials have established that lowering LDL-C with statins favourably modifies progression of atherosclerosis and reduces cardiovascular events in the primary and secondary prevention setting [53, 54]. The degree of benefit directly associates with the extent of LDL-C lowering and the greatest absolute risk reduction is observed in those patients at highest baseline risk of experiencing a cardiovascular event. While posthoc analyses of statin trials demonstrated an independent association between lowering of the inflammatory marker, C-reactive protein (CRP), and benefit, suggesting potential pleiotropic effects, the clinical significance remains uncertain [55]. This may contribute to findings that high

intensity statin therapy has an early clinical benefit in patients with acute coronary syndromes, which has provided the evidence for guideline-based management in the hospital setting.

However, a number of challenges remain despite the widespread use of statins for prevention of atherosclerotic cardiovascular disease. A substantial residual risk of clinical events is observed, even in patients treated with high intensity statin therapy or achieving current guideline targets. This suggests that additional strategies, including greater lowering of LDL-C, may be required in these patients. Many patients, particularly those with genetic hypercholesterolemia or those unable to tolerate high statin doses, are unable to attain treatment goals. These factors, in addition to reductions in adherence on long term follow up, will contribute to ongoing cardiovascular risk in patients and the need to develop new lipid lowering therapies [56].

Ezetimibe is a cholesterol absorption inhibitor, which lowers LDL-C by 15–20% as monotherapy or in combination with statins. Clinical trials have demonstrated that when used in combination with statins, ezetimibe produces incremental plaque regression [57] and reductions in cardiovascular events [58]. Proprotein convertase subtilisin/kexin type 9 (PCSK9) plays an important role in regulation of LDL-C, through its role in degradation of the LDL receptor in the liver. Gain of function PCSK9 mutations have been identified in some patients with familial hypercholesterolemia, while loss of function polymorphisms have been demonstrated to associate with lower levels of both LDL-C and cardiovascular risk. Development of monoclonal antibodies, administered subcutaneously every 2–4 weeks, has been demonstrated to be well tolerated, to lower LDL-C by up to 60% on top of statin therapy and reduce cardiovascular event rates in large clinical trials. The clinical benefit of these agents appears to be greatest in patients with either the highest baseline LDL-C levels or those at highest risk of future cardiovascular events, including those with atherosclerosis involving multiple vascular territories, multivessel coronary disease and recurrent ischaemic events [59]. Given the high cost of these agents, considerable efforts are underway to determine how to optimally triage their use to patients where their cost effectiveness will be greatest. Alternative approaches to PCSK9 inhibition involve impairing RNA synthesis within the liver, which will enable more durable biochemical effects and potentially much less frequent administration. Clinical trials of the RNA inhibitor, inclisiran, administered twice yearly are currently in progress to determine its clinical efficacy and safety [60]. An additional oral agent, bempedoic acid is being developed primarily for patients with statin intolerance. This agent reduces cholesterol biosynthesis in the liver, in a manner similar to statins, yet is not biologically active within muscle and theoretically may produce less myalgia, which can be problematic for many statin-treated patients. While the degree of LDL-C lowering is relatively modest (15–25%) [61], when combined with ezetimibe and potentially low doses of statins, this agent may have the potential to produce highly effective LDL-C reductions. The clinical efficacy and safety of bempedoic acid is currently being investigated in a large clinical trial of high cardiovascular risk patients with established statin intolerance.

4.7 Additional Lipid Modifying Therapies

While a generation of clinical development has produced a range of cardioprotective agents targeting LDL-C, there continues to be interest in use of therapies that modulate other circulating lipoproteins. Population and animal studies have suggested that high-density lipoproteins (HDL) are protective [62]. Accordingly, there has been considerable interest in development of HDL raising agents [63]. However, multiple clinical trials involving novel formulations of niacin and cholesteryl ester transfer protein (CETP) have proven to be disappointing, despite substantial HDL-C elevation [64]. This is supported by observations from genetic studies that polymorphisms that produce differences in HDL-C levels do not associate with alterations in cardiovascular risk. In contrast, multiple studies have suggested that the functionality of HDL, as opposed to quantitative measures of its cholesterol content, may be a more important determinant of cardiovascular risk. This is evidenced by reports that greater ex vivo cholesterol efflux capacity associates with protection from incident cardiovascular events [65]. Trials utilising HDL infusions, which stimulate cholesterol efflux, without any discernible long term increase in HDL-C levels, have proven to exert variable effects on plaque and are currently being investigated for their impact on cardiovascular events [66].

In parallel, the role of triglyceride rich lipoproteins (TRLs) in cardiovascular disease has varied. Contemporary analysis on the basis of both population and genetic studies implicates TRLs in the causal pathway for atherosclerosis. This is particularly important as hypertriglyceridemia is highly prevalent in the settings of abdominal obesity and type 2 diabetes, with elevations in both fasting and postprandial TRL levels. In the absence of evidence that triglyceride lowering results in cardiovascular protection, treatment guidelines have largely focused on use of more intensive LDL-C lowering strategies in the patient with hypertriglyceridemia. A number of observations provide some insight for use of approaches beyond LDL-C lowering in the patient with elevated triglyceride levels. Fibrates have a range of effects including lowering of triglycerides and LDL-C and elevation of HDL-C. While agents have produced variable effects on cardiovascular events in large outcomes trials, meta-analyses have demonstrated that any potential benefit of these agents is predominantly observed in the patient with baseline hypertriglyceridemia [67]. This has prompted the design of clinical trials that specifically target high risk patients with elevated triglyceride levels. Administration of high dose omega-3 fatty acids, in the form of eicosapentaenoic acid, has been demonstrated to reduce cardiovascular event rates in patients with hypertriglyceridemia at study entry [68]. This finding contrasts with prior data of omega-3 fatty acids, in which administration of low doses that failed to substantially elevate tissue levels, to patients with normal triglyceride levels, did not reduce cardiovascular risk. Ongoing studies of other omega-3 fatty acid preparations [69] and selective peroxisome proliferator activated receptor modulators in similar patients will determine whether a number of strategies will be effective when tailored to patients with a specific atherogenic dyslipidemia phenotype. Future studies of agents targeted to inhibit factors that impair

metabolism of TRLs will determine whether directly lowering triglyceride levels will be atheroprotective.

Lipoprotein (a) [Lp(a)] has received increasing attention as an individual lipid target in cardiovascular prevention [70]. This atherogenic lipoprotein has a structure including an apoB particle bound to apo(a) with considerable homology to plasminogen. Accordingly, this lipoprotein is thought to play an important role in atherogenesis, calcification and thrombosis. Lp(a) levels are genetically regulated with increasing evidence from Mendelian randomization studies that Lp(a) plays a causal role in both atherosclerotic disease and calcific aortic stenosis [71]. While statins do not lower Lp(a) and in some cases can increase its levels, use of high intensity statin therapy is advocated in high risk patients with elevated Lp(a) levels in efforts to reduce LDL-C levels as low as possible. Existing therapies that lower Lp(a) include niacin and oestrogen, although these agents have failed to produce cardiovascular benefit in contemporary clinical trials. PCSK9 inhibitors lower Lp(a) by up to 30%, with some evidence that this contributes to their cardiovascular benefit [72]. However, increasing interest has focused on the development of specific therapies that reduce Lp(a) synthesis, with several of these agents proceeding in clinical development. Given the role of Lp(a) in risk stratification and additional ability to identify patients with familial hypercholesterolemia, there is increasing support for widespread measurement of Lp(a) levels in clinical practice, in order to determine which patients require more aggressive risk factor modification.

4.8 Glucose Lowering Therapies

With an increasing prevalence of obesity, type 2 diabetes has become a global public health challenge and is a major factor underscoring the worldwide spread of premature cardiovascular disease. In addition to its high prevalence in patients with manifest atherosclerotic disease, the presence of diabetes associates with adverse outcomes and the presence of diffuse, systemic atherosclerotic disease [14]. Despite the clear association between dysglycaemia and cardiovascular risk [73], clinical trials of glucose lowering therapies for decades failed to demonstrate macrovascular benefit. With reports of potential cardiovascular harm with the PPAR-γ agonist, rosiglitazone, the regulatory requirements for approval of novel glucose lowering agents has changed, with the need to demonstrate cardiovascular safety in larger trials. This has paved the way for a large number of clinical trials of agents, which not only have proven to be safe, but have finally demonstrated cardiovascular benefit.

The sodium glucose cotransporter-2 (SGLT2) inhibitors act primarily via urinary excretion of excess glucose and are highly effective at improving glycaemic control in the setting of type 2 diabetes. Large outcomes trials of three agents have demonstrated cardiovascular benefit [74]. While the benefits in these trials have variably involved mortality, heart failure and atherosclerotic specific events, the reduction in fluid volume and improvement in cardiac dimensions suggests that this may be the

major mechanism underlying their benefit. This is being directly tested in the settings of heart failure with either reduced or preserved ejection fraction. Additional data has subsequently reduced progression to adverse renal outcomes, suggesting widespread benefit [75]. Caution should be taken in patients with a history of recurrent urinary or genital tract infection and a small, but significant increase in the rate of ketoacidosis and in the case of canagliflozin, lower limb amputations, has been reported. The latter suggests that caution should be taken with use of these agents with prior amputation or severe small vessel disease involving the extremities.

Glucagon-like peptide-1 (GLP-1) receptor agonists have also been demonstrated to improve glycaemic control, in addition to promoting weight loss by up to 10%, when administered via subcutaneous injection. Cardiovascular outcomes trials have demonstrated a reduction in clinical events with a number of these agents [76]. The major tolerance issue observed with these agents involves the development of nausea. More recent developments include the ability to administer GLP-1 receptor agonists orally and in combination with glucose-dependent insulinotropic polypeptide receptor agonists, the latter having the potential to achieve profound lowering of glycated haemoglobin by more than 2% [77]. The clinical impact of these advances remain to be determined in large clinical trials. As a result of these findings, treatment guidelines for management of high-risk atherosclerotic disease patients with type 2 diabetes advocates use of additional glucose lowering agents (preferentially SGLT2 inhibitors or GLP-1 receptor agonists given their cardiovascular benefit) in combination with background metformin in patients with glycated haemoglobin levels greater than 7% [78]. Whether administration of these agents will produce cardiovascular benefit when used in patients with better glycaemic control remains to be determined.

4.9 Anti-Inflammatory Therapies

Increasing evidence implicates inflammation at all stages of the atherosclerotic disease process. This is supported by mechanistic observations of plaque formation, progression and rupture and reports that greater circulating levels of inflammatory markers associate with prospective cardiovascular risk. The report that lowering C-reactive protein (CRP) levels independently associates with the benefits of statins suggests that anti-inflammatory properties may contribute to their cardiovascular benefit. As a result of these findings, considerable efforts have been undertaken to develop novel therapeutic approaches that primarily target the inflammatory nature of atherosclerosis. Early studies that have targeted specific downstream mediators of inflammation (phospholipase inhibitors, lipoxygenase inhibitors) have failed to reduce cardiovascular event rates or favourably modulate atherosclerotic plaque.

More recent efforts with agents that target the role of the inflammasome, a more upstream coordinator of the inflammatory cascade within the artery wall, have yielded promising results. A small study of patients with stable coronary artery disease demonstrated a clinical benefit with administration of low-dose colchicine [79].

The potential cardiovascular benefits of this agent have been further demonstrated via reports of favourable effects on plaque imaging and are being evaluated in large prospective studies. Cannakinumab is an interleukin-1β antagonist, thought to play a role in modulating inflammasome activity. A large cardiovascular outcomes trial of statin-treated patients with elevated CRP levels demonstrated that administration of canakinumab reduced the incidence of cardiovascular events, albeit with a predictable excess in infection [80]. In parallel, a reduction in lung cancer was observed, underscoring the significant burden of smoking related lung disease in patients with clinically manifest atherosclerotic disease. The findings of this study provide the first, large scale validation that specifically targeting inflammation can have a protective benefit in terms of reducing residual risk beyond statin therapy.

4.10 Obesity Targeted Therapies

The rising global prevalence of cardiometabolic risk factors parallels the spread of abdominal obesity. Efforts to curb obesity have the potential to reduce both associated risk factors, but more importantly cardiovascular complications. When introduced at an early stage in life, weight loss has been demonstrated to result in regression of early changes within the vessel wall. The benefits on cardiovascular risk in older individuals has proven more challenging to demonstrate. While improving the cardiac risk factor profile, weight loss strategies have not yet proven to reduce cardiovascular event rates in large outcomes trials. This is further supported by use of a range of pharmacological agents, specifically designed to achieve weight loss, in which a lack of clinical benefit, and in some circumstances, an excess in adverse events has been observed. Whether the weight loss and observed improvement in risk factor profile with GLP-1 receptor agonists produces clinical benefit in the setting of overweight or obesity, but not diabetes, remains to be determined in clinical trials. An additional approach involves the use of surgical interventions for extreme levels of obesity. While use of lap band techniques have not proven to be successful. More extensive techniques involving Roux-en-Y gastric bypass or sleeve gastrectomy produce robust and durable metabolic benefits, which may have a greater potential to translate to less cardiovascular events [81].

Beyond its effects on conventional metabolic risk factors, obesity is also associated with an excess rate of sleep disordered breathing [82]. Numerous reports have demonstrated an increase in cardiovascular risk with worsening degrees of obstructive sleep apnoea. A range of mechanisms may underscore this association leading to potentially an increase in atherosclerotic, heart failure and arrhythmia related events. While there is considerable interest in the potential role for sleep apnoea targeted interventions to reduce vascular events, the one large outcomes trial performed to date in this space failed to demonstrate a reduction in cardiovascular risk [83]. Whether specific triage of patients and dedicated efforts to promoting greater adherence to therapy during the night results in a greater chance of success remains to be determined.

4.11 Cardiac Rehabilitation

With a large body of evidence supporting both lifestyle and pharmacologic approaches to secondary prevention of atherosclerotic cardiovascular disease, there has been increasing interest in the provision of rehabilitation services to patients, particularly in the early weeks following an acute ischaemic event. This has the potential to reinforce information for patients about living with cardiovascular disease and the rationale for ongoing compliance with preventive therapies. Such clinics have proven to result in greater adherence with therapy, better risk factor control and several reports of potential reductions in recurrent clinical events [84, 85]. More efforts are required to determine how to maximise a patient's chance of attending and completing these programs, which are likely to be achieved by offering services in a range of formats and attempts to integrate digital technologies into the long term monitoring of a patient's risk factor control.

4.12 Conclusion

Atherosclerotic cardiovascular disease continues to present a major challenge throughout the world. While advances in lifestyle and pharmacologic approaches have made a substantial impact in reducing risk, many patients are either not offered the full complement of established therapies or their adherence with these approaches declines over time. There are ongoing efforts to develop new therapies and to maximise use of established approaches in the patients that are most likely to benefit from their use.

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Chapter 5
Pathophysiology of Angiogenesis and Its Role in Vascular Disease

Nathan K. P. Wong, Emma L. Solly, Christina A. Bursill, Joanne T. M. Tan, and Martin K. C. Ng

Key Learning Points

- Angiogenesis is the process of new blood vessel formation from pre-existing vessels that is critical for growth and development and in response to tissue ischaemia such as that seen during a myocardial infarction or in peripheral artery disease.
- Uncontrolled angiogenesis is a key contributor to the development and progression of malignant cancers and atherosclerotic plaque formation. Angiogenesis-associated diseases are the leading causes of mortality and morbidity worldwide. Impaired angiogenic responses underpin the mechanisms associated with diabetes- and age-related vascular complications.
- The intricate balance between desirable physiological angiogenesis and unwanted pathological angiogenesis involves the regulation of a suite of signalling

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pathways, regulatory factors and cell-to-cell interactions. Physiological angiogenesis is primarily mediated by the hypoxia transcription factor HIF-1α while pathological angiogenesis is driven by the inflammatory transcription factor NFκB. Numerous angiogenic mediators can be driven by both hypoxia and inflammation.

5.1 Introduction

The human circulatory system is comprised of a complex branching network of blood vessels designed to transport oxygen and nutrients to cells, remove waste products and facilitate immune surveillance. Given its diverse functions, this vascular network must be responsive and capable of adapting to a range of tissue microenvironments, stressors and changing metabolic demands. In the physiologic state, this is achieved through dynamic yet highly coordinated processes of blood vessel growth and remodelling, which are under the balanced control of both stimulating and inhibiting factors. In disease states, however, these processes are frequently dysregulated, with inadequate, excessive or abnormal vessel growth potentially leading to a broad range of clinical pathologies [1].

Angiogenesis refers to the process in which new blood vessels, in particular capillaries, are formed from the pre-existing vascular network. Angiogenesis is distinguished from vasculogenesis, which refers to the assembly of a primary vascular plexus, typically in the developing embryo, that arises *de novo* from the differentiation of mesoderm-derived precursors called angioblasts [2]. Recent decades of research have begun to reveal some of the cellular and molecular mechanisms that underpin the contribution of angiogenesis to the pathophysiology of vascular diseases. Pro-angiogenic stimuli activate endothelial cells (ECs) to detach from their basement membranes, then migrate and proliferate to form branching tubular structures, driving the sprouting of new capillaries from the primary plexus [2, 3]. Further remodelling involves the recruitment of mural cells such as vascular smooth muscle cells (VSMCs) and pericytes, as well as the laying down of extracellular matrix (ECM) to provide structural stability and facilitate vessel maturation [3]. This process continues until pro-angiogenic cues subside or are inhibited, at which point vessel growth becomes quiescent and anti-angiogenic factors predominate.

It is well recognised that angiogenesis is a critical process in normal postnatal growth and development. It is crucial in providing nourishment to granulation tissue during wound healing, as well as in the formation of collateral vessels as part of an adaptive response to vascular occlusion and ischaemia [4]. The failure of adequate angiogenesis plays an important role in conditions such as ischaemic heart disease, peripheral arterial disease (PAD), delayed wound healing and ischaemic stroke. Conversely, excessive pathological angiogenesis driven by inflammation is a key contributor to the development and progression of malignant cancers, atherosclerotic plaques, proliferative retinal disease and inflammatory arthritides, as well as many other pathologies [5]. A detailed and holistic understanding of the factors

which promote or suppress angiogenesis in these contexts could prove therapeutically useful. Indeed, several pharmacological, gene- and cell-based approaches for modulating angiogenesis have been trialled for various vascular conditions, though with mixed success to date [6].

In this chapter, we outline a basic mechanistic framework of the process of angiogenesis and summarise the key molecular factors that regulate it, particularly those that have current or potential clinical relevance. We will highlight the importance of angiogenesis in health, as well as in the pathophysiology of various ischaemic and inflammatory vascular diseases. In addition, we review the current range of therapeutic strategies that are designed to target angiogenesis and examine the barriers and pitfalls that have limited more successful clinical translation so far. Finally, we explore several emerging therapies that are showing great promise in pre-clinical models and offer some insights on future directions of research inquiry.

5.2 Basic Mechanisms of Angiogenesis

We will first overview the cellular mechanisms that underlie blood vessel formation and the key molecular factors that are known to regulate it. Primitive blood vessels are first formed through vasculogenesis, a process that occurs most prominently during embryonic development. Vasculogenesis involves the differentiation of mesoderm-derived angioblasts into ECs, which then establish a primary vascular plexus [2, 7]. Animal models suggest that this process requires a threshold level of vascular endothelial growth factor (VEGF)A expression from the embryonic endoderm [2]. VEGFA is the most well-characterised of all known vascular growth factors. It binds to and activates tyrosine kinase VEGF receptors (VEGFR)1 and VEGFR2, stimulating them to dimerise and become autophosphorylated to initiate intracellular signalling transduction [8]. During vasculogenesis, VEGFA acts in a paracrine manner on VEGFR2 on the surface of angioblasts to induce their differentiation [2]. The resulting ECs proliferate and assemble into primitive vascular cords that undergo differentiation to form arteries or veins [3]. Following vasculogenesis, new blood vessels arise from the pre-existing network through two major mechanisms: sprouting and intussusceptive (or non-sprouting) angiogenesis (Fig. 5.1).

5.2.1 Sprouting Angiogenesis

Vessel sprouting is the better characterised “classical” form of angiogenesis that typically occurs in the early stages of development, though intussusceptive angiogenesis can occur concurrently [2]. In response to VEGFA and other pro-angiogenic factors which are predominantly triggered by hypoxia, ECs are activated to release a range of proteolytic enzymes, particularly matrix metalloproteases (MMPs), that initiate breakdown of the underlying basement membrane [2, 3, 8]. These facilitate

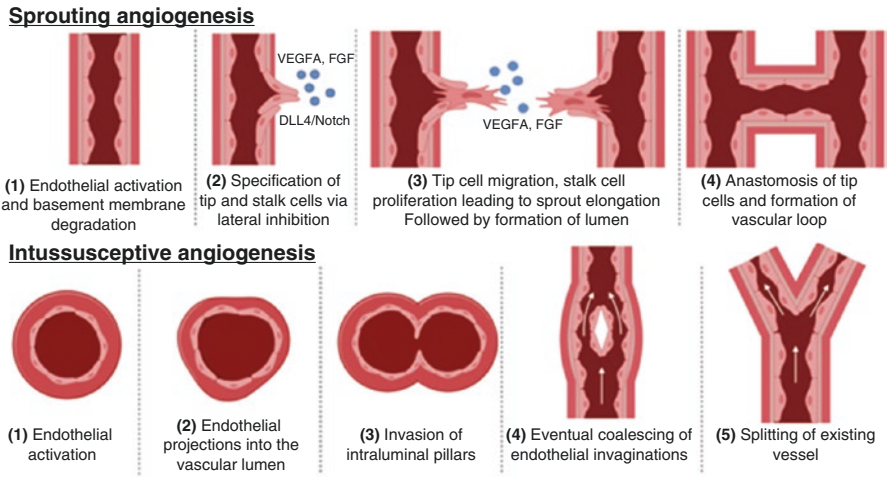

Fig. 5.1 Sprouting and intussusceptive angiogenesis. New blood vessels can form from pre-existing vessels by either sprouting angiogenesis or intussusceptive angiogenesis. Sprouting angiogenesis occurs in response to VEGFA and other pro-angiogenic factors, triggering the activation of endothelial cells (ECs) to release a range of proteolytic enzymes, that initiate basement membrane degradation. Migrating ECs then become either tip cells, which continue to direct sprout outgrowth or stalk cells which proliferate behind tip cells to support sprout elongation. Vascular loops are then formed when the tip cells anastomose. Intussusceptive angiogenesis involves the splitting of existing perfused blood vessels by intraluminal tissue pillars. These are formed as ECs undergo morphological rearrangement of their cellular junctions resulting in endothelial invaginations that extend into the vascular lumen. These protrusions usually develop on opposite sides of the vessel wall and eventually coalesce. Expansion of the intraluminal pillar ultimately results in vessel duplication as the parent vessel is split into two new capillaries

the detachment and chemotactic migration of ECs, pericytes and VSMCs towards angiogenic cues. Migrating ECs are then specified to become either "tip cells", which spearhead new sprouts via motile invasive filopodia, or "stalk cells" which proliferate behind tip cells to support sprout elongation [3, 7]. The specification of tip cells and stalk cells is dynamic and occurs via competitive lateral inhibition. Activation of VEGFR2 by VEGFA induces the expression of delta-like ligand 4 (DLL4) and the ECs that express this most efficiently are specified as tip cells [3]. In adjacent ECs, DLL4 acts on Notch receptors, leading to downregulation of VEGFR2 and upregulation of VEGFR1, which inhibits tip cell-like behaviour and instead promotes a stalk cell phenotype [3]. Tip cells continue to direct sprout outgrowth by probing for both attractive and repulsive guidance cues; several classes of ligand-receptor interactions are implicated in this such as ephrins which bind Eph receptors and semaphorins which activate neuropilin receptors [3, 6, 7]. Meanwhile, stalk cells undergo proliferation to extend the sprouts, eventually forming a lumen. This commonly occurs as cords of ECs become morphologically modified and flatten out to open up a lumen (cord hollowing), or via the development of pinocytic intracellular vacuoles which coalesce in adjacent ECs to form a lumen (cell hollowing) [3, 7].

The sprouting process continues through a dynamic balance of VEGFA/VEGFR2 and DLL4/Notch signalling. Eventually, the filopodia of adjacent tip cells interact and anastomose with each other to form a vascular loop, strengthened by intercellular junctions comprised of vascular endothelial (VE)-cadherins [3]. These proteins provide structural stability but also have a crucial role in maintaining EC survival [8]. Stalk cells also gradually transform into "phalanx cells", a monolayer of ECs with reduced proliferative capacity, that re-establish the basement membrane and form tight junctions [3]. To develop functional vessels, VSMCs and pericytes must also be recruited to the vessel wall to facilitate vessel stabilisation, maturation and deposition of ECM. This process is regulated by transforming growth factor (TGF)-β signalling as well as platelet-derived growth factor (PDGF)B released from ECs [3]. PDGFB activates PDGF receptor (PDGFR)β on VSMCs and pericytes, stimulating their migration, proliferation and incorporation into the vascular wall. These mural cells also produce angiopoietins (Ang), Ang-1 and Ang-2, which bind to tyrosine kinase with immunoglobulin-like and epidermal growth factor-like domain (TIE)-2 receptors on ECs [3, 7, 8]. In mouse models, deficiency of TIE receptors is embryonically lethal [2]. Ang-1 promotes pericyte adhesion and the maintenance of vascular barrier function by stabilising inter-endothelial junctions, while Ang-2 partially antagonises these effects leading to relative vascular permeability [3, 7, 8].

As angiogenic cues subside, further vascular remodelling is regulated by mechanical and metabolic influences. The initiation of blood flow in these new vessels exposes ECs to shear stress, activating PDGFB/PDGFRβ signalling pathways and transcription factors such as Krüppel-like factor (KLF)2 that promote luminal patency and contribute to adaptive remodelling [3]. ECs that line well-perfused vessels are maintained and become quiescent, while hypoperfused vessels conversely undergo regression. The delivery of adequate oxygen and nutrients to ECs also helps to establish quiescence by reducing both glycolytic and oxidative metabolism [9]. The prolyl hydroxylase domain (PHD) proteins use available oxygen to suppress hypoxia-inducible factors (HIFs), leading to reduced expression of VEGFA and other pro-angiogenic factors [4]. Vascular integrity is further maintained by strengthening cell-cell junctions and cell-ECM adhesion, as promoted by fibroblast growth factors (FGF) including FGF1 and FGF2, Ang-1, and receptors for various ECM components called integrins [3, 8]. EC survival is also maintained by intracrine (internal) VEGFA signalling, which induces the expression of the anti-apoptotic factor B-cell lymphoma (BCL)-2 [3].

5.2.2 Intussusceptive Angiogenesis

Non-sprouting or intussusceptive angiogenesis (IA) is also an important, yet often under-recognised, mode of vessel growth. Its primary function appears to be remodelling and pruning of vascular networks that were previously formed by vasculogenesis or sprouting angiogenesis [10, 11]. As opposed to the outgrowth of blind-ended capillary sprouts that are not initially perfused, the defining feature of IA is the

splitting of existing perfused blood vessels by intraluminal tissue pillars. These are formed as ECs undergo morphological rearrangement of their cellular junctions resulting in endothelial invaginations that extend into the vascular lumen [10]. These protrusions usually develop on opposite sides of the vessel wall and eventually coalesce as they are invaded by pericytes and myofibroblasts which deposit collagen-rich ECM. Expansion of the intraluminal pillar ultimately results in vessel duplication as the parent vessel is split into two new capillaries or, if it occurs at a point of bifurcation, the branching angle of the vessel may be altered [11]. Asymmetric pillar growth can also lead to pruning and regression of redundant vessels [11]. In contrast to sprout growth, the basement membrane remains intact during IA and ECs do not undergo significant migration or proliferation [10]. IA can therefore occur more rapidly and expends less metabolic energy compared to sprouting.

Although intussusception contributes substantially to angiogenesis in a variety of tissues under both physiological and pathological settings, the exact molecular factors that initiate and maintain it are less well understood due to a lack of appropriate experimental models. Unlike sprouting angiogenesis, the importance of VEGFA and VEGFR2 is ambiguous, as some studies suggest that VEGFA has a necessary role in IA, while others show that VEGFA is in fact downregulated and selective blockade of VEGFR2 does not significantly affect the occurrence of IA [10, 12, 13]. It may be that other VEGF isoforms are responsible, or VEGFA may be binding to alternative receptors such as VEGFR1. Other angiogenic factors, however, may also be important such as Ang-1 and their TIE-2 receptors. Targeted TIE-2 deletion in mice has been found to compromise pillar formation [14], while Ang-1 over-expression produces enlarged vessels with numerous small invaginations, a phenotype that resembles IA [10, 15]. FGF2 is also thought to stimulate PDGFB and PDGFRβ expression leading to the recruitment of pericytes into the intraluminal pillars during IA [10]. Beyond these factors, haemodynamic forces such as shear stress and cyclic stretch are also implicated in the remodelling of vascular networks through IA. This is suggested by observations that the extent of IA in chick chorioallantoic membranes *in vivo* was enhanced in response to increased blood flow induced experimentally by clamping off side branches [16].

A more detailed understanding of the occurrence, mechanisms and regulation of IA is clearly necessary to provide insight into its role in health and disease. This may inform the development of therapies directed specifically at this common, yet often overlooked, form of angiogenesis.

5.3 Angiogenesis in Health and Disease

Having reviewed the basic cellular and molecular mechanisms underlying sprouting and non-sprouting vessel growth, we will now overview pertinent aspects of angiogenesis and its regulation in various physiological and pathological contexts. The principal drivers of angiogenesis include hypoxia and inflammatory stimuli, and these can interact significantly through overlapping

pathways. Inhibitors of angiogenesis provide crucial counter-balances to prevent excessive angiogenesis, and these may be lost in pathological conditions.

5.3.1 *Physiological Angiogenesis*

In health, angiogenesis is an important contributor to normal homeostatic processes, such as growth and development, the menstrual cycle, wound healing and the adaptive response to ischaemia. Hypoxia is a potent trigger for angiogenesis and typically may occur due to vascular occlusion or when an expanding tissue mass outgrows its blood supply. Under such conditions, the key transcription factor HIF-1α is activated and serves as the master regulator of downstream signalling, upregulating the expression of numerous pro-angiogenic genes with promoter regions containing hypoxia-response elements (Fig. 5.2) [4]. These include VEGFA,

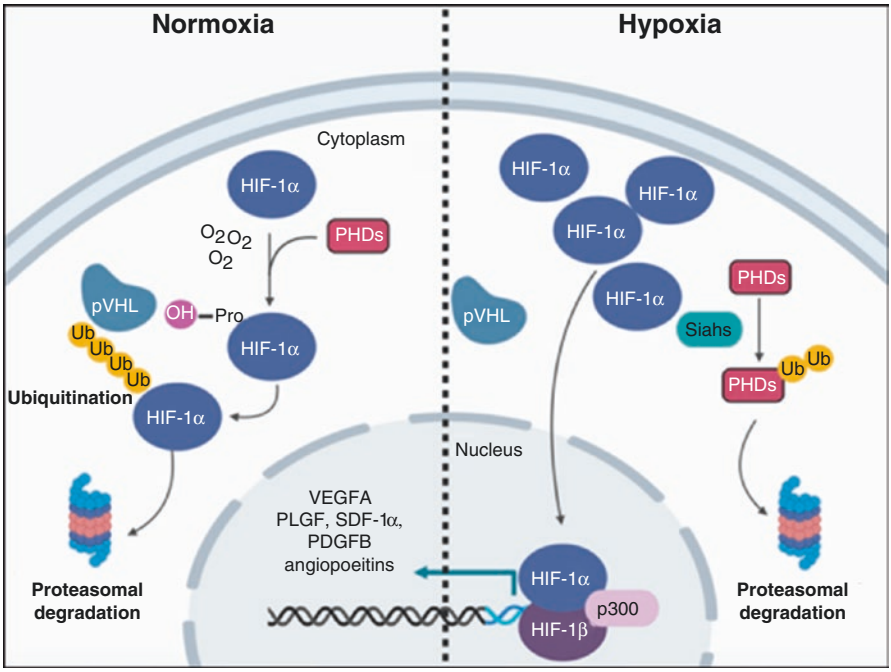

Fig. 5.2 Key hypoxia-driven cellular mechanisms that drive physiological angiogenesis. The hypoxia-driven transcription factor, HIF-1α is the master regulator of physiological angiogenesis. In normoxia, HIF-1α is inhibited post-translationally by the PHD proteins, which use oxygen as a co-substrate to hydroxylate proline residues on HIF-1α, targeting HIF-1α for ubiquitination and proteasomal degradation by the von Hippel-Lindau (VHL) complex. In hypoxia, transcriptional activation of the ubiquitin ligases Siahs target and degrade the PHDs. This prevents HIF-1α from being degraded, allowing it to accumulate and translocate into the nucleus where it binds and activates numerous pro-angiogenic genes including VEGFA, PLGF, SDF-1α, PDGFB and angiopoietins

the angiopoietins, a homologue of VEGFA called placental growth factor (PLGF), stromal-derived growth factor (SDF)-1α and PDGFB [4, 17]. In normoxia, the activity of HIF-1α is inhibited post-translationally by the PHD proteins, which use oxygen as a co-substrate to hydroxylate proline residues on HIF-1α [4]. This enables HIF-1α to be recognised by the von Hippel-Lindau ubiquitin ligase complex, which then targets it for ubiquitination and proteasomal degradation [17]. A drop in intracellular oxygen conversely leads to transcriptional activation of Siah1 and Siah2, ubiquitin ligases which target and degrade the PHDs, thereby relieving the inhibition of HIF-1α and promoting its stability [18]. HIF-1α is then able to promote the expression of proteins, particularly VEGFA and SDF-1α, which induce angiogenesis locally and/or stimulate postnatal vasculogenesis via the mobilisation of precursor cells including mesenchymal stem cells and endothelial progenitor cells (EPCs) from bone marrow [4, 17]. These mechanisms are crucial in supporting the adaptive hypertrophic response of skeletal muscle to exercise training. They also underpin the development of a collateral circulation that can help to salvage ischaemic tissue, maintain its function and delay symptom onset and progression. This is particularly pertinent in the context of angina due to ischaemic myocardium in coronary artery disease (CAD), claudication from the compromised vascular supply to skeletal muscle in PAD, and neurological deficits from ischaemic neuronal or glial cells in stroke and vascular dementia.

Wound healing represents yet another critical function of angiogenesis in health. The rapid expansion of a dense capillary network provides the necessary substrates to support tissue repair and regeneration. Once wound closure is complete, angiogenesis is inhibited, and these vessels must be pruned back and remodelled into a mature network [19]. Though traditionally viewed as a process occurring along with granulation tissue formation during the proliferative phase, angiogenesis is in fact closely linked with all stages of wound healing [20]. A number of pro-angiogenic factors are stimulated in the initial haemostatic phase. Thrombin directly upregulates receptors for VEGFA on ECs while also inducing the release of MMPs that initiate basement membrane degradation [20]. Tissue injury also liberates factors such as FGF2 sequestered within the ECM and activated platelets release various mediators including VEGFA, Ang-1, PDGFB and TGF-β [20, 21]. The hypoxic gradient between healthy and injured tissue also enhances HIF-1α signalling to promote angiogenesis [4]. In the inflammatory phase, vascular permeability is increased in response to cyclooxygenase (COX)2 [20]. This facilitates the recruitment of peripheral blood monocytes into damaged tissue and their differentiation to macrophages, which further amplifies the release of pro-angiogenic factors. During the proliferative phase from about 3–5 days post injury, the neovascular network starts to form in earnest through sprouting and non-sprouting angiogenesis [21]. These new vessels support the migration of macrophages and fibroblasts into the wound space, forming granulation tissue through the deposition of ECM rich in fibrin, fibronectin and vitronectin as regulated by TGF-β signalling [21]. In turn, endothelial tip cells interact with these ECM components by increasing their surface expression of $\alpha_v\beta_3$ integrins, helping to direct EC invasion through the ECM and expansion of the capillary network [21]. As normoxic conditions return and the acute

inflammation subsides, inhibitors of angiogenesis predominate, and the wound matures as fibrinous ECM is gradually replaced by collagen [21]. ECM components also give rise to many endogenous inhibitors of angiogenesis such as thrombospondin-1, the tissue inhibitors of MMPs, angiostatin, tumstatin and endostatin, the latter two being cleavage products of collagen types IV and XVIII respectively [6, 20]. These factors ensure that angiogenesis does not persist unnecessarily, allowing for excessive redundant vessels to be pruned through EC apoptosis as the wound undergoes continuous remodelling [21].

5.3.2 *Pathological Angiogenesis*

In contrast to the critical role of angiogenesis in development, ischaemia and wound healing, maladaptive and excessive angiogenesis are hallmarks of various pathological chronic inflammatory states. The molecular signalling pathways involved in inflammation are known to be intimately linked with the activation of angiogenesis (Fig. 5.3). In turn, these new vessels help to sustain the inflammatory response as they provide additional conduits for the delivery of immune cells and cytokines. In

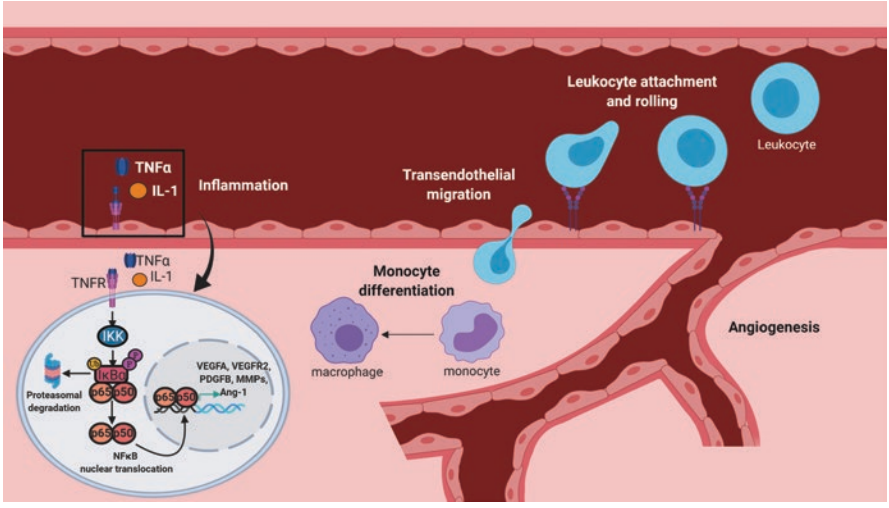

Fig. 5.3 Key inflammatory-driven cellular mechanisms that drive pathological angiogenesis. In response to local inflammatory stimuli such as TNFα and IL-1, the key inflammatory-driven transcription factor NFκB is activated in ECs which upregulate the expression of cell surface adhesion molecules that facilitate the tethering and rolling of leukocytes along the vascular endothelium. Within inflamed tissue, circulating monocytes differentiate into macrophages and produce a myriad of mediators that can directly and indirectly promote angiogenesis. Central to this is the activation of nuclear factor (NF)κB, a key transcription factor that not only switches on genes involved in orchestrating the inflammatory response, but also stimulates angiogenesis through the upregulation of VEGFA, VEGFR2, MMPs, PDGFB and Ang-1

response to local inflammatory stimuli such as various interleukins (IL-), tumour necrosis factor (TNF)α and interferon (IFN)γ, activated ECs upregulate the expression of several transmembrane glycoproteins including the P- and E-selectins, intercellular cell adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [5]. These surface molecules facilitate the tethering and rolling of leukocytes along the vascular endothelium. Coupled with factors that increase vascular permeability such as VEGFA, COX-2 induced prostaglandins, histamine from mast cells, and nitric oxide (NO) produced by inducible NO synthase (iNOS), these molecules promote the transmigration of leukocytes across the endothelium [5]. Within inflamed tissue, circulating monocytes differentiate into macrophages and produce a myriad of mediators that can directly and indirectly promote angiogenesis. Central to this is the activation of nuclear factor (NF)κB [22], a key transcription factor that not only switches on genes involved in orchestrating the inflammatory response, but also stimulates angiogenesis through the upregulation of VEGFA, VEGFR2, MMPs, PDGFB and Ang-1, among others [5]. Various chemokines including monocyte chemoattractant protein (MCP)-1 are also released leading to the further recruitment of monocytes that propagate and sustain the inflammatory and angiogenic response [5]. These factors drive ECs to detach from their basement membrane and undergo migration and proliferation. Proteolysis of the ECM also releases sequestered factors such as FGF2 that can further potentiate angiogenesis [5].

The maintenance of an unbalanced pro-angiogenic state appears to be a common pathological feature in a range of chronic inflammatory conditions. Rheumatoid arthritis (RA), for instance, is characterised by progressive and destructive inflammation of the joint synovium driven by a network of cytokines such as IL-1, IL-6 and TNFα [23]. These mediators, along with relative tissue hypoxia, strongly induce pro-angiogenic factors to establish new vessels that augment the inflammatory response. Compared to healthy controls, patients with RA have markedly elevated levels of VEGFA in the serum and synovial fluid [24], and their synovial fibroblasts demonstrate increased expression of Ang-1 and Ang-2 [25]. A similarly pathological interaction of hypoxic and inflammatory signalling triggers neovascularisation in proliferative ocular diseases such as wet age-related macular degeneration (AMD), retinal vessel occlusion and diabetic retinopathy. A central feature of these conditions is the over-expression of VEGFA leading to rapid formation of new vessels in the choroid and retina [5]. These vessels are typically fragile and leaky, predisposing to haemorrhage, oedema and the deposition of exudates that can obscure central light perception. The upregulation of adhesion molecules and chemotactic factors further allows the infiltration of inflammatory cells which propagate the vicious angiogenic cycle [5].

Similar mechanisms are involved in the pathogenesis of several vasculitic syndromes. Dysregulated angiogenesis has been linked to granulomatosis with polyangiitis, Takayasu's arteritis, Kawasaki disease, Giant Cell Arteritis and thromboangiitis obliterans, among many others [26]. Regardless of the initiating factors, these vasculitides are characterised by vigorous inflammation occurring in the vessel wall leading to intimal VSMC proliferation, fibrosis and luminal thrombosis [26]. This is driven by an array of cytokines and chemokines produced by activated macrophages

and T-lymphocytes, in particular various interleukins, TNFα, IFNγ, MCP-1, VEGFA and MMPs [26]. These factors also potently enhance angiogenesis, which in turn facilitates recruitment of more inflammatory cells and is integral to sustaining the metabolic demands of the inflamed tissue. As these vessels are progressively occluded by vascular wall thickening, scarring and thrombosis, resulting in tissue ischaemia which further promotes an angiogenic response that can drive inflammation [26].

In malignant neoplasms, activation of a highly dysregulated "angiogenic switch" is also a well-known contributor to tumour growth, survival and metastasis [1]. As tumour cell clones acquire oncogenic mutations and the expanding tumour mass outgrows its blood supply, local hypoxia potently stimulates angiogenesis through HIF-1α signalling [6]. This occurs on a background of tumour-associated inflammation mediated via TNFα and NFκB pathways in ECs, myeloid cells, fibroblasts and other stromal cells [5]. There is therefore uncontrolled amplification of pro-angiogenic factors such as VEGFA, FGF2, PDGFB, hepatocyte growth factor (HGF) and Ang-2, resulting in a highly disorganised vascular network characterised by leaky, irregular vessels with limited basement membrane coverage and mural support from pericytes [1, 3, 6]. The lack of reliable perfusion through these vessels also impairs oxygen and nutrient delivery, in turn triggering further pathological angiogenesis and providing optimal conditions for tumour intravasation into the vasculature and subsequent dissemination. In addition, factors such as PLGF are thought to recruit bone-marrow derived precursors to facilitate tumour-associated vasculogenesis by acting on VEGFR1 [27]. Besides sprouting and non-sprouting angiogenesis, it is now known that tumour cells also have the ability to establish alternative aberrant forms of vascularisation. These include vascular mimicry, a mechanism whereby tumour cells can form pseudo-vessels by adopting an EC-like morphology [1, 3], as well as vessel co-option, where tumours bypass angiogenesis by hijacking and utilising pre-existing vessels within adjacent non-malignant tissue [28]. Although the mechanisms underlying these processes are less well understood, they are recognised to be poor prognostic factors and confer resistance to current anti-angiogenic therapies [6].

5.3.2.1 Angiogenesis and Atherosclerosis

Given the central importance of atherosclerosis in vascular diseases, an overview of the angiogenic mechanisms that drive the development of atherosclerotic plaque deserves specific attention. Atherosclerosis is a progressive inflammatory process that typically spans decades. It is initiated by a range of factors including haemodynamic stress that can cause endothelial injury and dysfunction, often first manifest as thickening of the arterial intima. This disruption allows atherogenic lipids, particularly cholesterol delivered by low-density lipoprotein (LDL) particles, to be deposited into, and accumulate within, the sub-endothelial space, where they become oxidised by reactive oxygen species (ROS) [5, 29]. Oxidised LDLs are potent stimulants of the inflammatory response, in which circulating monocytes are

recruited into the damaged intima and are activated to become macrophages. As these macrophages attempt to scavenge oxidised LDLs, they become lipid-laden foam cells, in turn releasing numerous pro-inflammatory cytokines and chemokines, which promote plaque growth by stimulating the proliferation and migration of VSMCs from the arterial media into the intimal layer [29]. The VSMCs produce a poorly developed ECM and form a fibrous cap that encapsulates the expanding lipid- and debris-filled necrotic core. These plaques become clinically evident as they cause progressive stenosis of the arterial lumen or are acutely occluded by thrombus following erosion or rupture of the fibrous cap, crucially leading to end-organ ischaemia, typified by myocardial infarction (MI), PAD and ischaemic stroke syndromes.

There is now a well-recognised relationship between plaque growth, plaque instability and the extent of plaque angiogenesis. Indeed, in advanced atherosclerotic plaques, vulnerable regions are associated with greater degrees of inflammation and neovascularisation [30]. In the early stages of plaque growth, there is angiogenic expansion of the vasa vasorum, a network of microvessels in the adventitial and outer medial layer of medium and large arteries whose function is to provide oxygen and nutrients to the vessel itself (Fig. 5.4) [30, 31]. This occurs in

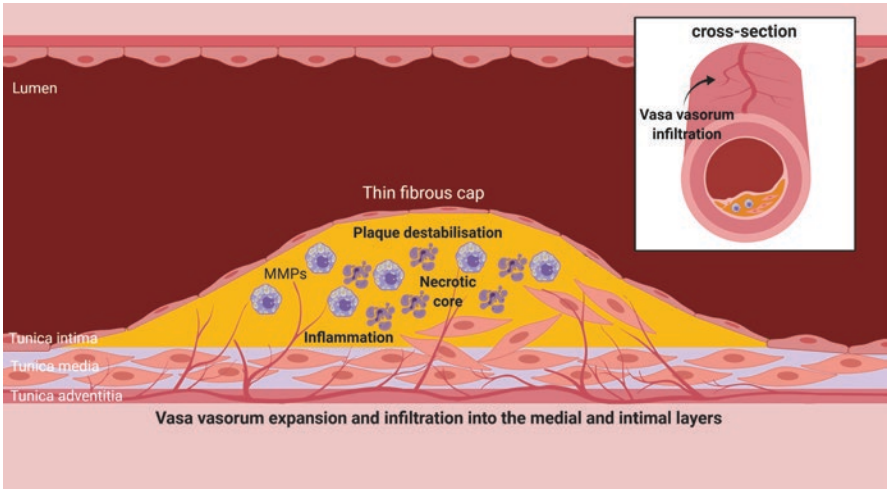

Fig. 5.4 Angiogenesis in the atherosclerotic plaque. In the early stages of plaque growth, there is angiogenic expansion of the vasa vasorum, a network of microvessels in the adventitial and outer medial layer of medium and large arteries which provide oxygen and nutrients to the vessel itself. As the plaque advances, progressive intimal thickening, lipid deposition and medial hyperplasia contribute to relative hypoxia, strongly driving vasa vasorum angiogenesis and the ectopic extension of adventitial neovessels into the intima to support ongoing plaque growth. The highly inflammatory milieu created by plaque macrophages further stimulates angiogenesis. These new vessels accentuate plaque inflammation by providing additional routes for the infiltration of inflammatory cells and cytokines. The release of MMPs and other proteases that facilitate invasion of ECs through the ECM also contribute to plaque instability through breakdown and thinning of the fibrous cap

response to several synergistic factors. Arterial hypertension leads to increased cyclical compression of the vasa vasorum especially during systole. As the plaque advances, progressive intimal thickening, lipid deposition and medial hyperplasia contribute to relative hypoxia, strongly driving vasa vasorum angiogenesis and the ectopic extension of adventitial neovessels into the intima to support ongoing plaque growth [30, 31]. The highly inflammatory milieu created by plaque macrophages further stimulates angiogenesis through numerous factors as previously outlined, particularly VEGFA, the TNFα and NFκB pathways, PDGF, FGF1 and FGF2, and the angiopoietins [29, 30]. Oxidised LDL and phospholipids themselves can also directly promote pathological angiogenesis [29]. New vessels are also thought to arise from the arterial lumen, though to a much lesser extent than via the vasa vasorum [31]. Regardless of the source, these new vessels accentuate plaque inflammation by providing additional routes for the infiltration of inflammatory cells and cytokines. The release of MMPs and other proteases that facilitate invasion of ECs through the ECM also contribute to plaque instability through breakdown and thinning of the fibrous cap [30, 31]. In a similar way to angiogenesis occurring in malignant tumours, plaque neovessels also have a paucity of tight junctions, along with discontinuous basement membrane and pericyte support. Such fragile intimal neovessels predispose to intraplaque haemorrhage [31]. This is a well-established marker of plaque instability as the extravasation of erythrocytes with cholesterol-rich membranes adds to the growing lipid load, and the abundance of free haemoglobin containing iron further activates macrophages and enhances oxidative stress within the plaque [29, 31].

Vascular calcification (VC), particularly occurring within the intima, is also recognised as a prominent feature of advanced atherosclerotic plaque, though the role and mechanisms of calcification remain rather poorly defined. In both CAD and PAD settings, calcium scores of atherosclerotic lesions are known to be associated with a higher risk of adverse cardiovascular events [32, 33]. VC in the form of spotty patchy intimal microcalcifications is typical of atherosclerosis [34]. It is thought to arise from macrophages and VSMCs that are stimulated to differentiate into osteoblast-like cells, acquiring an osteogenic phenotype by increasing the expression of bone-forming factors such as bone morphogenetic protein-2 and osteoprotegerin [34]. This is likely driven by a combination of factors including endothelial injury, increased generation of ROS, the accumulation of oxidised lipids and apoptosis of VSMCs providing nucleation sites for calcium and phosphate deposition [32, 34]. The role of angiogenesis in this process is still unclear, though it has been proposed that new vessels born out of the atherosclerotic inflammatory milieu may serve as highways for the delivery of osteogenic cytokines and the migration of osteogenic precursors into the plaque body [22, 35]. A clear relationship between angiogenesis and VC, however, has not been demonstrated *in vivo*, and the role of VC as a stabilising or precipitating factor for plaque rupture remains controversial.

Tobacco smoking, a strong risk factor and causative agent for atherosclerosis and tumorigenesis, is also known to enhance pathological angiogenesis. Nicotine, the principal addictive agent in tobacco, has potent mitogenic, pro-migratory and

tubulogenic effects on ECs at clinically relevant concentrations [36]. Nicotine acts on nicotinic acetylcholine receptors on ECs and VSMCs to promote the expression of key pro-angiogenic factors such as VEGFA and the FGFs [36]. Nicotine also stimulates the release of catecholamines which contribute to arterial hypertension by increasing heart rate and peripheral vascular resistance, contributing to endothelial dysfunction as an initiating step to atherosclerosis [36].

Despite the currently available repertoire of procedural interventions for atherosclerosis such as angioplasty, endovascular stenting and vein grafting, angiogenesis can still pose significant challenges. Indeed, angiogenesis has been implicated in the development of neointimal hyperplasia and neoatherosclerosis, which can lead to complications such as in-stent restenosis and thrombosis [37]. While it is clear that angiogenesis has a critical role in the pathophysiology of atherosclerosis, efforts to modulate it for therapeutic benefit have been largely unsuccessful, due in part to its highly complex and still incompletely understood biology.

5.3.2.2 Angiogenesis in Aneurysmal Disease

Angiogenesis is also believed to contribute to the pathogenesis of aneurysmal disease, which is of obvious interest in vascular surgery. Although the precise aetiology of aneurysms remains elusive, they are typically characterised by an inflammation-driven degeneration and weakening of the vascular wall, resulting in abnormal dilatation and an elevated risk of vessel rupture or thromboembolic complications [38]. There is increased degradation and turnover of ECM components such as elastin and collagen, which appear to be related to the increased expression of MMPs in the setting of a dense inflammatory infiltrate [38]. Much of the evidence implicating angiogenesis comes from the study of aortic aneurysms, though it is conceivable that similar mechanisms may be at play in the development and progression of aneurysms at other sites such as the cerebral, popliteal and renal arteries. In the healthy aorta, the vasa vasorum is usually rather sparse, but several histopathological studies of both thoracic and abdominal aortic aneurysms (AAA) have demonstrated angiogenic expansion of the vasa vasorum extending from the adventitia into the medial layer [39–41]. This occurs in close association with the presence of activated inflammatory cells such as macrophages and mast cells, which produce a host of cytokines including VEGFA, TNFα, FGF2 and various ILs that have pro-angiogenic actions [38, 39]. In thoracic aortic aneurysms, Ang-1, Ang-2 and FGF1 have also been found to be enriched within the medial layer [40]. During the process of vessel sprouting and EC migration, the ECM is continually degraded by MMPs, collagenases and plasminogen activators, contributing to structural weakening of the vascular wall [38]. The upregulation of adhesion molecules and chemokines in these immature and highly permeable neovessels also recruits more inflammatory cells, further promoting pathological angiogenesis [38, 39]. Hypoxia also has a postulated role. In the normal aortic wall, particularly infra-renally, the relative lack of vasa vasora means much of the nourishment is dependent on the diffusion of oxygen and nutrients from the lumen across the intimal layer [38]. However,

this is impaired as the aneurysmal wall expands and may be exacerbated by the presence of intraluminal thrombus, which is relatively common in AAA. As such, hypoxia-driven HIF-1α/VEGFA-dependent signalling drives further neovascularisation within developing AAAs [38], though this mechanism appears not to be as relevant for thoracic aortic aneurysms [40].

Importantly, the extent of aneurysmal neovascularisation is not only correlated with medial degeneration, elastin destruction and greater inflammatory infiltration, but studies have also demonstrated increased angiogenesis at sites of aneurysmal rupture concomitant with elevated expression of pro-angiogenic factors [42, 43]. Indeed, it has been suggested that anti-angiogenic therapies may be able to prevent aneurysmal progression and complications. Such agents are yet to be realised in human patients despite some encouraging results from rodent models of AAA, which have investigated the use of a soluble decoy VEGFA receptor, and an inhibitor of mast cell degranulation, tranilast, showing reduced aneurysm formation associated with decreased angiogenesis and inflammation [44, 45].

5.3.2.3 Diabetes- and Age-Impaired Angiogenesis

Diabetes mellitus [20] is a rapidly expanding global health epidemic known to be associated with a range of vascular complications. The pathophysiological hallmarks of these include dysregulated angiogenesis and accelerated atherosclerosis driven by inflammation, hyperglycaemia, insulin resistance and dyslipidaemia [46]. In patients with type 1 or type 2 DM, CAD represents the major cause of death and there is an estimated two- to four-fold increase in CAD mortality compared to non-diabetic individuals [47]. In addition, an extensive association exists between DM and PAD, with significantly higher incidence rates of claudication, critical limb ischaemia, lower extremity ulcers and major limb amputations [48]. These are likely due to a combination of factors including impaired immune function, reduced vascular collateral formation, diffuse atherosclerosis and peripheral neuropathy in those with DM. This is further compounded by microvascular dysfunction and inflammation, which is important in chronic kidney disease (CKD) and proliferative retinopathy.

Ample evidence indicates that diabetes-related vascular complications are related to significant impairment of physiological angiogenesis. Exposing cultured ECs to high glucose conditions *in vitro* reduces their capacity to migrate and form a capillary tubule network [49]. In mouse models of DM, hyperglycaemia also results in significantly delayed wound healing, as well as impaired recovery of blood flow in the setting of hindlimb ischaemia [49]. The mechanisms underlying these effects are complex and multi-faceted. Chronic hyperglycaemia leads to the generation of ROS and advanced glycation end-products (AGEs) [50]. These contribute to endothelial dysfunction by antagonising the protective effects of NO signalling, which usually helps to regulate vascular tone via VSMC relaxation, but also prevents platelet aggregation and thrombosis, abates excessive immune responses, and enhances endothelial growth and repair [50]. Diabetic hyperglycaemia is also linked

to diminished ischaemia-induced stability of HIF-1α and reduced production of NO via impaired endothelial NO synthase (eNOS) activity [49]. These pathways culminate in decreased production of angiogenic growth factors such as VEGFA, FGF2 and TGF-β, while AGEs can also downregulate or inactivate VEGFR2 leading to defective VEGF signalling [50]. Expression of PDGFB and the activation of Ang-1/Tie2 signalling in ischaemia is also reduced in various models of DM [50]. From a vasculogenic perspective, increased oxidative stress and disrupted NO signalling in DM has been linked to reduced numbers of circulating EPCs, which in turn produces fewer ECs with impaired proliferative and migratory capacity [50, 51]. This decrease in the mobilisation, homing and delivery of EPCs from the bone marrow to sites of injury is due to inhibited VEGFA and SDF-1α signalling in DM [50]. These changes ultimately lead to a defective neovascularisation response to ischaemia.

Additionally, impairment of wound healing is a key contributor to diabetic morbidity and mortality. This is most commonly manifest as ulceration in the distal limbs and is often complicated by concurrent peripheral neuropathy, which severely compromises the sensing of pressure and pain such that cuts and developing blisters go unnoticed by patients for prolonged periods. Peripheral neuropathy arises from the additive effects of oxidative stress, enhanced non-enzymatic glycation and dysfunction of neural proteins, reduced angiogenic and neurotrophic growth factors, and structural microangiopathic changes to neural blood vessels [52]. Once formed, diabetic wounds are also significantly predisposed to infectious complications, particularly cellulitis and osteomyelitis, as a result of diminished immune defences. Indeed, monocytes and granulocytes from individuals with DM characteristically exhibit defective phagocytic function and responses to chemotactic signals [50]. Moreover, the wound perfusion and oxygenation required to support the metabolic demands of immune cells is often severely reduced by the presence of diffuse atherosclerotic stenoses in the major arteries of patients with DM, leading to necrotic cell death. As a result, the ability to mount an angiogenic and vasculogenic response to wound hypoxia is strongly attenuated in DM, leading to delayed formation of wound granulation tissue and disrupted ECM remodelling [50].

Abnormal angiogenic processes also play a role in the development and progression of CKD, for which DM is one of the leading causes, along with hypertension. In the early stages of diabetic nephropathy, hyperglycaemia is thought to induce the over-expression of VEGFA, Ang-2 and TGF-β signalling, leading to glomerular EC proliferation and the formation of immature vessels with deficient basement membrane and aberrantly high permeability [53]. Combined with the presence of glomerular hypertension and impaired eNOS signalling, this promotes extravasation of plasma proteins resulting in albuminuria, arteriolar hyalinosis and nodular deposits in the mesangial matrix which are characteristic of diabetic CKD [53]. As the nephropathy advances, ongoing ROS and AGE formation causes progressive destruction to the glomerular filtration apparatus accompanied by widespread fibrotic changes [53]. Further injury to the endothelium, podocytes and the renal tubular epithelium eventually leads to frank proteinuria and reduced VEGFA expression in the late stages of diabetic nephropathy [53].

Excessive pathological angiogenesis is also a hallmark of diabetic retinopathy. In the ocular vasculature, chronic hyperglycaemia induces the production of ROS and AGEs, while microvascular occlusions promote HIF-1α signalling, both leading to over-stimulation of VEGFA [52–54]. This initiates a dysregulated angiogenic response characterised by leaky vessels that allow the extravasation of fluid, lipid and protein exudates, often clinically manifest as diabetic macular oedema [5]. There is also upregulation of pro-inflammatory cytokines such as TNFα, IL-1β and MCP-1 along with various adhesion molecules and integrins, which together recruit inflammatory cells, contribute to ECM breakdown via MMPs, and potentiate the VEGFA-mediated angiogenic response [53]. Concurrently, there is activation of the polyol metabolic pathway in which excess glucose is reduced to sorbitol. Accumulation of intracellular sorbitol is toxic to retinal neurons and pericytes as a result of increased osmotic and oxidative stress [55]. The combined mechanisms of progressive retinal injury and proliferative angiogenesis in DM ultimately lead to vision loss perpetuated by oedema, exudates and haemorrhage.

Beyond the impact of DM, there is emerging evidence that angiogenic processes are linked to obesity and can be substantially altered with ageing. These are issues with increasing prominence particularly in developed countries. Obesity and metabolic syndrome are now conceptualised as states of chronic low-grade inflammation, accompanied by elevated serum levels of pro-inflammatory factors such as TNFα, IL-6, resistin and C-reactive protein [5]. These are secreted in response to signalling from adipocytes and associated macrophages in visceral adipose tissue and likely facilitate inflammatory angiogenesis in other tissues [5]. Yet, adipose tissue itself, with its ability to rapidly expand and regress, is also highly angiogenic. Relevant mediators include VEGFA and FGF2, which can be induced by insulin, as well as leptin, a key regulator of energy balance that is elevated in obesity and has demonstrated pro-angiogenic actions [1]. It is not yet known whether an anti-angiogenic therapeutic approach would prove useful for obesity.

Conversely, ageing is related to significant impairment of physiological angiogenesis. Poorer wound healing and a blunted angiogenic response to ischaemia likely account for the higher risk of vascular complications with increasing age. Indeed, diminished ischaemia-driven expression of VEGFA, VEGFR2 and reduced HIF-1α stability has been reported in aged patients and animal models compared to younger controls [56, 57]. Similar to DM, ageing is also associated with senescence of ECs and EPCs, resulting in reduced production of angiogenic growth factors and impaired capacity for proliferation, migration and vasculogenesis. Cellular senescence is thought to be related to the accumulation of ROS with ageing, leading to inhibition of cyclin-dependent kinases that regulate the cell cycle, as well as decreased telomerase activity and telomere shortening in ECs. Age-dependent loss of sex hormones, particularly oestrogens, may also contribute as these are well-known to be protective against senescence and have pro-angiogenic actions. Conversely, increased ROS and attenuated NO signalling with age likely contribute to endothelial dysfunction, atherosclerosis and exacerbation of pathological angiogenesis [58].

5.4 Targeting Angiogenesis in a Clinical Setting

Given the ubiquity of angiogenic dysfunction in vascular disease, there has been long-standing interest in developing therapies that are able to appropriately suppress pathological angiogenesis or augment physiological angiogenesis. To date, only anti-angiogenic therapies have demonstrated sufficient benefits in randomised trials to justify their routine clinical use, particularly in the setting of cancer chemotherapy and proliferative eye disease. Therapeutic stimulation of angiogenesis by way of genetic manipulation, cell transfer or recombinant agents remains an attractive possibility for the management of ischaemic conditions, though no such therapies have yet achieved widespread clinical translation. Promisingly, various alternatives are also emerging from pre-clinical models that are capable of exerting both pro- and anti-angiogenic effects depending on the conditional context.

5.4.1 Pharmacological Inhibition of Angiogenesis

Anti-angiogenic therapies that primarily target VEGF signalling pathways are currently being used clinically in the therapeutic inhibition of tumour angiogenesis for several malignancies. These therapies primarily target VEGF signalling pathways and include monoclonal antibodies directed against VEGFA or VEGF receptors and various small molecule tyrosine kinase inhibitors (TKIs) that inhibit ligand-activated autophosphorylation of multiple VEGF and PDGF receptors [3, 6, 58]. Bevacizumab, a humanised monoclonal antibody against VEGFA, is the first-line therapy for metastatic colorectal cancer (CRC) [6, 58] and is also effective in the treatment of metastatic non-squamous non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC) and epithelial ovarian cancer when combined with standard chemotherapy regimens [58, 59]. Several TKIs including sunitinib, sorafenib and pazopanib have shown efficacy as monotherapy for metastatic RCC, a tumour type known to express high levels of VEGFA, which renders them rather sensitive to anti-VEGF approaches [6]. Other notable anti-angiogenic agents include aflibercept, a soluble VEGF receptor [58] that has been successfully trialled in addition to standard chemotherapy as second-line therapy for patients with metastatic CRC [60]. Ramucirurab, a monoclonal VEGFR2 antibody, is effective in the treatment of advanced gastric and gastro-oesophageal junction adenocarcinomas, and as second-line therapy for metastatic NSCLC [58, 61].

Beyond cancer therapeutics, anti-angiogenic agents have also been used extensively to treat ocular neovascularisation diseases. In conditions such as wet age-related macular degeneration and diabetic retinopathy, aberrant VEGF signalling plays a central role in stimulating excessive growth of new vessels that are fragile and abnormally permeable [5]. Locally-directed intravitreal anti-VEGFA therapies, such as pegaptanib and ranibizumab, have been effective in slowing the progression of these retinal diseases, and in many cases, leading to

improvements in visual acuity [6]. Intravitreal forms of bevacizumab and aflibercept are also routinely used in this setting [3].

Several non-VEGF-directed anti-angiogenic therapies are also undergoing early phase clinical trials, again predominantly in the cancer setting. These therapies target other key regulators including Ang-2 [58], HGF and its receptor c-MET [58] and the $\alpha_v\beta_3$ integrins [62]. Beyond these, a recombinant form of endostatin, one of the endogenous inhibitors of angiogenesis, as well as several anti-angiogenic gene transfer approaches are also currently being tested as cancer therapies [63].

5.4.2 Limitations of Current Anti-angiogenic Therapies

Despite the successful uptake of anti-angiogenic therapies in cancer therapeutics and proliferative eye diseases, the currently available agents do have several limitations. Anti-VEGF therapies have been associated with a range of adverse effects including hypertension [61, 64], proteinuria [64], higher incidences of haemorrhagic events [64], increased rates of impaired wound healing [64] and a significantly increased risk of both venous and arterial thromboembolic events [65]. This likely reflects inhibition of the physiological role of VEGF in reducing endothelial activation and the attenuation of the adaptive angiogenic response to ischaemia. Beyond these adverse effects, many patients are refractory to current anti-angiogenic therapies. Several cancer types are unresponsive to anti-VEGF treatments altogether, or can develop resistance to VEGF signalling leading to progressive disease [6] while some tumours are able to switch to non-sprouting modes of vascularisation or develop VEGF-independent vessel growth [3, 6]. Tumour-associated fibroblasts and macrophages release chemokines and factors such as SDF-1α, FGF2 and HGF that recruit bone marrow-derived cells to facilitate tumour neovascularisation, bypassing the reliance on VEGF signalling [6]. Moreover, the extended use of anti-angiogenic therapies is postulated to promote the selection of tumour cell clones that are more resistant to hypoxia and less dependent on angiogenesis [3].

For these reasons, there are now renewed research efforts into developing more effective therapies that target multiple angiogenic pathways rather than VEGF alone. Multi-functional agents capable of both promoting and suppressing angiogenesis under specific conditions are also being explored with the hope of avoiding the adverse effects of non-selective anti-angiogenic therapies.

5.4.3 Therapeutic Stimulation of Angiogenesis

While anti-angiogenic therapies have enjoyed relative translational success so far, therapies designed to stimulate angiogenesis in ischaemic cardiovascular conditions are still in their relative infancy. By enhancing vessel growth, these therapies have the potential to improve tissue perfusion and facilitate tissue repair and recovery.

The unmet demand for such therapies is immense. There are many patients who may be refractory to pharmacological risk factor-based therapies or are unsuitable for either surgical or catheter-based revascularisation procedures.

Several approaches have been tested particularly in the setting of myocardial or peripheral limb ischaemia. These include the administration of recombinant angiogenic proteins such as FGF1, FGF2 and various isoforms of VEGFA [66, 67]. Early studies in animal models of MI demonstrated augmentation of collateral blood flow and functionally significant myocardial angiogenesis [66]. The findings in placebo-controlled human trials, however, have been more ambivalent. While intra-arterial delivery of recombinant FGF2 in patients with intermittent claudication modestly improves peak walking time [68], similar intra-coronary infusions of FGF2 in patients with CAD did not produce lasting improvements in myocardial perfusion or exercise tolerance [69]. Intracoronary and intravenous delivery of recombinant VEGFA also failed to significantly alter symptom- and perfusion-based outcome measures in patients with CAD [70].

With the recognition that such recombinant proteins have a relatively short tissue half-life and are generally less targeted therapies, there has been keen interest in developing gene-based approaches instead. These involve transgenes encoding various pro-angiogenic proteins that are delivered via plasmids or adenoviral vectors, the latter allowing for higher transduction efficiency at the expense of potentially greater immunogenicity [66]. In the setting of PAD, intramuscular administration of transgene constructs containing various VEGFA isoforms as well as FGF1, FGF2, HGF and HIF-1α have yielded largely disappointing results in randomised trials [71]. A possible exception to this may be HGF plasmids, which have shown improvements in tissue oxygenation, ulcer healing rates and pain at rest compared to placebo in patients with chronic limb-threatening ischaemia (CLTI) [72, 73]. For patients with CAD, the landscape for angiogenic gene therapy is similar. Numerous trials have been conducted with plasmid and adenoviral-delivered VEGFA isoforms, VEGFC, FGF4, HGF and HIF-1α, but these have not generated consistently successful outcomes [74, 75]. It is at least encouraging that gene therapies have not produced significant adverse safety signals to date, and this has supported the resolve for ongoing development. Proposed ideas for more successful translation include more judicious patient selection, more specific anatomical targeting of ischaemic tissues, the use of adeno-associated or retroviral vectors that may promote more durable transgene expression, and the repurposing of gene therapies as adjuncts following revascularisation procedures [71, 74, 75].

Cell-based therapies have also been keenly investigated as strategies for stimulating angiogenesis. These involve the transplantation of precursor cells with vasculogenic potential, which can then home to sites of ischaemic injury where they differentiate into ECs that can form vessels *de novo* [76]. A number of randomised placebo-controlled trials have been conducted in patients with CLI using autologous bone marrow-derived mononuclear cells injected either intra-muscularly or intra-arterially [71]. These have had mixed success, as earlier studies demonstrated promising improvements in ankle-brachial index, tissue oxygenation, rest pain and maximal pain-free walking time [77, 78], while the largest and most recent of these

trials was negative [79]. Trials of cell therapies have also been undertaken in patients following acute MI or during elective percutaneous coronary intervention. In these settings, intra-coronary transplantation of precursor cells have led to significant improvements in left ventricular ejection fraction and myocardial perfusion in various trials [66]. However, some larger clinical trials have struggled to replicate these findings [76].

At present, the utility of stimulating therapeutic angiogenesis in ischaemic vascular diseases is limited by relative inefficacy and discrepancies in trial findings. There also remains theoretical concern that repeated use of these pro-angiogenic therapies may unintentionally stimulate pathological inflammatory or tumour-associated angiogenesis, though fortunately these effects have not been borne out in clinical trials to date, likely because these therapies are only administered on a short-term basis. Nevertheless, these recombinant proteins, gene and cell-based therapies hold great promise. Further refinement and testing of these agents in large-scale trials will be required before they can be successfully translated to the clinic.

5.4.4 Emerging Angiogenesis-Modulating Therapies

In light of the issues encountered with targeted pro- and anti-angiogenic therapies, attention has shifted towards identifying agents that might have differential effects on angiogenesis depending on the context. Indeed, there is evidence from *in vitro* and *in vivo* studies that some of the currently available lipid-lowering therapies have such effects. Statins, in particular, are mainstays of therapy for cardiovascular disease and are known to have pleiotropic effects independent of their lipid-lowering functions. They have been shown to modulate angiogenesis in a dose-dependent manner, with low doses promoting EC migration, proliferation and capillary tubule formation, while high doses conversely have anti-angiogenic actions in models of atherosclerotic plaque development and tumour growth [80, 81]. Fenofibrate is commonly used to treat patients with dyslipidaemia but has also demonstrated conditional angiogenic effects; inhibiting tumour-associated angiogenesis [82] but can conversely rescue diabetes-related impairment of angiogenesis in murine models of peripheral limb ischaemia [83]. These findings suggest that there may be considerable scope to repurpose currently available pharmacological therapies to take advantage of their beneficial modulation of angiogenesis for a range of vascular diseases.

A wealth of epidemiological evidence has established that serum HDL concentrations are inversely correlated with the rates of cardiovascular disease [84] and cancer [85] while elevated HDL levels are associated with improved survival and prognosis following MI [86, 87]. Recent studies have shown that HDL conditionally regulates angiogenesis; promoting physiological vessel growth in ischaemia but suppressing it in pathological inflammatory contexts [88, 89]. Despite this, therapies designed to increase endogenous serum HDL levels have been largely disappointing in clinical trials with respect to reducing the risk of major adverse

cardiovascular events. This is thought to be related to endogenous HDL particles that have been rendered dysfunctional via chemical modifications such as oxidation or glycation in the setting of atherosclerosis or diabetes [90]. There is now keen interest, therefore, in the use of purified rHDL infusions and analogues of apoA-I instead. Embedding of apoA-I or rHDL onto endovascular stent platforms is also being investigated, as these moieties may improve stent biocompatibility by supporting rapid re-endothelialisation while inhibiting neointimal hyperplasia, stent thrombosis and neoatherosclerosis [91, 92].

microRNAs (miRNAs), small non-coding RNAs that post-transcriptionally regulate gene expression, are the leading next generation biopharmaceuticals for the treatment of complex multi-faceted diseases [93]. The importance of miRNAs in vascular development and angiogenesis was first observed when the critical miRNA processing enzyme Dicer was inhibited with embryonic lethality observed due to an underdeveloped vascular system [94]. miRNAs that regulate angiogenesis by targeting angiogenic genes include miR-34a, miR-124, miR-29, miR-126, miR-150, miR-221/222 and miR-17–92 cluster [95]. miRNAs that are modulated by pro- or anti-angiogenic factors include miR-483-3p, miR-21, miR-210, miR-296, miR-93, miR-206, miR-26, miR-155, miR-424, miR-27b and miR-130a [95]. It is therefore likely that the pleiotropic action of targeting specific pro- or anti-angiogenic miRNAs will give significant therapeutic advantages over single gene-targeted therapies currently in clinical use. Indeed, miRNA targeting drugs are already showing promise in Phase I and II clinical trials in a wide range of diseases [96]. Furthermore, miRNAs can be released from the cell into the bloodstream and are extremely stable in the extracellular environment, where they can be taken up within tissues [97]. Circulating miRNAs have emerged as a new class of disease biomarkers [98]. Patients with CAD had reduced levels of angiogenesis-associated miRNAs including miR-126, miR-17 and miR-92a and miR-155 [99]. In individuals with diabetes-associated PAD, lower circulating miR-126 levels were found to be associated with lower ankle-brachial index while levels of the anti-angiogenic miRNAs miR-15a and miR-16 were elevated and predicted the occurrence of amputation in CLI patients [100]. It is likely that the role of miRNAs as both potential therapeutic targets and clinical diagnostic markers will provide an alternate approach that can either complement current therapies or open new avenues for better informed clinical diagnosis and targeted personalised therapies.

5.5 Conclusion

Significant advances have been made in our understanding of angiogenesis and the cellular and molecular factors that modulate it under both physiological and pathological conditions. While angiogenesis is a fundamental and adaptive process in development, wound healing and ischaemic conditions, dysregulated angiogenesis is also a pathological hallmark of cancer, atherosclerosis, proliferative eye disease and many other inflammatory conditions. Targeting angiogenesis as a therapeutic

approach for vascular disease has so far been fraught with issues, not least of which is the risk of inadvertently exacerbating pathological angiogenesis while trying to augment physiological angiogenesis, and vice versa. Indeed, therapeutic angiogenic stimulation is not yet a clinical reality, and anti-angiogenic therapies, though more common, still have numerous limitations. New strategies therefore demand consideration. These include more detailed characterisation of the molecular factors and agents capable of differentially modulating angiogenesis in different contexts, refining novel methods to deliver existing therapies in a more targeted fashion, and the discovery of new gene- and cell-based technologies. Appropriately harnessing these will be crucial for the ongoing battle against a range of vascular diseases which contribute immensely to morbidity and mortality worldwide.

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Chapter 6
Vascular Biology of Smooth Muscle Cells and Restenosis

Victoria Nankivell, Khalia Primer, Achini Vidanapathirana, Peter Psaltis, and Christina Bursill

Key Learning Points

- Vascular smooth muscle cells (VSMCs) are present in the wall of the artery and regulate the constriction and dilatation of blood vessels.
- VSMCs play multiple roles in vascular pathologies such as atherosclerosis and restenosis and can be derived from different origins including progenitor cells
- In atherosclerosis, VSMCs can perform both plaque stabilising and deleterious roles that lead to plaque expansion.
- In restenosis, the most predominant role of VSMCs is inflammation-induced rapid proliferation that happens in response to vascular injury.
- Interventional strategies such as balloon angioplasty and stent deployment commonly use anti-proliferative agents to suppress inflammation to prevent restenosis and vessel re-narrowing.

6.1 Introduction

Smooth muscle plays an important role in the vasculature. Vascular smooth muscle cells (VSMCs) are essential for providing shape and withstanding mechanical forces. In the vasculature, VSMCs can be dynamically regulated to provide contraction and dilatation in response to specific neuro-hormonal and haemodynamic signals. The functions and regulation of VSMCs, however, extend far beyond these physical force attributes. VSMCs are highly plastic and can undergo significant changes in their phenotype, which causes substantial alterations in their function.

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VSMCs play key roles in diseases of vascular inflammation such as restenosis and atherosclerosis. Whilst VSMCs have extremely influential roles in the growth and stability of atherosclerosis, they dominate the development of restenosis, which is a focus of this Chapter. Understanding the multiple roles of VSMCs in restenosis and atherosclerosis is vital for improving the success rate of interventional strategies such as balloon angioplasty and stenting. Accordingly, this Chapter will describe the VSMC mechanisms that underlie their functional effects and their contributions to atherosclerosis and restenosis. Other mechanisms of restenosis will also be discussed as well as current interventional strategies for overcoming restenosis with their associated advantages and disadvantages.

6.2 Vascular Smooth Muscle Cells

6.2.1 Role in Vascular Function

VSMCs characteristically have an elongated spindle shape. Their principle function is to contract and generate mechanical output for the function of a particular organ. They are extremely heterogenous in phenotype, which is dependent on a range of factors including their location and embryological origin [1]. VSMCS are stromal cells of the blood vessels. They reside in the important 'middle layer' of the vessels also known as the media. The media is responsible for regulating pulsatile blood flow and vascular tone and is comprised of alternating layers of VSMCs and elastic connective tissue (Fig. 6.1).

VSMCs are non-striated. To regulate contraction and relaxation, VSMCs have actin and myosin filaments attached to their cell membrane which criss-cross the

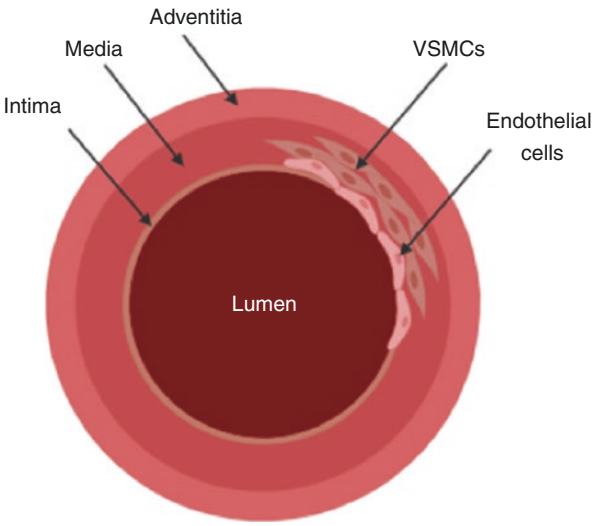

Fig. 6.1 Layers of an artery. The middle layer or media is composed primarily of VSMCs. The inner lining of the blood vessel or intima is composed of a monolayer of endothelial cells. The outer layer of the vessel is called the adventitia and is composed of multiple cell types including fibroblasts, macrophages and adipocytes

entire cell body. The contraction machinery of VSMCs is regulated by dynamic alterations in cytosolic calcium concentrations. The key effector in VSMC contraction/relaxation signalling is the 20 kDa myosin light chain protein (MLC_{20}). When active, MLC_{20} activates myosin and enables it to bind and slide along the actin filament, resulting in contraction. The system is activated when there is an increase in cytosolic calcium concentrations. Calcium is delivered from extracellular sources via plasmalemmal calcium channels or from the sarcoplasmic reticulum through sarcolemmal calcium channels. The calcium complexes with calmodulin, a calcium-binding messenger protein, to initiate contraction. Examples of vasoactive ligands that increase contraction include: endothelin-1, norepinephrine, angiotensin II, vasopressin and prostaglandins [2].

VSMC relaxation occurs by removal of the contractile stimuli. Vasodilators can either: close calcium channels, activate outward calcium pumps or other signalling pathways that reduce cytosolic calcium levels. Examples of vasodilator agonists include: nitric oxide (NO), adenosine, natriuretic peptides, adrenomedullin and insulin [2].

6.2.2 Regulation of Vascular Function by VSMCs

Smooth muscle tissue found in hollow organs (i.e. vasculature) is generally split into two types: single-unit or multi-unit smooth muscle. Despite this categorisation, there are usually combinations of these types. Multi-unit smooth muscle is primarily regulated via autonomic sympathetic innervations whereby there is a release of neurotransmitters along the length of the axon, rather than coupling to individual cells. Diffusion of the neurotransmitters activates a voltage dependent ion channel such as Ca^{2+} channels via membrane depolarisation. This type of cellular activation is known as electromechanical coupling [3].

With little innervation, single-unit smooth muscle is activated predominately by para- and autocrine hormones such as adrenalin, noradrenalin and angiotensin II, functioning via interaction with G protein coupled receptors. The activation of these receptors triggers sarcoplasmic reticulum Ca^{2+} release or activation of ion channels via membrane depolarisation with this activation known as pharmaco-mechanical coupling [3].

6.2.3 Atherosclerosis

VSMCs play multiple roles in the development of atherosclerosis (Fig. 6.2). It has long been thought that, as atherosclerotic plaques develop and increase in size, VSMCs are then recruited from the media and migrate towards the top of the plaque near the lumen. This migration is directed by platelet-derived growth factor (PDGF) and other inflammatory proteins such as the chemokines CX_3CL1 and CCL5 [4].

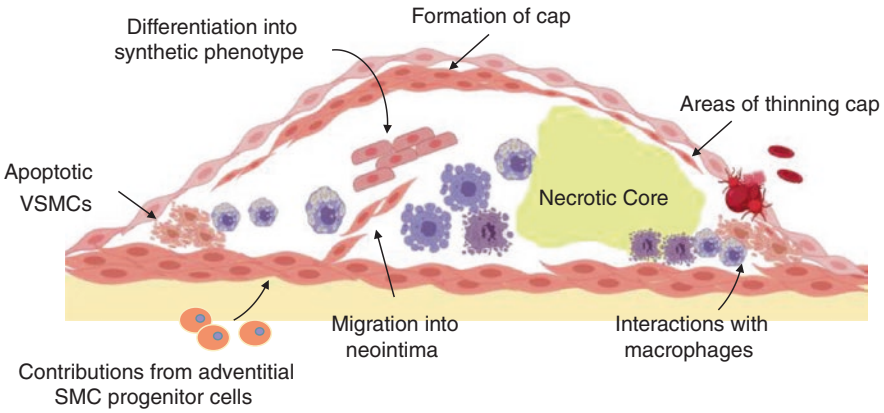

Fig. 6.2 Multiple roles of SMCs in the development of atherosclerosis

When at the top of the plaque, the VSMCs secrete and lay down collagen, a structural protein, that eventually leads to the formation of a 'cap'. The cap has a stabilising effect on the plaque that prevents it from rupturing, an event that can lead to myocardial infarction or stroke [5].

By stabilising atherosclerotic plaques, VSMCs play a beneficial role. However, VSMCs also contribute in multiple ways to the expansion of the plaque and the narrowing of the lumen. In the earliest stages of atherosclerosis, the appearance of a neointima occurs before the presence of lipid-laden fatty streak deposits. The growth of the neointima is in fact driven by the rapid proliferation of VSMCs, stimulated by growth factors and inflammatory cytokines. These VSMCs also produce a large amount of extracellular matrix (ECM) that further adds to the plaques size [6]. As plaque growth progresses further and becomes more complex with more inflammation, VSMCs in the cap can undergo apoptosis (programmed cell death). Proteases that become more prevalent in more advanced plaques will also degrade the ECM of the cap. Combined, these factors lead to a thinning cap and increase the likelihood of plaque rupture [7].

VSMCs within a plaque also express a host of inflammatory cytokines that can contribute to plaque expansion. This is particularly through the enhancement of monocyte recruitment. VSMCs express PDGF, transforming growth factor (TGF)-β, macrophage inhibitory factor (MIF) and interferon gamma (IFN-γ), which can all assist with the recruitment of monocytes from the circulation. Whilst endothelial cells are major players in the recruitment of monocytes into the intima of a developing plaque, there is immunohistochemical evidence that SMCs and monocytes are in direct contact [8]. This process is mediated by interaction with adhesion molecules vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [9]. Interestingly, VCAM-1 is only found to be expressed on VSMCs in diseased aortas but not in healthy aortas, thereby suggesting a role for VCAM-1 in VSMC monocyte recruitment and disease progression. The chemokine, fractalkine (CX_3CL1) is a unique membrane-bound chemokine and the ligand

for CX_3CR1 [10]. It is expressed on VSMCs and it has been found, that when monocytes are incubated with oxidised LDL, they upregulate their expression of CX_3CR1. This then increases monocyte binding to membrane-bound CX_3CL1 on VSMC that serves to anchor the monocytes to the VSMCs [11].

6.2.4 VSMC Apoptosis

VSMC are believed to play a central role in stabilising the plaque through the development of the cap. VSMC apoptosis is therefore likely to influence the stability of the plaque. In early-stage plaques, the level of apoptosis is found to be very low and starts to increase with increasing plaque size [12]. Plaque rupture occurs most-commonly at the shoulder regions of the plaque in which there are lower numbers of VSMCs and higher number of macrophages. This suggests that VSMC apoptosis is induced by macrophages at the shoulder region sites, triggering plaque rupture. In support of this, it has been shown that there are increased levels of VSMC apoptosis in symptomatic plaques when compared with stable plaques [13].

Another complication of VSMC apoptosis in atherosclerosis is that it is associated with increased inflammation. Interestingly, this increase in inflammation does not occur for VSMC apoptosis that is associated with vascular ageing or medial degeneration. The reason underlying this discrepancy is thought to be due to a lack of efficiency in the clearance of apoptotic VSMCs in atherosclerosis as well as a slower clearance of inflammatory cytokines released from dying cells [5]. Dying apoptotic VSMCs release IL-1β and necrotic VSMCs release IL-1α. In a healthy vessel, VSMCs are very efficient at clearing apoptotic VSMCs within ~48 h. However, in the presence of hyperlipidaemia, the phagocytotic capacity of VSMCs is impaired, thereby delaying phagocytosis and apoptotic cell and cytokine clearance [14].

6.2.5 VSMCs Origins; Role in Atherosclerosis

Lineage tracing studies using genetically modified mice that enable tracking of cells have revealed that VSMCs arise from a number of different developmental origins. For example, the VSMCs in the ascending aorta and aortic arch as well as the head and neck vessels originate from the neural crest. Coronary VSMCs are generated from the epicardium and the descending aorta VSMCs arise from somatic mesoderm precursors [15]. The importance of this is that these different developmental origins can have significant effects on the development of atherosclerosis. For example, the descending thoracic aorta is relatively resistant to atherosclerosis when compared to the aortic arch that rapidly develops plaque. The thoracic aorta has a significantly higher level of expression of the developmental genes, the Homeobox genes, and a reciprocal relationship with NF-κB, a transcription factor that drives

inflammation and atherosclerosis [16]. This suggests that there is a relationship between developmental origin and the susceptibility to developing atherosclerosis.

Other data supports this concept. When VSMCs from neutral crest origin are exposed to TGF-β, they increase their production of collagen. This does not occur, however, in VSMCs from the mesodermal zone. Furthermore, homocysteine, a protein that is important in cardiovascular disease, will induce the proliferation of VSMCs from the neural crest but not from the mesoderm origin [17]. There are also variations in the propensity to develop atherosclerosis, undergo aortic aneurysm development and develop calcification [18].

VSMCs contribute to plaque development and stability by: (1) forming a stabilising 'cap', (2) differentiating in a highly proliferative synthetic VSMC phenotype, (3) undergoing apoptosis and (4) through VSMC progenitor cell contributions originating from the adventitia.

6.2.6 Restenosis

VSMCs play a major role in the development of restenosis which is the process of rapid expansion of a neointima after vascular injury, also called neointimal hyperplasia (Fig 6.3). Restenosis is driven predominantly by inflammation, as a result of the vascular injury, and the inflammation causes dysregulated VSMC proliferation and migration. It is a significant problem following balloon angioplasty and stent deployment. The mechanisms and processes of restenosis are biologically distinct from atherosclerosis. Restenosis occurs over shorter time frames (months-years) compared to the decades that it takes for an atherosclerotic plaque to develop. A detailed discussion of restenosis will be included later in this Chapter.

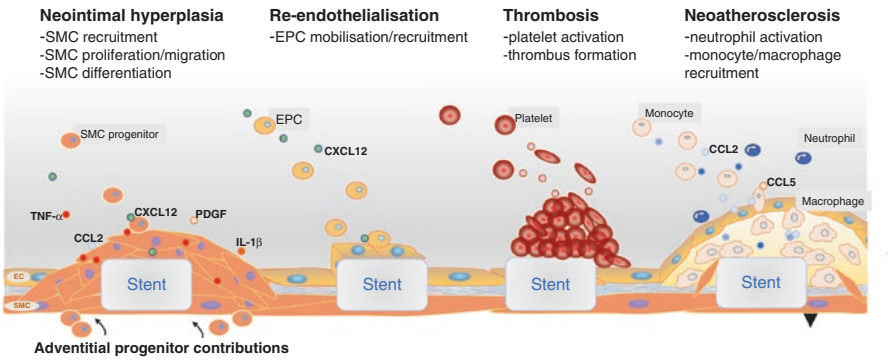

Fig. 6.3 Multiple biological mechanisms associated with in-stent restenosis

6.2.7 Smooth Muscle Cell Phenotype

6.2.7.1 Contractile Versus Synthetic Phenotype

VSMCs are highly plastic in nature which can play a significant role in their contributions to atherosclerosis and restenosis. In a healthy adult vascular wall, VSMCs are able to regulate vasoconstriction and dilatation, and are known as the 'contractile' phenotype. However, in response to injury, inflammation or different growth factors, the phenotype of these VSMCs can change. During this switch, VMSCs down-regulate contractile functions and de-differentiate into a 'synthetic' VSMC phenotype that is highly proliferative and can have deleterious effects such as promoting atherosclerosis and restenosis growth. Synthetic VSMCs have diminished responses to vasodilators and constrictors and thereby exhibit reduced vascular function [2].

Changes in the cell surface marker expression profile is the most important way to characterise changes in VSMC phenotype and this correlates with changes in function. The contractile phenotype expresses markers that are vital to the SMC contraction machinery and cell anchorage including: SM-α-actin, SM-myosin heavy chain (MYH11), calponin, SM22α, smoothelin, *h*-caldesmon and others [19]. As VSMCs start to de-differentiate to the synthetic phenotype they start to lose these important contractile markers and increase their expression of proteins involved in cell cycle progression (cyclins, calmodulin) and ECM remodelling (matrix-metalloproteinases and collagen), and increase their expression of cytokines [19]. There are also significant changes in morphology. The contractile elongated spindle-shaped phenotype starts to take on a rhomboid-like shape. There is, however, a broad range of morphological possibilities in between these two classically described VSMC morphologies and also a large number of different combinations of phenotypic marker possibilities [20]. This diversity is caused through different stimuli or environmental cues, disease milieu and embryonic origin. In atherosclerosis, one example of the VSMC phenotype switch is their significant loss of α-actin, an important marker of the contractile phenotype, with disease development. This has been validated in rigorous studies using transgenic mice that track SMC origins and fate. For example, it was demonstrated that greater than 80% of VSMC-derived cells within an established atherosclerotic plaque do not express alpha actin [21, 22].

Recent studies have helped to clearly define the role of phenotypic switching in atherosclerosis and there is a growing realisation that inhibition of the differentiation to the synthetic VSMC phenotype may suppress the progression of atherosclerosis. For example, mice heterozygous for myocardin (myocardin$^{-/+}$), that supports the expression of most VSMC contractile genes and therefore promotes the contractile VSMC phenotype, have increased atherosclerosis when crossed with atherosclerosis-prone apolipoprotein (apo)E$^{-/-}$ mouse. The loss of a single allele of myocardin (i.e. myocardin$^{-/+}$) also increased inflammatory pathways and macrophage recruitment to the plaques. Interestingly, these effects were reversed in a gain of function myocardin mouse [23].

The role of Krϋppel-like factor-4 (KLF4) in phenotypic switching has been studied extensively. KLF4 is required in VSMCs for PDGF and IL-1β-induced differentiation [24]. KLF4 is also found to regulate genes that control and inhibit myocardin. Deletion of KLF4 is able to prevent and delay phenotypic switching *in vivo* in VSMCs in a murine vascular injury model [25]. Interestingly, using a VSMCs-specific KLF4 knockout mouse, no change in phenotypic switching was detected but there was a reduction in plaque size and increased cap thickness [21].

There is accumulating evidence that micro-RNAs (mi-RNAs), short stretches (20–30 bases long) of non-coding RNA, play a role in phenotypic switching from a contractile to a synthetic VSMC phenotype. For example, bioinformatics analyses have predicted that miR let-7g modulates the switch. The target genes for let-7g were found to be the genes in the PDGF/mitogen-activated protein kinase kinase 1 (MEKK1)/extracellular signal-regulated kinase (ERK)/KLF4 signalling pathway, known to regulate phenotypic switching. Increased levels of let-7g were found to protect against PDGF and MEKK1-induced differentiation from the contractile to the synthetic VSMC phenotype. Furthermore, *in vivo* studies revealed that over-expression of let-7g in apoE$^{-/-}$ mice decreases atherosclerosis in the aorta.

6.2.7.2 Trans-differentiation of VSMCs to Macrophages

It is well-established that circulating monocytes infiltrate the developing plaque and differentiate into macrophages that take-up modified low-density lipoproteins (LDL). More recently in both *in vitro* and *in vivo* studies, it has been discovered that VSMCs are also able to engulf modified LDL. It has been demonstrated that VSMCs are able to take up lipid and form foam cells. Upon internalisation of LDL, the VSMCs start to acquire a more macrophage-like phenotype and lose their SMC phenotype [26]. For example, lipid-loaded VSMCs will express macrophage markers including CD86, Mac-2 and ABCA1. Concurrently they lose their expression of α-actin, tropomyosin, MYH11 and calponin. Accompanying this, lipid-loaded VSMCs express higher levels of inflammatory proteins and are considered therefore to contribute to the expansion of the plaque in a deleterious fashion [27]. It should be noted that although VSMCs are able to engulf lipid, the overall phagocytic capacity of VSMCs is much lower than that of a professional phagocytic cell like the macrophage. These findings were more recently confirmed *in vivo*. It was found that, in apoE$^{-/-}$ mice, the cells lining the necrotic core of atherosclerotic plaques were positive for both VSMC and macrophage markers. Bioinformatic analysis also confirmed that lipid-loaded VSMCs expressed macrophage markers but overall expressed markers that were closer to the traditional VSMC state [5]. This, however, has the potential to also be deleterious. In this trans-differentiated state, macrophages not only perform some of the unfavourable functions of macrophages and express more inflammatory cytokines, they also have impaired beneficial macrophage functions including phagocytosis and efferocytosis (process by which apoptotic/necrotic cells are removed from the plaque).

High-density lipoproteins (HDL) play a key role in effluxing cholesterol from cells. A recent study has demonstrated that incubation with the HDL sub-particle, HDL_3 is able to reverse the trans-differentiation of VSMCs to macrophages following cholesterol-loading. This is likely to be via the ability of HDL to remove the excess cellular cholesterol. This was confirmed through the use of VSMCs with a deletion in the cholesterol transporter, ABCA-1, that mediates cholesterol efflux from cells. Interestingly, the ability of HDL to reverse trans-differentiation was almost completely lost in these ABCA-1 knockout VSMCs. HDL_3 was also able to restore the expression of myocardin and KLF4, key regulators of differentiation, to baseline levels following cholesterol loading [27].

Mi-RNAs are known to regulate the trans-differentiation, or conversion, of VSMCs to macrophages. For example, miR-143/145 regulates myocardin and is down regulated with cholesterol loading. Conversely, if miR-143/145 levels are maintained it is able to reverse cholesterol-loading-induced phenotypic change [27].

6.2.8 Smooth Muscle Progenitor Cells

There is increasing evidence indicating the presence of smooth muscle progenitor cells (SMPCs) in the vasculature. In fact, SMPCs have been located in each layer of the vessel wall including the intima/neointima, the media and the adventitia [15]. It is highly likely that these tissue resident vascular progenitor cells also make contributions to the development of both atherosclerosis and restenosis. There is also evidence, although controversial, that bone marrow-derived SMPCs can contribute to disease development that will be discussed in this section.

The disease milieu surrounding sites of atherosclerosis or restenosis including inflammatory factors, reactive oxygen species (ROS), hyperlipidaemia and hypertension, are all likely to affect the migration, proliferation, mobilisation and differentiation of SMPCs. In hypertension, for example, there is intimal thickening and expansion of the media of the blood vessels. There is also an increase in the expression of α-actin. This is suggestive of the possibility that progenitor cells may have contributed to this adaptive response through enhanced migration and proliferation in the media, stimulated by inflammatory cues. In support of this concept, it has been demonstrated that SMCs in the neointima of vessel allografts originate from a local source of progenitor cells that were not from a bone marrow-derived source [28]. Furthermore, it has been shown that there is a local vessel source of SMCs that contributes to atherosclerotic plaque development [29]. Other studies have shown that, after femoral artery injury, more than 50% of the SMCs in the neointima are derived from adventitial progenitor cells. These findings were supported further by a mouse model that enabled specific lineage tracing of the SMC origin and showed definitively that for an injured artery the main source of SMCs are adventitial progenitor cells. In the $apoE^{-/-}$ model of atherosclerosis it has also been shown using fate-tracking that adventitial cells migrate into the media and the developing neointima of the plaque and differentiate into SMCs. In summary, local progenitor populations

from the vasculature, particularly the adventitia, differentiate into SMCs and can contribute to the development of restenosis and atherosclerosis disease processes.

It has been reported that bone marrow-derived SMPCs make contributions to the development of atherosclerosis and restenosis. Apoptotic VSMCs at sites of vascular injury have increased expression of the chemokine stromal cell-derived factor 1α (SDF-1α). SDF-1α has a well-established role in promoting the mobilisation, migration and recruitment of bone marrow-derived progenitor cells to sites of injury or repair. This suggests that apoptotic VSMCs, through their expression of SDF-1α, may be attempting to recruit bone marrow-derived SMPCs to the injured site. Studies have reported the existence of SMPCs in mouse models of arterial injury and neointimal hyperplasia [30]. It is also reported that bone marrow-derived SMPCs are present in atherosclerotic plaque, albeit very few. Subsequent SMC-specific lineage-tracking studies in transgenic mice have, however, failed to reproduce these studies. The overall current view is that whilst SMPCs can make significant contributions to atherosclerosis and restenosis, these cells are from local tissue sources and not from a bone marrow-derived source.

6.3 Restenosis

Restenosis is the re-narrowing of the arterial lumen. It is induced by vascular injury that causes an inflammatory-driven VSMC hyperproliferation and overproduction of ECM proteins resulting in excessive neointimal hyperplasia.

6.3.1 Causes and Mechanisms of Restenosis

Percutaneous angioplasty with or without stent deployment is the major mode used to re-open arteries blocked by atherosclerosis. Both cause significant damage to the vessel wall which can result in the expansion of the neointima due to the uncontrolled proliferation of medial SMCs. A combination of important factors including thrombosis, intimal hyperplasia, re-endothelialisation and remodelling contribute to the development of restenosis. There have, however, been significant transformative improvements in the design of current generation drug-eluting stents (DES) that have reduced the rates of stent-related major adverse cardiovascular events (MACE). These MACE include: stent restenosis, stent thrombosis and end-organ infarction.

The initial insult caused by the expansion of an angioplasty balloon catheter (with or without stent deployment) severely damages the endothelial layer and initiates inflammatory events. This causes a significant impact on the endothelial cells resulting in injury or complete removal of the endothelial cell layer at the site. In addition, after injury, medial VSMC apoptosis occurs (e.g. balloon injury causes up

to 70% mortality of SMCs within 30 min [31]). This injury initiates a cascade of pro-inflammatory events which stimulate VSMCs to de-differentiate to a more proliferative and synthetic phenotype which promotes increased re-entry into the cell cycle, thereby increasing proliferation and migration. VSMC proliferation increases dramatically in the first few days following injury leading to the rapid expansion of a neointima which is SMC-rich. After 4–6 weeks, re-endothelisation of the injured site occurs, however, there is evidence to show that these cells are dysfunctional and have decreased vascular integrity and increased permeability [32]. At 8 weeks post injury, the proliferation of the VSMCs returns to normal levels. There may, however, still be areas that have not undergone sufficient re-endothelialisation in which low levels of VSMC proliferation continue. Endothelial cells play an important role in regulating SMC proliferation and migration. *In vivo* studies have found that areas of early re-endothelialisation had less neointimal growth after vessel injury than those areas with low re-endothelialisation [33]. In an attempt to increase endothelialisation and decrease neointimal hyperplasia after stent deployment, stents coated with VEGF have been tested in rabbits. However, after 28 days the stents failed to increase re-endothelialisation and reduce neointimal hyperplasia. This indicates that local delivery of VEGF is not a viable strategy [34].

6.3.1.1 Mechanisms of Inflammation-Driven Neointimal Hyperplasia

The inflammatory response that causes neointimal hyperplasia and restenosis following vascular injury has not been fully elucidated. The degree of inflammation induced by stent deployment or angioplasty has been associated with the extent of restenosis as well as the prevalence of macrophages in the initial lesion. Leukocytes can be recruited to the site of vascular injury which is initiated by binding to platelets and cell adhesion molecules (e.g. ICAM-1 and V-CAM-1). Following injury to the endothelium there is a release of a host of cytokines including TGF-β1, PDGF and fibroblast growth factor (FGF) 2, TNF-α, VEGF, macrophage colony stimulating factor (MCSF), IL-4, IL-1α, IL-1β, IL-8 and CCL2. The release of these cytokines induces the subsequent proliferation and migration of medial VSMCs into the intima, resulting in expansion of a now SMC rich neointima.

There is also a growing body of evidence that inflammatory chemokines are critically important in the promotion of neointimal hyperplasia and vascular repair processes. *In vitro*, incubation with a range of chemokines (CCL2, CCL5 and CX3CL1) increases VSMC proliferation [35–37]. The transcription factor, NF-κB, the driver of inflammatory processes and chemokine expression, has been implicated in this process [37]. The involvement of chemokines in neointimal development has been supported in an *in vivo* study that showed deletion of CCL2 reduced neointimal formation in a murine model of arterial injury [38]. Furthermore, deletion of TNF-α, a cytokine that stimulates inflammation and NF-κB, was found to prevent neointimal hyperplasia in a murine model of carotid artery injury [39].

6.3.1.2 Growth Factors

PDGF is one of the key SMC mitogens that stimulates the growth of SMCs during vessel repair. However, when present at excessively high levels, such as the case following angioplasty-induced vascular injury, it can strongly stimulate proliferation and cause neointimal hyperplasia [40]. FGF2 also stimulates medial SMC proliferation and migration to the intima of injured vessels. Interestingly, FGF2 will not stimulate the proliferation of intimal SMCs as the intimal hyperplastic response is normal in FGF2 knockout mice. FGF2 is, however, also important in remodelling following angioplasty as FGF2 knockout mice display decreased SMC contractility [41]. One study has also identified a role for IL-11 in neointimal hyperplasia. IL-11 is an anti-inflammatory growth factor known to inhibit NF-κB. *In vitro* studies have demonstrated that incubation with IL-11 causes dose-dependent decreases in bFGF-induced VSMC proliferation as well as a reduction in pro-inflammation cytokine expression from VSMCs including IL-8 and IL-6 [42].

6.3.1.3 Extracellular Matrix (ECM)

The pro-inflammatory environment that promotes the proliferation and migration of VSMCs also results in the release of extracellular matrix proteins that contribute to intimal expansion. ECM contributes to restenosis in the later stages post-angioplasty. Histological analysis of human samples has revealed that in tissues with advanced restenosis, there is a lower cell density surrounded by substantial amounts of ECM. The ECM proteins induced by angioplasty include type I collagen, as well as the proteoglycans versican, perlecan, biglycan and hyaluronan, without the decorin that is present in primary plaques [43]. Some forms of ECM can stimulate SMC proliferation, whilst other types prevent further ECM accumulation and deposition [44].

6.3.1.4 Percutaneous Arterial Interventions That Cause Restenosis and Their Evolution

Whilst balloon angioplasty can be successful in restoring arterial luminal patency, the procedure is not without its limitations. Vessels have a high likelihood of re-narrowing due to the elasticity of the lamina, and any damage to the vessel caused by the balloon is found to trigger restenosis [45]. Many approaches have been trialled in an effort to reduce both vessel recoil and restenosis. These include drug strategies such as anticoagulants and antiplatelet agents and growth factor inhibitors, in addition to mechanical strategies including rotational cutting devices and lasers mounted on catheters [46]. Stents, however, have proven to be very successful in reducing vessel re-narrowing after angioplasty. Due to the sustained mechanical support provided to the vessel by the stent, it eliminates vascular recoil which occurs

once the balloon catheter has been removed. Over the past 50 years stent design, metal composition, coating and strut diameter have been extensively modified to optimise the effectiveness of stents. Two main classes of stent have dominated, the bare metal stent (BMS) and the drug eluting stent (DES). Whilst BMS are still routinely used, particularly for peripheral arterial disease, it is the DES which are predominantly used for coronary artery disease.

6.3.1.5 Bare Metal Stents (BMS)

Whilst BMS deployment reduces vessel recoil and increases vessel lumen area (particularly when a sub-optimal angioplasty result is obtained), acute stent thrombosis is a major complication following BMS deployment. Stent thrombosis after BMS deployment most commonly occurs within the first month, particularly in coronary intervention, with the risk decreasing after re-endothelialisation [47].

To prevent acute thrombus formation, patients are prescribed dual antiplatelet therapies (DAPT) for a period of time and then single antiplatelet therapy to reduce acute stent thrombosis following intervention. DAPTs combine aspirin with a P2Y12 inhibitor (e.g. Clopidogrel or Ticlopidine) and have proven to be the most effective regime to decrease coronary stent thrombosis, reducing incidence to 1%. The Stent Anticoagulation Restenosis Study (STARS) showed that the incidence of death, target lesion revascularisation, vessel thrombosis or MI after 30 days was 3.6% for patients treated alone with aspirin, however, this was significantly reduced to 0.5% with DAPT (aspirin + ticlopidine) [48].

While DAPTs are necessary to reduce thrombosis risk, they are not without their limitations. It is estimated that approximately 4–8% of patients require non-cardiac surgery within the first year after stent deployment [49]. Patients relying on DAPT to reduce their thrombotic risk are faced with the dilemma of continuing DAPT and risking both minor and major bleeding during surgery and post-operatively. Alternatively, patients can stop one or both antiplatelet agents, which increases their risk of a thrombotic event and is dependent on the length of time post-intervention. Despite antiplatelet therapy, BMS still fail as a result of restenosis or thrombosis.

6.3.2 Strategies to Reduce Neointimal Hyperplasia

Initial studies aimed at reducing neointimal hyperplasia following arterial intervention focused on the systemic delivery of anti-proliferative drugs to reduce SMC proliferation. One study investigated whether the anti-proliferative drug Tranilast could reduce restenosis rates after BMS deployment. Initial animal studies found that the agent was effective in reducing restenosis rates in rabbits and pigs [50, 51]. Furthermore, when Tranilast was studied in a small clinical trial, a modest reduction in restenosis rates were seen. However, once the drug moved into a large randomised clinical trial (Prevention of RESstenosis with Tranilast and its Outcomes-PRESTO)

there was no significant difference in restenosis formation between Tranilast and the placebo group [52]. Similar outcomes have been seen with other systemic anti-proliferative drugs trialled to reduce neointimal hyperplasia.

The main reason cited for the failure of larger clinical trials with systemic anti-proliferative therapy was the inadequate level of the drug at the angioplasty site. As a result, local drug delivery using perfusion balloons was explored. This method also failed due to the poor uptake of drugs at the site of inflation due to the minimal vessel contact time. Efforts then turned to investigate the potential of drug-eluting stents (i.e. anti-proliferative drugs released from stents) to reduce restenosis.

6.3.2.1 Drug Eluting Stents

Drug eluting stents (DES) were designed to deliver a sustained dose of anti-proliferative drugs to the local area and aimed to overcome the issue of restenosis experienced in BMS. The stents were coated with a polymer such as phosphoryl choline silicon carbide which allowed an anti-proliferative drug such as Sirolimus or Paclitaxel to be coated on and slowly released into the surrounding tissue [53].

Early randomised clinical trials showed promising results, with the first-generation DES causing a reduction in SMC proliferation and restenosis rates post-implantation at both 6 and 12 months-post deployment, compared to BMS [54]. Initial clinical testing of this first DES in 2003 used the CYPHER™ stent (Johnson and Johnson), which was coated with the drug Sirolimus. This was closely followed with TAXUS™ (Boston Scientific) which used Paclitaxel. Both Sirolimus and Paclitaxel work via inhibition of the cell cycle, however, each drug does this by a different mode of action. Sirolimus works by halting cell division in the initial phase (G-phase) of the cell cycle. In contrast, Paclitaxel arrests cells during the mitosis phase when they are about to divide. While CYPHER and TAXUS DES reduced restenosis rates when compared to BMS, a new complication of delayed healing and re-endothelialisation at the deployment site arose.

First-generation DES are prone to late (30 days–1 year) and very late (>1 year) stent thrombosis as a result of delayed healing, likely caused by the anti-proliferative agents released at the stent site. DAPT therapies for BMS were initially recommended for the 4–6-week period following stent deployment, but for DES this was increased to 12-months in an effort to reduce complications associated with late stent thrombosis [55].

Due to the necessity for a prolonged use of DAPTs, some patients experience mild to severe side effects from the medications. For example, continued use of aspirin can cause gastrointestinal upset and tinnitus. However, prolonged used of DAPT appears to be essential following coronary DES deployment. Clinical studies have found that stent thrombosis is significantly elevated following the cessation of DAPT at 12-months. Furthermore, the incidence of late stent thrombosis continues to rise at a rate of 0.6% per year in the following 3 years post-procedure resulting in a high mortality rate of 25% [56]. First generation DES therefore exacerbated the

vascular biological biocompatibility issues of intervention and highlighted the need for improved stent technologies and designs.

Second generation DES have subsequently been developed that significantly overcome many of the issues exhibited by first-generation DES. Second-generation coronary DES incorporate more biocompatible polymers. They are also thinner with more flexible cobalt-chromium or platinum struts and release newer anti-proliferative drugs. In the second-generation stents two limus analogs (Zotarolimus and Everolimus) have replaced paclitaxel, which have a wider toxic-therapeutic ratio. Combined, these changes have markedly lowered, although not completely eliminated, the rates of late-thrombosis. Whether event-free survival can be further improved by more enhancements in stent design is uncertain. Bioresorbable stents are the latest stent technology in which drugs are eluted from bioresorbable polymer-free systems. They are designed to gradually disappear over the course of a year, a time at which vessel remodelling should be complete. They therefore offer theoretical advantages over DES but are yet to show improved clinical outcomes and are thus far disappointing. Contemporary second-generation DES are therefore the leading stent and clinical outcomes following their deployment are generally very good, but have plateaued and have largely remained unchanged over the past decade. The principal remaining causes of failure include early and late inflammatory and hypersensitivity reactions to the drugs or stent polymers, mechanical problems such as strut fracture and longitudinal deformation, very late issues with a permanent metallic implant such as vessel straightening, loss of cyclic strain, vasomotion and adaptive vascular remodelling, and, in particular, neoatherosclerosis.

6.3.2.2 Drug-Coated Balloons

Drug-coated balloons (DCBs) are a newly developed device in which the anti-proliferative drugs found in DES are coated onto the surface of the balloon. The drugs are delivered to the site of stenosis following balloon inflation which causes contact with the vessel wall. DCBs display a number of advantages over DES. For example, the balloons allow for uniform delivery of the drugs. In DES, by contrast, it has been shown that different gradients of drug concentration by non-uniform strut distribution can trigger neointimal overgrowth in sirolimus-eluting stents. DCBs also avoid the issues related to the presence of a metallic stent and do not require a polymer carrier. They are therefore less likely to induce unfavourable responses and allow for complete restoration of the vessel to its original state with a functional endothelium. Finally, DCBs allow for more flexibility in future treatment options.

Paclitaxel is primarily used for coating DCBs. The reason for this is that paclitaxel is highly lipophilic and allows for passive absorption through cell membranes which improves treatment uptake and effect. More recently carrier excipients for DCBs have been developed that greatly facilitate drug transfer during the short period of contact between the inflated balloon and the vessel wall. The excipient prevents crystallisation of the drug on the balloon surface which hinders drug

transfer and absorption. The excipent also, therefore importantly, prevents the loss of drugs into the blood stream. A variety of exipients have been developed including iopromide, urea and shellac that display a diversity of responses, indicating that improvements in this technology still need to be made.

Clinical studies have shown early promise for the treatment of both coronary and lower extremity vascular disease. A number of recent studies report their use, in particular, for the femoropopliteal artery. DCBs show good efficacy when the atherosclerotic lesions are medium length and minimally calcified. Other indications that are being investigated include niche lesion subsets such as small vessels and bifurcations in which stents are not a viable alternative. Another interesting use is for the treatment of in-stent restenosis or for a restenotic site that previously received balloon angioplasty. Whilst DCBs are still in their infancy and procedural improvements (e.g. inflation pressure, duration) still need to be made, they do show excellent promise as the next revolution in PCI.

6.3.2.3 Emerging Role of miRNAs in Restenosis

The discovery of microRNAs (or miRNAs) has revealed a further layer to the regulation of restenosis, amongst an already large number of regulators. MiRNAs are short RNA molecules that repress gene expression by binding to the 3′UTR of mRNA (region of mRNA that immediately follows the translation termination codon) transcripts to either degrade the transcript or prevent translation completely or partially. MiRNAs have been investigated for their potential to either be a therapeutic target or a biomarker of disease. Recent evidence has revealed that miRNAs play roles in several aspects of the vascular response to injury and a vast number of miRNAs have been reported to influence VMSC proliferation, migration and apoptosis (reviewed in Gareri et al [57]). One of the more highly reported miRNAs is miR-21 which is highly expressed in both VSMCs and ECs and increases further following vascular injury [58]. This suggests that it plays a role in restenosis. In support of this, if VSMCs are serum starved to slow their proliferation, miR-21 levels decrease. Furthermore, inhibition of miR-21 increases VSMC apoptosis and reduces cell growth *in vitro* and after vascular injury *in vivo*. Intravenous infusion of an anti-miR-21 to reduce miR-21, caused dose-dependent suppression of luminal closure, without affecting re-endothelialisation [58]. Systemic delivery of miR-21 causes, however, a number of other unwanted side effects including increased serum creatine concentrations. Further studies with a local delivery approach in which the anti-miR-21 was coated onto the stents was found to effectively reduce restenosis without causing any unwanted side effects. Further experimentation revealed that PTEN (phosphatase and tensin homolog deleted on chromosome 10), an antiproliferative protein, was a direct target of miR-21, which may explain the mechanism for its action on VSMCs [58]. Another study also reported that circulating levels of miR-21 were a predictor of restenosis after interventional therapy in patients with lower extremity arterial occlusive disease [59]. Furthermore, miR-21 levels are significantly correlated with age, diabetes and hypertension.

MiR-146a is another miRNA of interest as it targets the pro-proliferative transcription factor KLF4. KLF4, in turn, binds to the promoter region on miR-146, thereby forming a feedback loop in which they regulate each other [60]. Other restenosis-related mechanisms of miRNAs have also been revealed. MiR-612 and miR-125a-5p, for example, act on PDGF that promotes the phenotypic switch and increases VSMC proliferation [61, 62]. Studies have found that overexpression of both miR-612 and miR-125a-5p inhibits VSMC proliferation and migration.

Therapeutic approaches using infusions of inhibitors (anti-miRNAs) or mimics that overexpress miRNAs have been utilised in animal models of vascular injury and restenosis. In a rabbit model of carotid balloon angioplasty, infusion of miR-140-3p to increase its circulating levels, was found to reduce restenosis. Similarly, infusions of miR-126 and miR-495 are found to reduce restenosis in rodent models [63, 64].

A number of circulating miRNAs have been found to have predictive value for in-stent restenosis. miR-195, miR-320a and miR-572 have all been associated with in-stent restenosis for patients with PAD [65, 66]. Furthermore, miR-93-5p has been found to be predictive of coronary in-stent restenosis [67].

The number of miRNAs reported to regulate and predict restenosis continues to increase. Future stent technologies may indeed incorporate miRNA release in an effort to further reduce the complications of DES-related restenosis.

6.4 Neoatherosclerosis

Neoatherosclerosis is an important contributing factor to the late stent-related cardiovascular events after deployment of DES. It may be the major final common denominator in late stent failure and is not decreasing in prevalence as stent technology improves. Indeed, autopsy series have shown no difference between the first- and second-generation DES in the prevalence of coronary neoatherosclerosis [68]. Neoatherosclerosis is time-dependent, with its prevalence increasing the longer the stent is in place. The histopathology of neoatherosclerosis is similar to native atherosclerosis, containing macrophage/foam cells, cholesterol clefts, areas of calcification and necrotic cores. Neoatherosclerosis occurs within much shorter time frames than native atherosclerosis and develops at 6 months–5 years post-stent deployment, rather than over a lifetime. Neoatherosclerosis accelerates late expansion of the neointima as a key cause of stent failure. The neointimal plaques can also become unstable, with ruptured thin-capped neointimal plaques acting as the primary cause of very late stent thrombosis [69]. Although the mechanisms of neoatherosclerosis are not entirely elucidated, the higher occurrence of neoatherosclerosis in DES may be the result of drug resistance, a reaction to the DES polymers or DES-induced delayed re-endothelialisation.

It has been widely hypothesised that the formation of neoatherosclerosis is closely related to the progression of native arterial atherosclerosis [70]. During stent deployment .the vascular wall undergoes expansion by stent struts, which

causes endothelial denudation, significant medial injury, plaque compression and rupture of the internal elastic lamina. These events trigger an inflammatory response that initiates the development of neoatherosclerosis. The original plaque that is compressed by the stent is also a source of growth factors and chemokines that further promote neoatherosclerosis. In support of this theory, in a serial intravascular ultrasound study, smaller atherosclerotic plaques behind the stent correlated with the extent of neointimal formation. A better understanding of biological processes that drive the early and late inflammatory reactions to stenting may therefore aid in further reducing the risk of in-stent restenosis and neoatherosclerosis.

6.5 Conclusions

VSMCs have a fascinating array of functions and phenotypes that are influenced by their surrounding stimuli and origin. They play an important role in regulating the constriction and dilatation of vessels and participate in the development of vascular inflammatory pathologies such as restenosis and atherosclerosis (Fig. 6.3). The reliance upon angioplasty and stents to treat atherosclerotic stenosis and occlusions in the last two decades has significantly increased, which has led to a rise in complications such as restenosis, stent thrombosis and neoatherosclerosis. The continuing development of new ways to prevent in-stent restenosis is therefore critically important. With the recent increase in knowledge regarding the involvement of miRNAs in the regulation of restenosis, it raises the possibility for miRNAs to be incorporated into future stent technologies. A continued understanding of the multiple roles of VSMCs and their regulation in atherosclerosis and restenosis will be important for the road ahead.

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Chapter 7
Vascular Haemodynamics

Shirley Jansen, Michael Lawrence-Brown, Siamak Mishani, Christopher Lagat, Brian Evans, Kurt Liffman, and Ilija D. Šutalo

Key Learning Points

- An appreciation of vascular haemodynamics is important for understanding the cardiovascular system in health and disease.
- Numerical models that explain realistic mechanical and physiological characteristics (non-Newtonian behaviour, pulsatile flow, nonhomogeneous and elastic blood vessel) are accurate but complex and difficult to describe. However using a simplified model may significantly affect the final results.
- The laws and governing equations that describe the behaviour of fluids in conduits play a critical role in our understanding of aneurysms, arterial dissections, atherosclerotic occlusive disease and behaviour of implanted grafts and stents.
- Vascular haemodynamics play a critical role in the development of new medical devices and to improvements in existing vascular interventions.

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7.1 Introduction

Complex parameters such as non-Newtonian fluids, turbulent flow, shear stresses within the blood and between the blood flow and the intima, as well as within the wall itself, are important for understanding the flow of blood, which is a particulate mixture of cells and plasma proteins. We will not try to be definitive for some of these parameters, especially when they are combined, but we do want to impart some understanding of how they may influence the fluid dynamics.

Accompanying the major advances in computing and imaging capabilities is the improvement in computational fluid dynamics (CFD) modelling which now includes patient-specific geometry, fluid-structure interactions, pulsatile flow and non-Newtonian flow. CFD and Finite Element Analysis (FEA) are now used to assist in the design of implantable medical devices and enhance understanding of the physics of vascular systems. They will influence future vascular disease management and are presented in the next chapter.

This chapter will summarise and discuss the following laws, equations and phenomena to give a basic understanding of the haemodynamic principles of the conduits and fluids with which we work:

- Darcy's law
- Poiseuille flow
- Laplace's law of wall tension
- Newtonian fluid
- Non-Newtonian fluid
- Reynolds number
- Womersley number
- Bernoulli's equation
- Young's modulus and pulsatile flow
- Mass conservation
- Shear stress and pressure
- Forces on graft systems
- Venous muscle pump mechanism

7.2 Pump Mechanisms in the Circulation

As Fig. 7.1 shows, the first pump is the heart and it works as a positive displacement pump to supply blood to the organs. For every cardiac cycle, the ventricles, which are pumps in series, push (displace) a fixed amount of blood into the aorta and pulmonary artery. The compulsory relaxation phase of cardiac muscle and its control of rate by regular physiological feedback systems ensures a regular

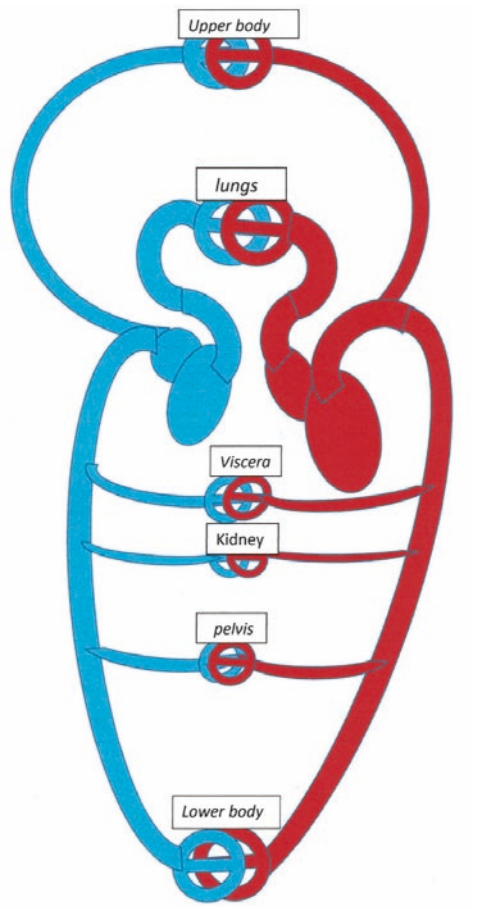

Fig. 7.1 Schematic diagram of cardiac pumps in series feeding parallel circuits

supply, and this rhythm and control give the heart the qualities of a servo pump. The aorta acts not only as a vessel to transfer blood but also as a modulator and dampener (Windkessel effect, as described by Otto Frank, a German physiologist) (see Glossary) to convert this pulsatile blood flow into a more uniform flow to supply blood to the tissues whilst ensuring diastolic flow to supply the heart and walls of the great vessels themselves.

Propulsion in the venous system is independent of the heart and relies on several mechanisms; the most important and powerful of which is the skeletal muscle pump where every muscle in the body is acting as a 'heart'. Contraction and relaxation of the skeletal muscles form a venous muscle pump to return blood from the

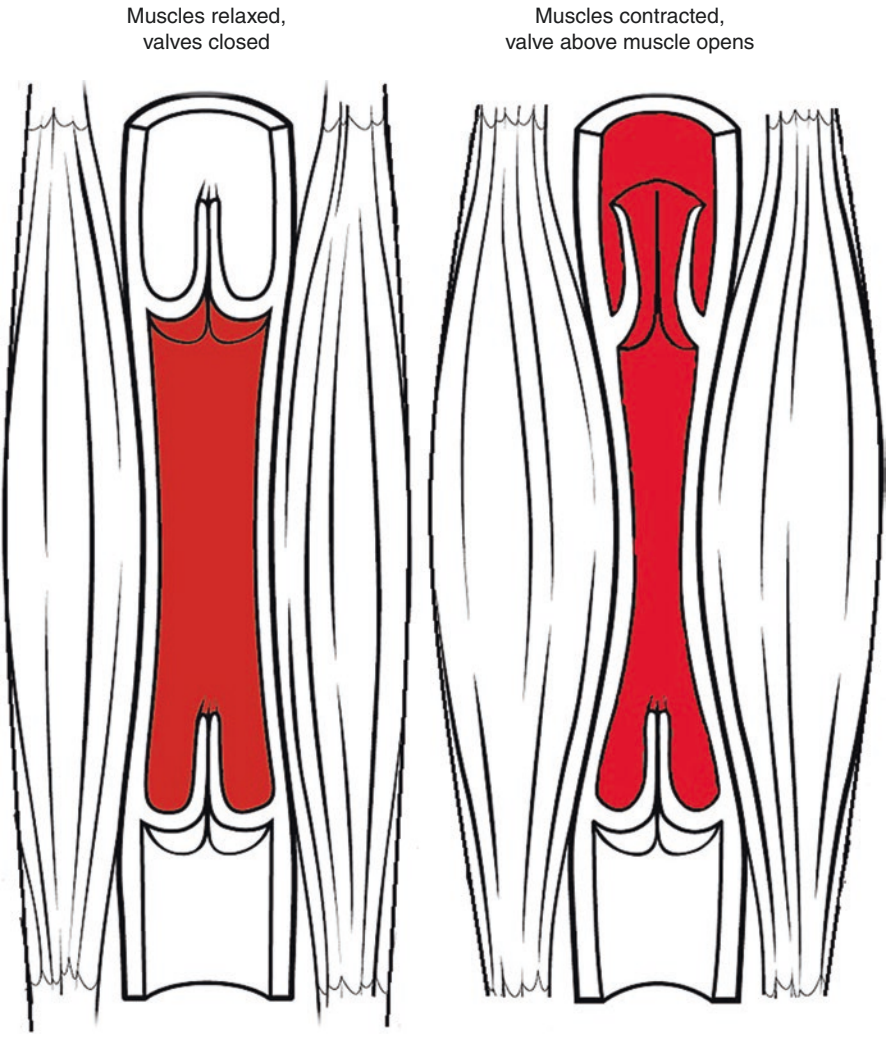

Fig. 7.2 Schematic diagram of the venous muscle pumping mechanism

deep veins to the heart—another form of displacement pump. Contraction of the muscles (increasing during exercise) push the blood to the heart from valve to valve (Fig. 7.2). The pumping mechanism in the muscles is similar to the sucker-rod lift pump in oil wells. The sucker-rod pump is based on the positive displacement progressive lift method. When the muscle relaxes, the fluid (blood) rushes into the vein refilling the deep veins prior to blood being pumped cranially with surface veins acting as fillers.

The synchronous movements of the chest wall and diaphragm are another displacement pump (Fig. 7.3). This affects the working of the other two pumps; heart and skeletal muscles. During inspiration, venous return to the right side of the heart

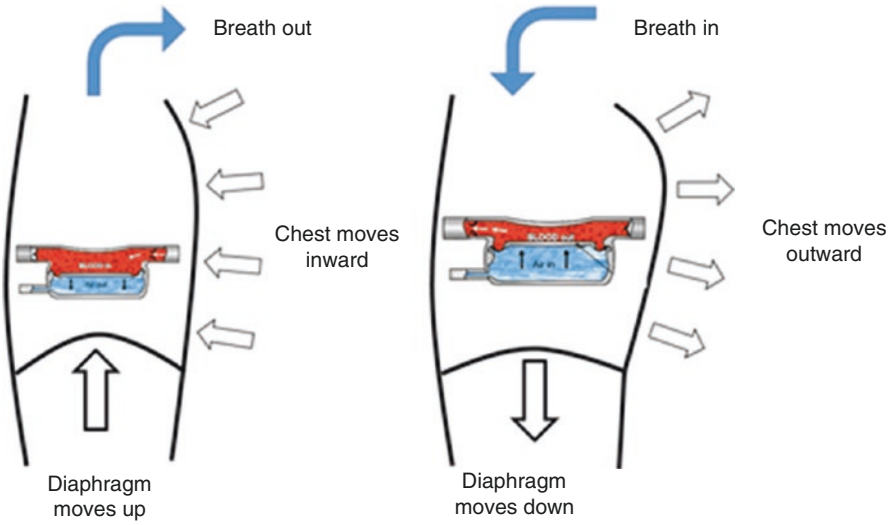

Fig. 7.3 Schematic diagram of respiratory pumping mechanism

is enhanced and expiration enhances pulmonary venous return. Note that the respiratory muscle and cardiac pumps have different rates; the interaction between the three pumps is complex but when they coincide the venous return is enhanced. For example, when we are startled, we contract our muscles and gasp ensuring cardiac filling for flight or fight response.

7.3 Darcy's Law

For those who understand electrical circuit theory, there is considerable similarity between electrical circuit theory and haemodynamics. The physics of blood flow is aided by the understanding of Ohm's law (Eq. 7.1). When considering fluid dynamics instead of:

$$\Delta V = IR, \tag{7.1}$$

where $\Delta V = (V_2 - V_1)$ is the potential difference (voltage) between two points, I is the electric current and R is the electrical resistance, we substitute this formula with Darcy's law:

$$\Delta P = QR, \tag{7.2}$$

with $\Delta P = (P_2 - P_1)$ the pressure difference, Q the volume flow rate and R the flow resistance.

Pressure is defined as the force exerted perpendicularly on the surface of an object (expressed as force per area). However, blood pressure readings are not reported in this way but expressed as millimetres of mercury (mmHg). Since the pressure is changing over the course of the blood vessel, the pressure parameter used is pressure difference (ΔP), also called pressure gradient, which is the difference between the pressure at the beginning of the blood vessel (P_1) and the pressure at the end of the blood vessel (P_2). Because the pressure at capillary level is practically zero, ΔP is effectively the blood pressure (P) in the arterial system. This means that there is no pressure left from the heart to drive the venous system.

The resistance equation is [3]:

$$R = \frac{8}{\pi} \frac{\mu L}{r^4} \tag{7.3}$$

where μ is the blood viscosity, L is the blood vessel length and r is the inside radius of the blood vessel.

Resistance is the main cause for maintenance of pressure in the blood vessel [4]. As seen in Darcy's law, the greater the resistance the lower the flow rate. Resistance has an inverse relation with the fourth power of r (inside radius of the blood vessel), so that with small changes in r, the overall resistance will change dramatically. Viscosity of a fluid measures the interaction (tensile and shear stresses) between flowing particles, which corresponds to the inter-molecular friction of fluid.

The organs and limbs can be thought of as resistors in parallel rather than in series and this is important when it comes to the ability to regulate organ flow and to cope with ischaemia and the contribution of collaterals [3]. The great vessels, like the aorta, are without muscle and their walls are composed of collagen and elastin fibres. This allows them to behave as capacitors and store some of the energy in systole to be released to power flow in diastole; this is important for vessels such as the coronary arteries. Elastic arteries stiffen with age [5] which explains the loss of phasicity of flow with aging. The flow to the heart and the vessel walls themselves is dependent on diastolic flow which is dependent on capacitance. Capacitance reduces when arteries stiffen, thus affecting blood flow to these organs.

In Eq. 7.2, $\Delta P = P_1 - P_2$, where P_1 is the pressure out of the heart and P_2 the pressure in the target organ or peripherally, an increase in P_2 results in a decrease of ΔP and blood flow. For example, if the peripheral arterial resistance increases, blood flow will decrease. With constant resistance (R), if pressure goes up, flow goes up. Flow can be regulated by varying resistance rather than by varying pressures.

$$P_1 \uparrow \Rightarrow \Delta P \uparrow \Rightarrow Q \uparrow \quad (\text{R is constant}) \tag{7.4}$$

Resistance is the sum of fixed resistance and variable resistance. Clinically this might equate to a fixed stenosis plus variable peripheral resistance. For example, in healthy young people, the pressure may be constant during exercise because the peripheral resistance falls to allow an increase in flow. In older people, or in diseased and therefore stiffened arteries, the resistance variability

is reduced and exercise results in increased blood pressure as flow demand increases. This can have pathological consequences. Once the resistance is fixed or at a steady state, any increase in flow is restricted and the pressure P_2 (beyond the stenosis) falls. This is the basis of the ankle/brachial index (ABI) and the effect of exercise on ABI.

7.4 Poiseuille Flow

Suppose that you have a Newtonian fluid flowing in a steady, non-pulsatile manner down a cylindrical, non-elastic pipe of length L and radius r. If the pipe (Fig. 7.4) is long enough (more than L_e, as shown in Eqs. 7.5 and 7.6) the flow will develop a parabolic velocity profile (Fig. 7.5), which is generally called a Poiseuille flow profile [6]. The flow takes its name from Jean Louis Poiseuille, a physician with training in physics and mathematics, who first described this flow structure in 1846.

$$\frac{L_e}{d} \approx 0.06\, R_e, \quad For\ laminar\ flow \tag{7.5}$$

$$\frac{L_e}{d} \approx 4.4 R_e^{\frac{1}{6}}, For\ turbulent\ flow \tag{7.6}$$

where L_e is the entrance length, which is the length of conduit that the flow should travel until the flow velocity profile becomes fully developed, d is the pipe inside diameter and R_e is Reynolds number (Eq. 7.11).

If in Eq. 7.2, we substitute R with Eq. 7.3, the volumetric flow rate (Q) for Poiseuille flow, *i.e.*, the volume of fluid flowing along the tube per unit time, is given by the formula (7.7), where $P_1 - P_2$ is the pressure difference between the two ends of the tube and μ is the viscosity of the fluid.

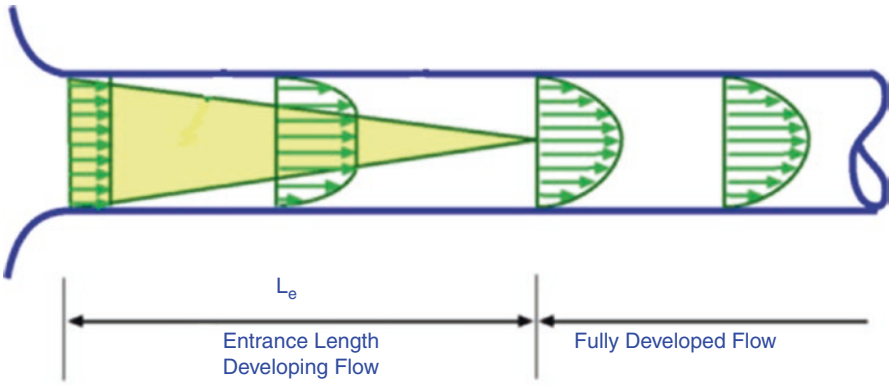

Fig. 7.4 Flow development in a pipe

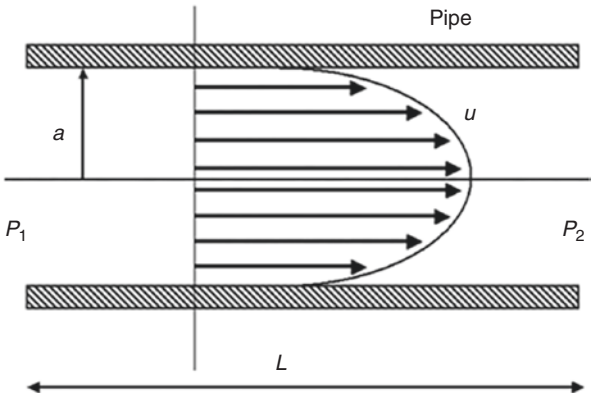

Fig. 7.5 Parabolic velocity profile for fully developed Poiseuille flow

$$Q = \frac{(P_1 - P_2)\pi r^4}{8\mu L} \tag{7.7}$$

The four main characteristics of the Poiseuille (known as the Hagen-Poiseuille) equation [7] are;

1. Constant fluid viscosity;
2. Rigid and cylindrical pipe;
3. Length of the pipe greatly exceeds its diameter;
4. Laminar, steady and non-pulsatile fluid flow.

The physics of the flow is nicely described by this equation. That is, flow is driven by the pressure gradient in the tube or conversely, when there is flow in a tube, then you must have a pressure gradient to drive the flow. Note that increasing length will increase friction and consequently reduce blood flow. Patency, therefore, such as in femoro-popliteal synthetic conduits, is related to the length of the conduit, as well as changes in cross-sectional area, kinking, bending and change in diameter. Therefore, below knee bypass is more prone to occlusion than above knee. This explains better patency in shorter bypass grafts. The internal surface and wall properties of the prosthetic graft should also be considered.

7.5 Laplace's Law of Wall Tension

Laplace's law relates the tension in an arterial or venous wall to the pressure that the elastic tube can apply to the material inside the tube [8]. To assist in understanding this law we consider Fig. 7.6. In this figure, w represents the thickness of the arterial wall, r is the inner radius of the artery, P the inward pressure force due to the elastic nature of the artery and T is tensional stress within the wall of the vessel: the tensional stress therefore points in a direction that is tangential to the vessel wall. Due to mass conservation (see Sect. 7.12), the wall thins as the vessel expands.

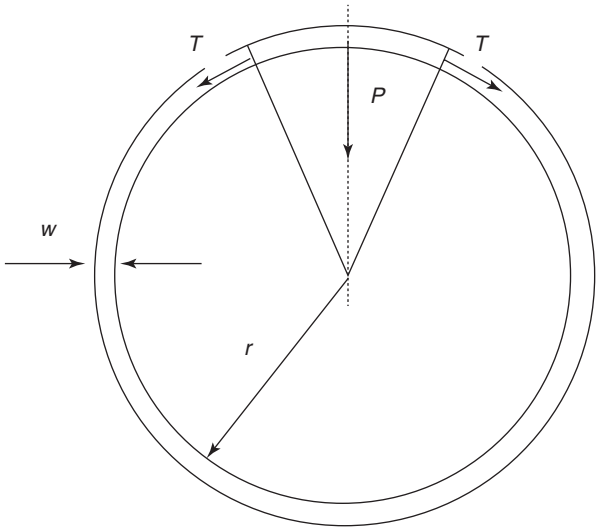

Fig. 7.6 Cross section of an artery showing the various physical components that make up Laplace's law

The formula for Laplace's law is given by Eq. 7.8;

$$P = \frac{w}{r}T, \tag{7.8}$$

where it is usually assumed that the wall thickness, w is small relative to r. This law tells us that the inward pressure that is exerted by the vessel wall on the blood is directly proportional to the tensional stress in the wall and inversely proportional to the radius of the wall. Thus the smaller the vessel, the larger the pressure it can apply on the blood.

Large thin-walled vessels are low pressure vessels. Increasing the pressure distends the vessel and increases the vessel volume which is a characteristic property of veins. For arteries to maintain pressure, the width of the wall must obviously be greater, so large veins are thin-walled and arteries are thick-walled.

One consequence of this behaviour is that, to a certain extent, an artery acts like a long cylindrical party balloon. When one attempts to blow up such a balloon, it is quite difficult to do at the first blow, however once the balloon reaches a particular radius, it usually becomes much easier to expand the balloon. That is, you require less pressure to increase the size of the balloon. This phenomenon is known as instability. If this happens to an artery, then we are dealing with an aneurysm and the relatively constant blood pressure will keep on increasing the size of the aneurysm.

The radius of the artery at which this instability occurs is difficult to compute accurately, but some fairly general arguments suggest that the following formula is a good guide;

$$r_c \sim 2r_0, \tag{7.9}$$

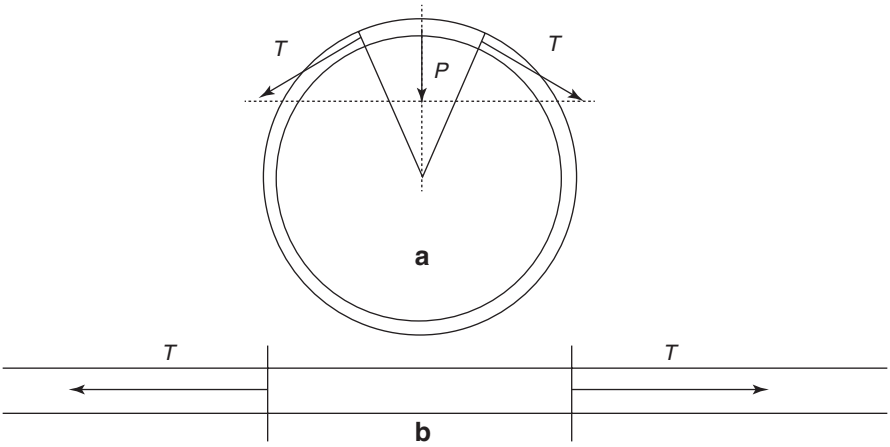

Fig. 7.7 Cross sections of a small artery (**a**) and a very large artery (**b**) showing the stress distribution within the artery wall

where r_c is the critical radius for the onset of the instability and r_0 is the initial radius of the artery. The median diameter of the aorta is 23 mm and the thus aortic rupture is very rare when less than 50 mm in diameter, which is consistent with clinical data [9, 10]. This guide also directs us to consider that the ratio of the diameters is probably more important than the absolute diameter and this should be taken into account when assessing aneurysms in the smaller diameter vessels of women. How arterial wall instability arises is illustrated in Fig. 7.7, where in Fig. 7.7a, we show the stress structure within a small artery. Here the tensile stresses have a component in the radial direction, where the letter *T* labels this component. In Fig. 7.7b the aneurysm/balloon has become very large, such that over a small segment of the wall the artery has hardly any curvature. This is an extreme case, but it does show that there is now no radial component to the tensile stresses. In such a case, the aneurysm can expand freely for just about any internal arterial pressure, hence rupture risk is increased at larger diameter.

7.6 Viscosity Behaviour

When we wish to describe the behaviour of a fluid it is necessary to know something about the frictional properties of the fluid. Consider the schematic depiction of a fluid shown in Fig. 7.8. In this figure, fluid is flowing from left to right along the x direction. For the purposes of illustration, we assume that the speed of the fluid, v, is increasing with increasing height (*i.e.*, increasing y). This means that elements of fluid are sliding past each other and so generating frictional shear stress τ. In a Newtonian fluid, the frictional shear stress is proportional to the rate at which the speed changes as a function of distance, where μ is the viscosity. Therefore, dv/dy in Eq. 7.10 corresponds to the shear rate [11, 12].

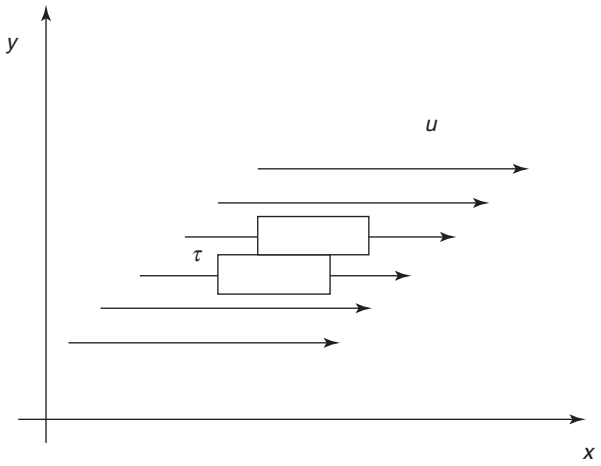

Fig. 7.8 Elements of fluid slide past each other and generate a frictional shear stress

$$\tau = \mu \frac{dv}{dy}, \tag{7.10}$$

Non-Newtonian fluids like blood do not obey Newton's law of viscosity; so the viscosity of blood varies with shear rate. As the blood flow increases (during exercise and peak systole) the flow shear rate increases and consequently the viscosity of blood decreases. This is called "shear thinning". This behaviour usually occurs because at rest, a shear thinning fluid typically has a tangled molecular structure, which makes the fluid relatively viscous. When force is applied, the molecules become ordered, the fluid viscosity decreases and the fluid begins to flow more easily.

In Fig. 7.9, we show the experimentally determined shear thinning behaviour of blood, where the haematocrit value for the blood is 45%. These data show that for high shear rates, which may occur in the large arteries of the body, the viscosity of blood is about four times that of water (where the viscosity of water is approximately one centipoise (*cP*)). However, for lower shear rates, the viscosity of blood can be over 100 times that of water.

This change in viscosity is mostly due to the collective behaviour of red blood cells. At low shear rates, red blood cells form aggregates where they stack one upon another, somewhat like a cylindrical pile of coins or "rouleaux" (Fig. 7.10). When the shear rate increases, the rouleaux are broken down and tend to line up with the flow of fluid and blood viscosity decreases.

However, the viscosity of blood does not increase as blood travels from the arteries through the arterioles and into the capillaries but is approximately constant throughout much of the body. The explanation for this is as follows;

Firstly, the viscosity of blood is dependent on the haematocrit. If the haematocrit decreases, then the blood viscosity decreases. For example, in Fig. 7.9, we show the viscosity of blood as a function of shear rate for 45 and 0% haematocrit. Reducing haematocrit essentially changes blood to a more Newtonian fluid. Secondly,

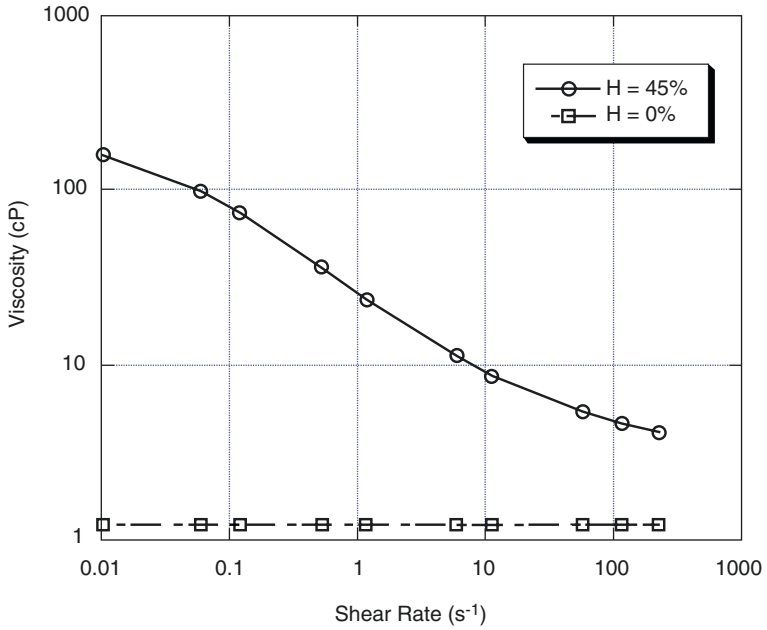

Fig. 7.9 Blood viscosity as a function of shear rate for 0 and 45% haematocrit [28]

haematocrit is dependent on the diameter of the blood vessel. As the blood vessel decreases in diameter, the haematocrit also decreases (Fig. 7.11). This effect occurs because the blood cells tend to move away from the vessel walls and travel where the flow velocity is a maximum in the centre of the blood vessel. This behaviour is known as the Fahraeus Effect and it has been shown to occur in tubes with a diameter as small as 29 μm [13, 14] in the context of a red blood cell diameter of 8 μm.

The combination of these two effects counteracts the effects of shear thinning to maintain constant viscosity of blood throughout the body. It is important to understand how these properties affect shear stress between blood and vessel walls—or more relevantly between blood and atheroma.

7.7 Reynolds Number

The Reynolds number is an important dimensionless parameter (it has no units) used in fluid mechanics to measure whether the fluid flow pattern in a conduit is laminar, transient or turbulent. The Reynolds number is related to blood density, viscosity, velocity, and the diameter of the blood vessel. Reynolds number can be defined as the ratio of inertia force ($\boldsymbol{\rho v D}$) to viscosity or friction force ($\boldsymbol{\mu}$). The Reynolds number for flow in a pipe is given by:

$$\mathrm{Re} = \frac{\rho \mathrm{v} D}{\mu} = \frac{\mathrm{v} D}{\nu}, \tag{7.11}$$

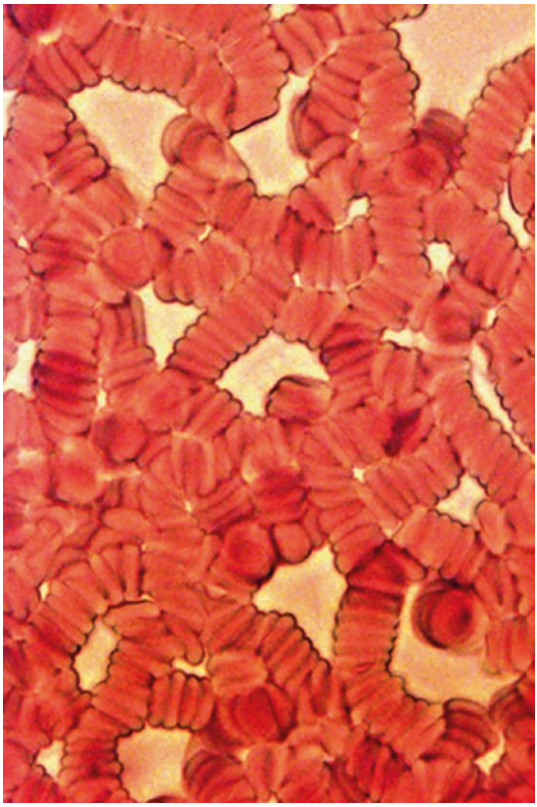

Fig. 7.10 Rouleaux blood cell formation seen in capillaries

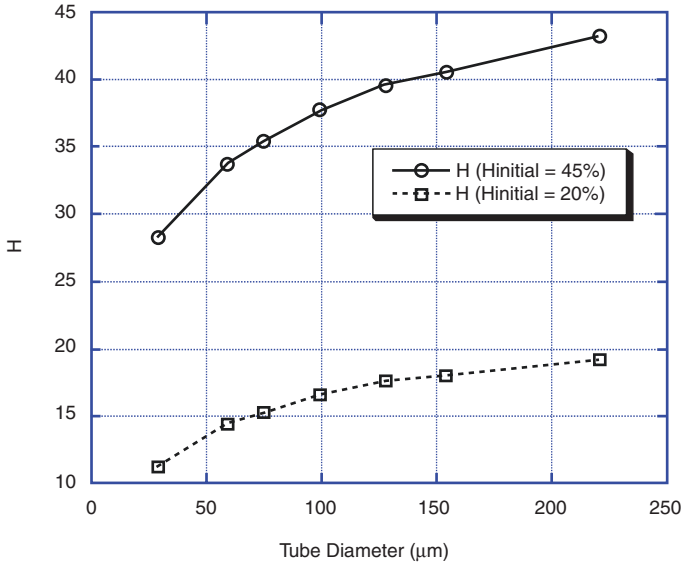

Fig. 7.11 Hematocrit as a function of tube diameter. The initial hematocrit value for each line is shown in the inset box [29]

where:

$\boldsymbol{\rho}$ = Fluid density (kg/m^3).
v = Flow velocity over cross-section area (m/s).
$\boldsymbol{D}$ = Hydraulic diameter (m).
$\boldsymbol{\mu}$ = Dynamic viscosity (N.s/m^2).
$\boldsymbol{v} = \boldsymbol{\mu}/\boldsymbol{\rho}$ = Kinematic viscosity (m^2/s).

In an artery, if the flow is assumed to be Newtonian then it is predicted to change from laminar to turbulent at a Reynolds number of approximately 2000, which is known as the critical Reynolds number. For less than the critical number, the viscous effects are dominant and flow is laminar. For numbers near to 2000, flow is called "transient", which is neither laminar nor turbulent [15]. Laminar or turbulent flow can be beneficial or detrimental. It is possible the properties in the composite nature of blood work in this transitional range to prevent turbulent flow. This would be important during high cardiac output especially in children and athletes.

For example, let us calculate Re the peak value of Reynolds number for an ascending aorta of diameter, $\boldsymbol{D}$ = 3 cm, peak blood flow speed $\boldsymbol{u}$ = 60 cm/s, blood density ρ = 1.06 g/cc and blood viscosity μ = 0.0036 ***Pa.s***. These values give ***Re*** = 5300 and turbulent flow would be expected but there is no evidence for this happening on ultrasound.

Fluid flowing in a laminar fashion is dominated by the viscosity and at a high Reynolds number by its inertia. When fluid has turbulent flow such as in an arterial stenosis, an audible bruit is heard called Korotkoff sounds [16]. This is the result of chaotic flow at high velocity which transforms energy to noise. Flow is inefficient and may be disruptive as in a carotid stenosis, where turbulence can increase the risk of dislodging material from plaque as embolus. Turbulent flow is less efficient relative to laminar flow. This means that more energy or a greater pressure drop is required to drive turbulent flow compared to laminar flow.

It is interesting to speculate that the particulate nature of blood and plasma composition may act to discourage the formation of turbulent flow. The bi-concave shape of the red cell could be crucial in affecting cell-cell interactions so that flow tends to remain laminar. This shape may also enhance the efficiency of blood flow by vortex shedding in addition to increasing surface area for oxygen delivery.

7.8 Womersley Number

Now we must consider the effect of pulsatile versus constant flow. The Womersley number (***W***) is a dimensionless parameter in haemodynamic and biofluid mechanics which typically characterises pulsatile flow within an artery. It denotes the ratio of inertial forces to viscous forces. It is named after John R. Womersley (1907–1958) [17], for his work on blood flow behavior in arteries. The Womersley number of blood flow can be measured by:

$$W = r\sqrt{\frac{2\pi f \rho}{\mu}} \tag{7.12}$$

where r is the radius of the artery, $\boldsymbol{f}$ is the pulsatile frequency (heart bpm = 60 times $\boldsymbol{f(Hz)}$), $\boldsymbol{\rho}$ = 1060 kg/m^3 is blood fluid density, and $\boldsymbol{\mu}$ = 0.00345 Pascal-Second (***Pa.s***) is the viscosity.

If $\mathbf{0 < W < 1}$ the flow is governed by viscosity effect, and if $\boldsymbol{W}$ is much greater than 1, the flow is governed by transient effect (unsteady flow with time-dependent velocity and pressure). When $\boldsymbol{W}$ is low, the velocity profiles are parabolic in shape. For $\boldsymbol{W}$ more than 10, the unsteady inertial force governs the flow motion.

From here one can see that blood flow steadies with change in diameter and flow divergence. The arterial system uses this to enable flow during diastole to organs that do not allow perfusion during systole such as the heart and great vessels themselves. This is enabled through the capacitance of the great vessels because of their elasticity. The Reynolds number (Eq. 7.13) and the Womersley number (Eq. 7.14) are nondimensional parameters that investigate the pulsatile flow pattern. They are useful in solving haemodynamic problems.

$$\mathrm{Re} = \frac{convective\ inertia\ force}{viscous\ friction\ force} \tag{7.13}$$

$$W = \frac{transient\ inertia\ force}{viscous\ friction\ force} \tag{7.14}$$

Utilizing Womersley and Reynolds numbers may provide insights into detrimental to-and-fro and turbulent flow patterns. For example, does connecting a bypass graft around a diseased native vessel that is still delivering blood cause the two parallel blood flows to compete, with the potential for thrombosis in one channel? An increase in Womersley and Reynolds numbers indicate significant increase in the complexity of the blood flow pattern and changes in the vortex flow position. Therefore, understanding quality of flow is crucial for good operative planning and choice of conduit. Some typical values for the Womersley number are shown in Fig. 7.12:

7.9 Bernoulli's Equation

Johann Bernoulli (1667–1748) was a professor in Basel and taught physics, anatomy and physiology. His understanding lies at the heart of vascular physics and relates pressure to motion and energy. For a fluid that has no viscosity, one can write;

$$P + \rho\frac{\mathrm{v}^2}{2} + \rho g y = constant \tag{7.15}$$

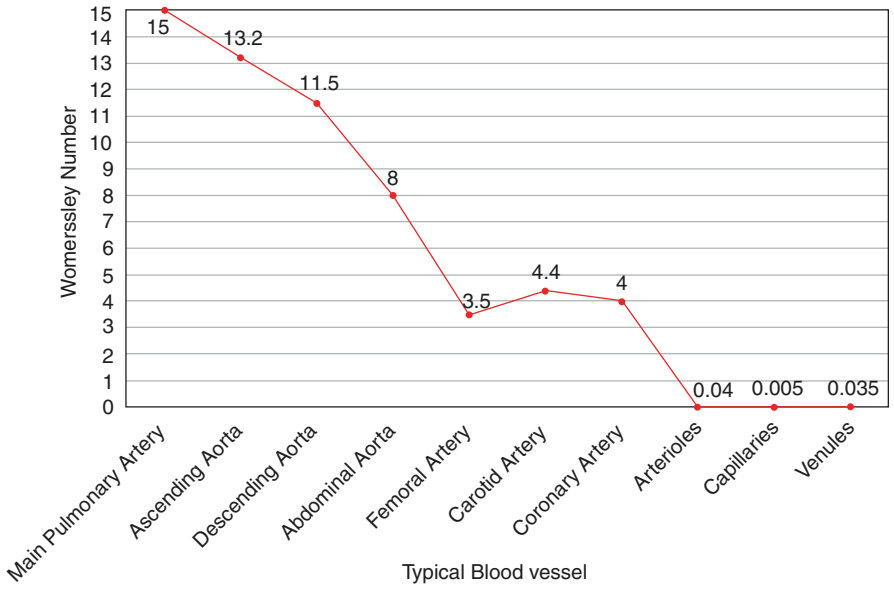

Fig. 7.12 Blood vessel type vs. typical Womersley number

where P is the pressure, ρ the mass density of the liquid, v the speed of the fluid, g the gravitational acceleration, and y the height. In other words, the Bernoulli equation states that the pressure plus the kinetic energy per unit volume $\rho\frac{v^2}{2}$, plus the potential energy per unit volume, ρgy, is a constant at any point along the blood vessel. The Bernoulli equation takes into account the effect of gravity as well as resistance, flow and pressure. This is the basis of Buerger's test, the Trendelenberg position and Roos test.

It should be understood that Eq. 7.15 is an approximation, as it ignores the loss of energy due to shearing friction between the flowing blood and the walls of the artery. Even so, it does provide us with an intuitive understanding of the physics of the arterial/venous system. For example, suppose we wish to measure the blood pressure of a person. Typically one places an external cuff around the upper arm which is approximately at the same level as the heart and so the pressure will not be affected by any difference in height. To measure the systolic pressure, the cuff pressure is increased until all blood flow ceases. From Eq. 7.15 we know that this "cut-off" pressure is the maximum pressure in the artery. The pressure in the external cuff is then reduced until the flow is a maximum. This is the diastolic pressure.

In practice, the arterial system has two sources of potential energy to drive the blood forward. The first is blood pressure and this is transformed into the kinetic energy of flow during the period between systole and diastole, and the second is stored energy in the wall of the artery, called capacitance. Consider what might happen when the kinetic energy meets a resistive obstacle—some energy is dissipated as heat as in electrical circuit theory and some is stored for use in diastole for onward flow in the period of heart filling by the elasticity of the large blood vessels

acting as capacitors. However, some energy is used up due to repetitive alterations in forward pressure and resistive back-pressure from pulsatile flow. This phenomenon is called water hammer. The injury and healing cycle effect of these water hammers on atherogenesis and aneurysm behaviour at stress points has yet to be fully determined.

7.10 Young's Modulus and Pulsatile Flow

Blood flows through the arteries in a pulsatile fashion. Arteries are semi-elastic tubes and expand and contract as the pulse of blood flows along them. The speed, $\boldsymbol{c}$, at which blood flows along an artery is determined by the speed that a pulse of fluid can travel along an elastic tube. This speed is given, approximately, by the Moen-Korteweg formula;

$$c \approx \sqrt{\frac{Eh}{\rho d}}, \tag{7.16}$$

where $\boldsymbol{E}$ is Young's modulus for the wall of the artery, $\boldsymbol{h}$ is the thickness of the artery, $\boldsymbol{d}$ is the inner diameter of the artery and $\boldsymbol{\rho}$ is the density of blood. A schematic depiction of how a pulsatile wave propagates along an artery is given in Fig. 7.13.

As can be seen from Eq. 7.16, the speed at which blood travels along an artery is partially dependent on the Young's Modulus of the arterial wall. To illustrate the definition of Young's Modulus it is useful to consider Fig. 7.14, where a block of material is being stretched due to an applied force on one end of the block.

The block has a natural length denoted by $\boldsymbol{L}$, when a force $\boldsymbol{F}$ is applied to one side of the block then the length of the block increases by $\boldsymbol{\Delta L}$. This change in length is known as a *strain*, $\boldsymbol{\varepsilon}$, and it is defined by the equation:

$$\varepsilon = \frac{\Delta L}{L}, \tag{7.17}$$

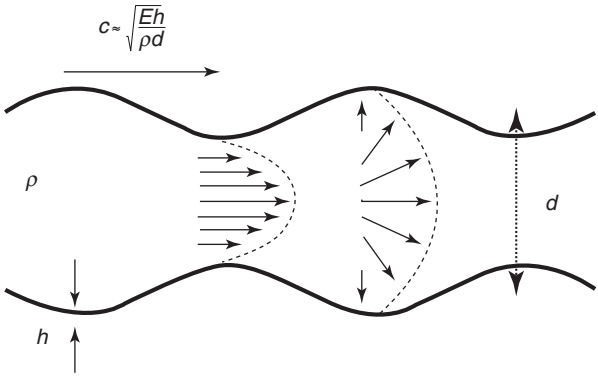

Fig. 7.13 An exaggerated, schematic view of blood flow in an artery

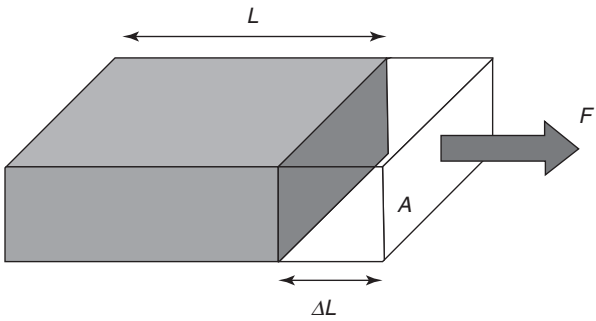

Fig. 7.14 A block of material with a length, L, and side area A is subject to a force F. The applied force stretches the block a distance ΔL

The *stress* ($\boldsymbol{\sigma}$) that the force applies to the block of material has the definition

$$\sigma = \frac{F}{A}, \tag{7.18}$$

Young's Modulus is defined as the stress over the strain, *i.e.*,

$$E = \frac{\sigma}{\varepsilon} \tag{7.19}$$

Young's modulus is a measure of how easy it is to stretch and compress a material. Thomas Young (1773–1829) was a medical physician who made significant contributions to fields of Physics (through his experiments which demonstrated the wave-like nature of light), medicine (with his studies of blood flow), and structural mechanics (e.g., Young's Modulus). Surprisingly however, despite being well aware of the elastic nature of arteries, he does not appear to have used Young's Modulus to describe their properties.

One consequence of ageing is increasing stiffness in the arteries. This means that the Young's modulus increases and this, as a consequence of Eq. 7.16, increases the speed of pulsatile flow within the arterial system.

7.11 Mass Conservation

In Fig. 7.15, we view a schematic depiction of an artery that is changing in shape as one travels along the artery. The blood flows in at one end with a speed u_1. The area at the inlet of the artery is given by A_1. In its simplest form, the mass conservation equation provides us with the relationship between the quantities at the proximal and distal ends of the artery:

$$\mathrm{v}_1 A_1 = \mathrm{v}_2 A_2 \;\; u_1 A_1 = u_2 A_2, \tag{7.20}$$

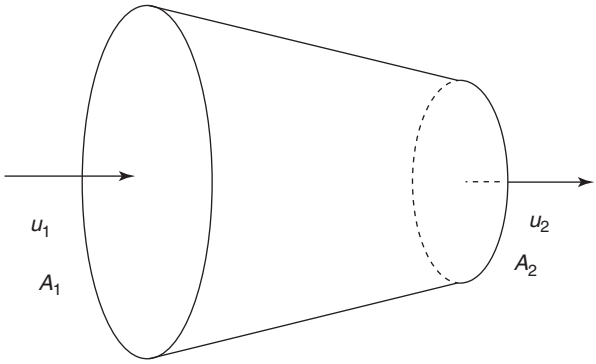

Fig. 7.15 A change in the diameter of an artery leads to a change in the blood flow speed

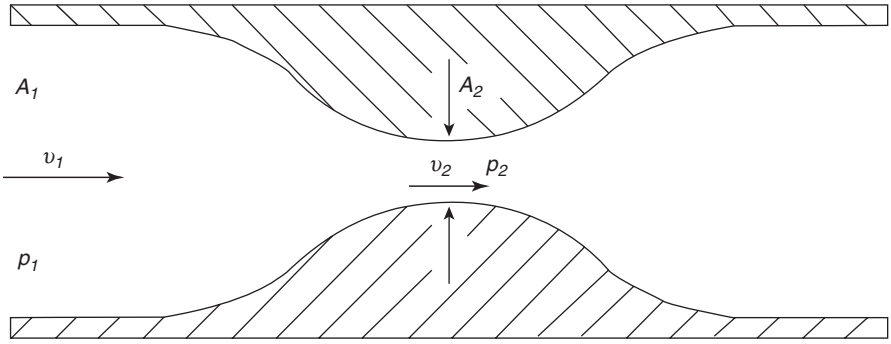

Fig. 7.16 Flow through a blood vessel with a stenosis

Here v_2 and A_2 are the outlet flow speed and area, respectively. In plain English, Eq. 7.20 is another way of saying "what goes in must come out".

We can see from Eq. 7.20 that if an artery becomes narrower, i.e., A_2 becomes smaller, then the flow speed, v_2, increases. This occurs because the mass flow cannot be created or destroyed and so if the tube becomes narrower, then the flow rate has to increase. Stenosis in a blood vessel wall may be caused by atherosclerosis or restenosis following an intervention (Fig. 7.16).

7.12 Arterial Dissection, Collateral Circulation and Competing Flows

Up to this point we have essentially discussed flow in series. Much of the normal circulation in the human and some pathological flow occurs in parallel. Examples of parallel flow include the collateral circulation in each segment of the body; the profunda system in the thigh, the geniculate system around the knee and the tibial system in the leg. Another good example is the carotid and vertebral systems

combining to form the cerebral circulation. In parallel circulation, the pressure at the separation of the two systems is theoretically the same for each, and the pressure at the re-union is also the same for each. The proportion of ongoing flow from the two systems is determined by the resistance of each system. These two therefore compete for the proportion of on-flow. This works well to direct or redirect the flow to different target tissues. The body may select priorities for flow, for example, the brain and heart in shock or the muscles during exercise. The branches of the great vessels and arteries to the tissues are resistance vessels and they have muscular walls for this purpose.

The formula for resistors in parallel circuits is:

$$\frac{1}{R_{total}} = \frac{1}{R_1} + \frac{1}{R_2} + \ldots + \frac{1}{R_n}, \tag{7.21}$$

where n is the number of parallel circuits.

These circuits also provide alternative channels should the dynamics change due to injury or disease. Not all parallel circuits are beneficial. Detrimental competing flows may occur with artificially created channels, for example, aorto-bifemoral bypass, when one iliac system is normal and the other occluded. The competing flows on the normal side predispose for either that limb of the graft or part of the iliac system on that side to occlude. Similarly, with femoro-popliteal bypass after long-standing superficial femoral artery occlusion when the profunda collateral flow has been well developed. If it is desirable to maintain patency of a vessel is may be better to do this early for longer term patency, before the collaterals develop. This is obviously debatable in clinical practice, but worthy of consideration.

In aortic dissection, the outflow from the false lumen is met with greater resistance than the outflow from the true lumen. The flows compete at fenestrations or where the intima has been torn off the origin of a branch vessel. The pressure is higher in the false lumen at any time in the cardiac cycle other than peak systole. Figure 7.17 shows the trace from true and false lumens of a dissected aorta. Note the systolic pressure is the same in each lumen at 138 mmHg. The diastolic pressure is higher in the false lumen at 93 mmHg compared to the diastolic in the true lumen of 82 mmHg. The area under the curve is the same and so the pulse wave in the false lumen is wider. The mean pressure in the false lumen is higher at 109 mmHg than the true lumen, where the mean is 91 mmHg.

This means that the false lumen is generally the larger of the two and is more likely to dilate. Flow of contrast injected into the true lumen is not seen to flow out to the false lumen through the holes in the membrane unless the pressure of the injection and the pressure of the lumen together exceed the pressure of the false lumen. The membrane that is the remnant of the intima oscillates as the pressure ratio between the true and false lumen changes during the cardiac cycle. This dynamic also applies for a Type 1 endoleak into the residual sac of an aortic aneurysm treated by an endovascular graft.

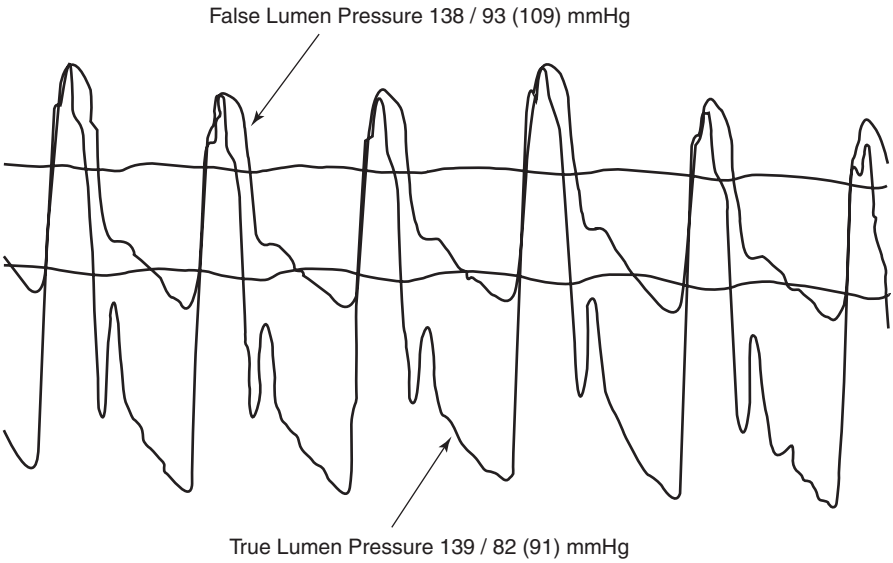

Fig. 7.17 Pressure readings from the true and false lumens of a dissected abdominal aorta (courtesy of Dr. John Anderson)

7.13 Shear Stress and Pressure

All vascular clinicians are familiar with the ultimate shearing force injury of high velocity impact when the mobile arch of the aorta and heart continue to move forward while the descending aorta is held by the intercostal and posterior mediastinum to the vertebral bodies. What of subtle persistent long-term shear stresses and the relationship with the greatest risk factor for arterial disease—age? There are known common sites for occlusive atheromatous plaques e.g. the carotid bifurcation, aortic bifurcation, origins of branches of the aorta and coronary arteries and shear stress points such as the adductor canal.

Atheroma is an arterial lesion. It is only seen in veins subject to long term pulsatile pressure when they are said to be "arterialised". Pressure and pulsatility are the forces involved here and the biochemical and biological responses act as accelerators and decelerators.

Shear stress on an arterial wall, τ_w, due to Poiseuille fluid flow is given by the formula; [18]

$$\tau_w = \frac{4\mu Q}{\pi r^3} \tag{7.22}$$

where r is the radius of the artery and Q is the volume flow rate of blood through the artery. From this formula, it can be seen that shear stress increases with the increase

of blood flow through the artery and tends to increase as the artery becomes smaller in diameter—provided that the volume flow rate and the viscosity are approximately constant.

Atherosclerotic lesions form at specific areas where low and oscillatory endothelial shear stress occur. High risk plaques have a large lipid core, thin and inflamed fibrous cap and excessive expansive remodeling [19]. Wall shear stress may rupture the established plaque. Plaque rupture and intraplaque haemorrhage are recognized causes of cardiac events. Computational models from CT scans of carotid bifurcations with atherosclerotic plaques showed that stresses in the fibrous cap and around the plaque shoulders affect plaque rupture risk, with higher stress and plaque rupture risk for thinner caps [20–23].

7.14 Forces on Graft Systems

The performance of stent grafts was found to be different to open repair with a sutured replacement of the artery because of unsuspected influences, as mentioned above, that relate to sustained physical forces [1]. The openly-sutured prosthesis binds the wall of the artery to the prosthesis with a transmural suture. The artery may expand above or below the prosthesis. However, at the point of attachment the artery wall is held to the fixed diameter by the through-wall suture for as long as the suture holds. Endoluminal grafts (ELG) to date do not bind the adventitia to the prosthesis—they merely attach. The ELG must continue to act to bridge the gap between the normal artery above and below until, if ever, the aneurysm's cavity shrinks right down. The diameters of the grafts used for the same Abdominal Aortic Aneurysm (AAA) differ markedly between the open and ELG methods. The common diameters used for tube replacement surgically of infrarenal AAA is 18 or 20 mm. The commonest diameter for a stent graft is 26 or 28 mm and 30+ mm is not uncommon. Why is there such a discrepancy when the surgeon judges the diameter for suitable fit? This discrepancy is due to the different types of attachment of an open graft and a stent graft. With the former, the aortic diameter is permanently fixed to the diameter of the graft in its pressurised state. The diameter of a crimped vascular graft is, by definition, the minimum internal distance between the crimps in the non-pressurised state. It is increased by approximately 10% when pressurized. With the ELG, a residual radial force is required for seal and the oversize allowance must accommodate elasticity and compliance while maintaining the seal between pulsations for the whole of the length of the sealing zone. With a stent graft the long-term function and durability demands are different and greater [1]. Understanding the forces involved is basic to the design and use of new technology, and the weaknesses that lead to aneurysmal disease provide an ongoing challenge because it is progressive [1, 24].

A mistaken clinical impression is that the forces on a thoracic ELG should be greater than those on an abdominal ELG. The flow and diameter of the thoracic aorta are greater and the haemodynamic forces potentially much larger. However,

because the diameter of the graft changes little, if at all, the downward displacement force in the thoracic ELG is small as the resistance in the graft is low—except on the curve of the aortic arch. The resistance of any graft that extends into the iliac vessels is much greater because of the significant change in diameter and high resistance with the graft acting like a windsock or sea anchor [24]. An aorto-uni-iliac device affords greater resistance than a bifurcated graft and detachment at the neck and migration is a common problem due to high displacement forces. The force applied to the thoracic graft is on the curve and centrifugal forces apply. Since every action has an equal and opposite reaction (Newton's third law), one must ask where is the reaction? The reaction is to pull the graft out from the top and the bottom almost equally. When stent grafts were first used in the thorax, unexpected upward migration of the distal end emerged as the problem, especially when there was a significant curve on the graft. For the same reason, this 'lift out' may also be seen from the iliac arteries when the graft fixation is weak because of ectasia and/or short length of distal attachment. Type 1B endoleak can be more dangerous than Type 1A if this factor is ignored and it is important to have an understanding of the possible forces that may be exerted on a graft.

To illustrate the steps used in determining the forces on a graft system, via analytic equations, we consider the steady flow of blood through a bent pipe (Fig. 7.18). In this figure, the proximal inlet entrance is labelled (1) and the distal exit by (2). D_1, A_1 and D_2, A_2 are the diameters and cross-sectional areas, respectively, of the graft at the points 1 and 2. The blood flow forces (A_1 and A_2) on the grafted system are at angles of θ_1 and θ_2 to the vertical, so can only be calculated when the inlet and outlet force components are in the same plane. Similarly p and $\boldsymbol{v}$ refer to the pressures and velocities at these points. R_x and R_y are the x and y components of the restoring force. The external pressure on the graft system is denoted by P_{ex}.

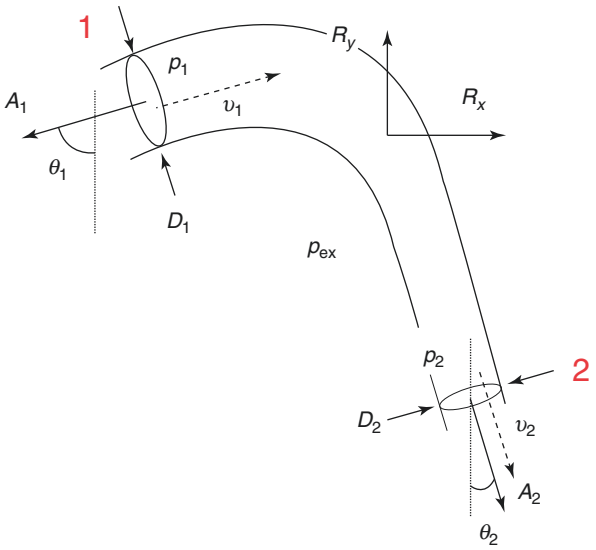

Fig. 7.18 The characteristic velocity, pressure, area and force vectors required to compute the restraining forces on a bent, single-tube graft system

In our analysis, we assume a steady-state, *i.e.*, non-pulsatile, flow. We do this as it gives us a basic idea of how the system is behaving. The first equation is the steady-state **mass conservation** equation, which we rewrite in the form:

$$v_1 A_1 = v_2 A_2 \tag{7.23}$$

One should note that v_1 and v_2 are average flow speeds, where the average is taken over the areas of A_1 and A_2 respectively.

The next analysis tool at our disposal is the **momentum conservation** equation, which can be expressed in the form;

$$R_x = \left(P_2 - P_{ex}\right) A_2 \sin\theta_2 - \left(P_1 - P_{ex}\right) A_1 \sin\theta_1 + \rho v_2^2 A_2 \sin\theta_2 - \rho v_1^2 A_1 \sin\theta_1 \tag{7.24}$$

and

$$R_y = -\left(P_1 - P_{ex}\right) A_1 \cos\theta_1 - \left(P_2 - P_{ex}\right) A_2 \cos\theta_2 - \rho v_1^2 A_1 \cos\theta_2 - \rho v_2^2 A_2 \cos\theta_2 \tag{7.25}$$

where in these formulae, we have ignored the weight of the graft and the weight of blood in the graft. These terms are easily included into the equations, if required.

Energy is the final conserved quantity that we can use in our analysis. The **energy conservation equation** has the form:

$$\frac{P_1}{\gamma} + \frac{\alpha_1 v_1^2}{2g} + Z_1 = \frac{P_2}{\gamma} + \frac{\alpha_2 v_2^2}{2g} + Z_2 + h_L, \tag{7.26}$$

where g is the gravitational acceleration, $\gamma = \rho g$ is the weight density of blood, z_1 and z_2 are the vertical heights of the proximal and distal ends of the graft, respectively, and h_L is the 'head loss' in the pipe, *i.e.*, the amount of pressure or energy that is lost due to frictional viscous effects as the fluid travels through the pipe. Head loss is usually given by the equation;

$$h_L = K_L \frac{v_2^2}{2g}, \tag{7.27}$$

where K_L is a constant, the value of which is usually dependent on the shape, length and diameter of the pipe. The coefficients α_1 and α_2 are kinetic energy correction factors that have different values depending on the type of flow. For example, for uniform flow $\alpha = 1$, turbulent flow has $\alpha \approx 1$, and laminar flow gives $\alpha = 2$.

By combining Eqs. 7.23, 7.26 and 7.27, one obtains

$$P_2 = P_1 + \frac{\gamma v_1^2}{2g}\left(\alpha_1 - \left(\alpha_2 + K_L\right)\left(\frac{A_1}{A_2}\right)^2\right) + \gamma\left(Z_1 - Z_2\right) \tag{7.28}$$

So, by using Eqs. 7.23 and 7.28, we can express p_2 and v_2 in terms of quantities at the entrance of the graft. This then allows us to compute the restraining forces on the graft system by then using Eqs. 7.24 and 7.25.

7.14.1 Case 1: The Cylindrical Graft

For this case (Fig. 7.19), the inlet and the outlet areas are the same, so, by Eq. 7.23, the inlet and outlet flow speeds are also equal. The angles θ_1 and θ_2 are equal and have a value of 90°. The inlet and outlet pressures are not equal due to the frictional, shear interaction between the blood and the graft (*i.e.*, the head loss as given by Eq. 7.27). This frictional interaction causes the outlet pressure, p_2, to be less than the inlet pressure, P_1. This is called the pressure drop.

In considering the restraint forces on the graft, we know there are no vertical forces generated by blood flowing through a horizontal graft in this case. The horizontal force on the graft is quite small, therefore one can conclude that straight, cylindrical grafts only feel a relatively small drag force in the direction of the flow.

7.14.2 Case 2: The Windsock Graft

Suppose now we consider a graft in the shape of a wind-sock, such as in Fig. 7.20. For this case, the inlet area is now larger than and the outlet area, so, by Eq. 7.23, the outlet flow speed is greater than the inlet flow speed as given by;

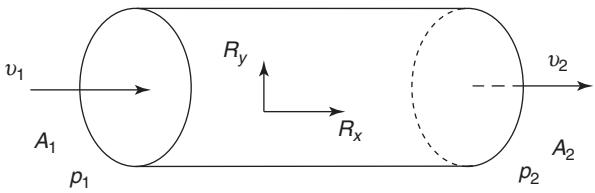

Fig. 7.19 Cylindrical graft

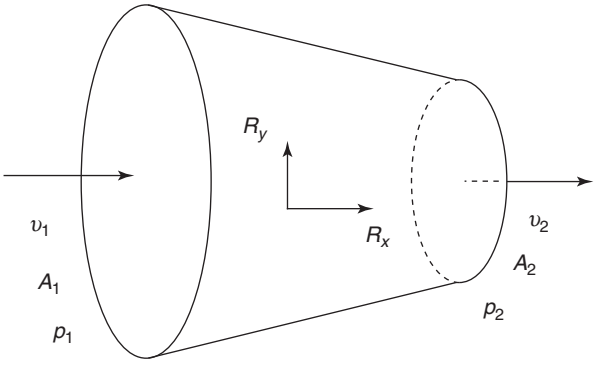

Fig. 7.20 A stent graft in the shape of a wind-sock

$$v_2 = \left(\frac{A_1}{A_2}\right) v_1 \tag{7.29}$$

As in the previous case, the angles θ_1 and θ_2 are equal and have a value of 90° and the inlet and outlet pressures are not equal due to the frictional, shear interaction between the blood and the graft. The restraint forces on this graft act to create a windsock which has a much larger drag force than a cylindrical graft.

7.14.3 Case 3: The Curved Graft (Fig. 7.21)

As with the cylindrical graft, the inlet and the outlet areas are the same, so, by Eq. 7.23, the inlet and outlet flow speeds are also equal. Due to the symmetry of the situation, the vertical restraint force is zero, the horizontal restraint force however is much greater. A tortuous stent graft which also narrows is subjected to both axial and radial forces.

7.14.4 Case 4: The Symmetrical Bifurcated Graft

Suppose that we consider a symmetric bifurcated graft, such as shown in Fig. 7.22, where the two outlet distal legs of the graft are at an angle α to the horizontal, the two distal ends are equal and gravity is ignored. The proximal end of the graft is labelled by the number 1, the symmetric distal ends by 2 and 3. We also know to satisfy mass conservation that the flow in has to equal the sum of the outflows.

By applying Bernoulli's equation and the mass conservation equations we can calculate that the horizontal restraint force is strongly dependent on inlet area, pressure and on the bifurcation angle (especially >15°). However, the blood inlet

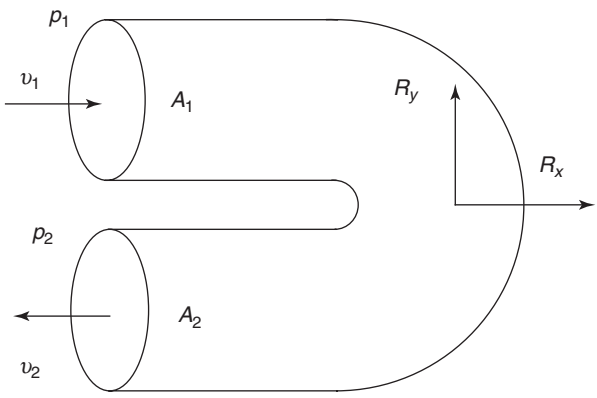

Fig. 7.21 Curved graft

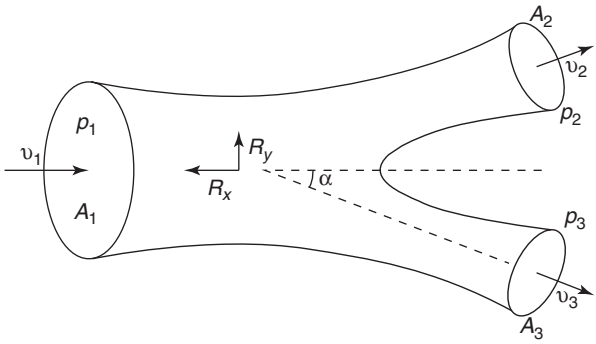

Fig. 7.22 Symmetric bifurcated graft

velocity or flow rate has negligible effect on the horizontal restraint force [25–27]. Naturally, a steady-state assumption is questionable, since pulsatile flow occurs in the human body. However, it was shown experimentally that a steady-state analytical model can be used, with variable pressure and flow rate inputs, to predict forces on a symmetric, bifurcated graft in pulsatile flow with reasonable approximation within design limits [26, 27].

7.15 Conclusion

Understanding the physics of the vascular system in health and disease influences vascular management. This is a rich field for research. Further clues to atherogenesis may lie in the differences in the fluid dynamics and stresses applied to the arterial system particular to branch points. Computational modelling will be of increasing importance to our understanding of vascular haemodynamics, the behaviour of aortic dissection, the impact of devices on arterial disease and prediction of injury and rupture risk.

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Chapter 8
Computational Fluid Dynamics in the Arterial System: Implications for Vascular Disease and Treatment

Siamak Mishani, Shirley Jansen, Michael Lawrence-Brown, Christopher Lagat, and Brian Evans

Key Learning Points

- Pulsatile blood haemodynamics and irregular geometry of arterial wall play an important role in the formation of vascular disease.
- Due to nonhomogeneous and anisotropic structural behaviour of the blood vessel and non-Newtonian shear thinning properties of blood flow, the response of the arterial wall to blood pressure is complex.
- Multidisciplinary numerical modelling (CFD, FEA and FSI methods) can simulate pulsatile blood flow in an artery to calculate force transferred from blood flow into blood vessel and determine the resultant stresses in the arterial layers (intima, media, and adventitia).
- The accuracy of the numerical model is influenced by geometries and meshing resolution, initial and boundary conditions, and fluid-solid coupling method.

8.1 Introduction

The arterial tree is a branching tube composed of three nonhomogeneous layers subject to pulsatile blood pressure. Biomechanical analyses of the strain and stress in, and between, the layers can provide a good understanding of the relationships between blood flow and the arterial wall and therefore, of arterial disease. Pulsatile

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blood haemodynamics and arterial wall geometry, which is irregular, play an important role in the formation of vascular disease especially in areas of complex blood flow [1, 2]. Disturbance of blood flow occurs in regions where arteries branch or vary in cross-sectional area [3, 4]. The response of the arterial wall to blood pressure is also complex, due to there being a nonlinear, time-dependent structural response to blood pressure, and nonhomogeneous and anisotropic structural behaviour of the blood vessel wall.

Blood pressure affects the arterial wall, and in turn, the elastic deformation of the arterial wall influences blood flow. The intima can transduce displacements and pressure from the blood flow to the arterial wall and vice versa. This phenomenon can be analysed using three numerical approaches based on coupling fluid flow dynamics with structural analysis:

1. CFD (Computational Fluid Dynamics) uses numerical methods to simulate and analyse blood flow behaviour.
2. FEA (Finite Element Analysis) uses numerical methods to predict stress and strain in the arterial wall.
3. FSI (Fluid-Solid Interaction) is modelling the interaction of the elastic blood vessel with blood flow and pressure.

These three approaches used together can help us understand the behaviour of blood flow and deformation of the arterial wall to investigate arterial disease and the behaviour of prosthetic devices. This chapter explains the modelling techniques for characterizing stress and strain within different layers of the arterial wall and blood flow patterns in an artery.

8.2 Terminology

We include the following glossary to explain terms used in this chapter;

- **Analytical method:** Solving the problem using ordinary analysis which can be obtained with pencil and paper.
- **Numerical method:** Using mathematical methods to solve complicated problems using computer programming.
- **Governing equation:** The mathematical statements of the three fundamental equations (continuity, momentum and energy) which describe the physical principles of fluid dynamics.
- **Anisotropic material:** A substance with different mechanical properties in different directions.
- **Validation in FEA:** Using equations to quantify the uncertainty of the results (get the right physics).
- **Verification in FEA:** Using equations to quantify the errors of the results (get the right mathematics).
- **Transient flow:** An unsteady flow with position and time dependent velocity and pressure.

- **Secondary Flow:** In non-uniform and non-circular conduits, there exists the phenomenon that relatively minor fluid particles flow away from main fluid stream, which flows parallel to intramural surface of the conduit, toward the surface of conduit at any flow section, this minor flow is called secondary flow.
- **Separation point:** In a conduit, when a fluid flows over a curved surface, such as a bifurcation or stenosis, the fluid may separate from the inner surface of the conduit (wall boundary layer). This point, at which the wall shear stress vanishes, is called the flow separation point. The characteristics of the flow after the separation point will be turbulent with eddies and vortices.
- **Poisson's ratio:** The ratio of the change in lateral strain to the axial strain of a material under uniaxial elastic stretching.
- **Control volume:** Creating a mathematical model of a volume in space which is fixed or moving with constant velocity through fluid flow. The surface enclosing the control volume is referred as the volume boundaries.
- **Boundary conditions (BC):** The physical conditions that are required in a model to set specific parameters for displacements and forces.
- **Discretisation (Meshing):** The process of subdividing the domain (body) of a finite element system with associated nodes and elements.
- **Mesh refinement:** Increasing the number of elements in a domain to improve the accuracy of a solution, but requiring longer processing time.
- **Element (Cell):** The small component of domain in a structure of fluid which is interconnected to other components within the domain.
- **Nodes:** The points on the corners of each element.
- **Edge:** Boundary of a face of an element.
- **Face:** Boundary of an element.
- **Domain:** A grouping of nodes, faces, and elements of a structure or fluid.

8.3 Finite Element Analysis (FEA)

Finite element analysis (FEA) is a numerical technique used to obtain an approximate answer to a complex engineering and physical problem in a specific domain. In the early 1940s, FEA was developed to solve complex elasticity and structural analysis problems such as buildings and bridges etc. [5]. FEA is based on building a complicated object by assembling small and manageable pieces, like a child's Lego® (Fig. 8.1). These small parts are rods, plates and blocks representing 1-D, 2-D and 3-D elements, respectively, whose material properties and behaviour are readily understood. The pieces or units are called elements. The domain is composed of a finite number of elements connected to each other in the end nodes. These finite elements are connected to neighbouring elements at 'nodes' [6]. Some of these nodes may be given limitations in degrees of freedom (DOF) to constrain them in specific positions and directions. Relevant force and pressure set in the boundary conditions are then imposed on the elements.

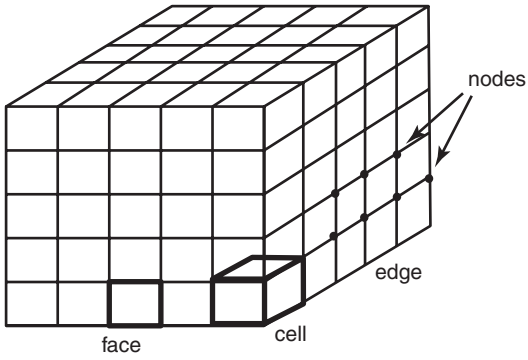


Fig. 8.1 The finite element grid

A simple mathematical and physical model can be solved through a number of equations using calculus or trigonometry analysis techniques. This is an analytical solution and there will be an exact answer. In complex equations, the analytical techniques become too complicated so numerical methods are used instead. However the result is an approximation, and consequently, engineers and scientists use FEA methods to build computational models and enhance the accuracy of the solution with the aim of converging around the exact value.

Finite element analysis starts with understanding the geometry and physics of the system. The geometry is represented using a 3D-model. A typical 3D-model will accurately describe the shape and structure. The FEA also considers the physics that is related to the modelling, such as the material properties, the forces and constraints, and other parameters which can affect the results. The physics of the model is not always precisely known, so the accuracy can vary, however it is easier to develop a simple model and then add complexity rather than use a complex model then simplify it.

The mesh is the partition of a given numerical model into elements, such that every point of the model is found in one of those finite elements. The size and shape of the elements is the challenging part of FEA. For example, smaller sized elements give more accurate results but increase the processing time. Apart from the appearance of the elements, the model must represent the material properties of the object, and imposed forces and constraints. Thus the finite element method analyses complex structures based on geometry and their physical properties [7].

There are three main steps to FEA (Fig. 8.2);

1. Build the model (Pre-processing)
2. Solve the model (Solver)
3. Display the results (Post-processing)

The finite element method used in engineering and research utilizes commercial software which comes with pre- and post-processing abilities e.g. ANSYS® [5]. The pre-processor is used to feed modelling and design data into the software and the post-processor is used to present the results in the form of a graph, table and contour map. However the ability to use finite element software (pre- and post-processing) depends on the user having the core knowledge and understanding of FEA needed to obtain accurate results [8].

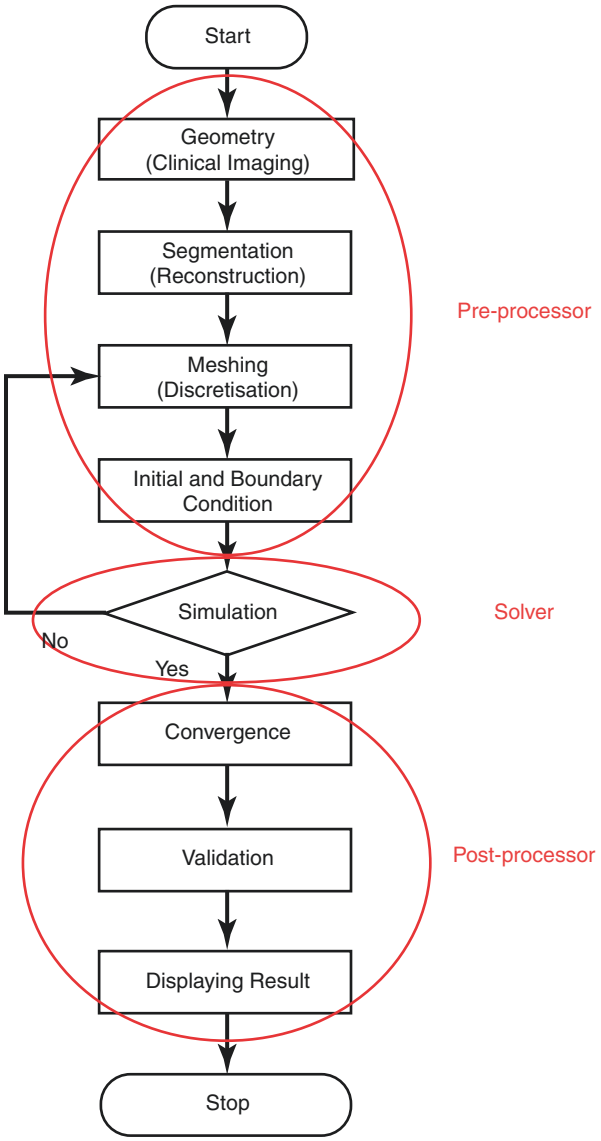


Fig. 8.2 Three steps of model construction

8.4 Computational Fluid Dynamics (CFD)

In the past, CFD has provided a powerful and popular tool for the study of haemodynamic and image-based modelling of blood flow in the development, diagnosis, and also treatment of cardiovascular disease. CFD is a flow measurement technique helping to make links between haemodynamic arterial wall shear stress (WSS) and the distribution of atherosclerotic plaque to explain why the plaque

develops at arterial junctions, for example [9]. Moreover, CFD can also be linked to medical images. These images can be extracted from in vivo vascular geometry and, along with flow data from ultrasound, can provide adequate inputs for CFD modelling [10].

The essential part of producing a CFD model is deriving volumetric (3D) images from lumen geometry, often derived from Magnetic Resonance Imaging (MRI) or Computed Tomographic (CT) imaging, with the inlet and outlet blood flow parameters taken from medical ultrasound. CFD analyses the pressure and WSS for every element. Therefore, the results can depict the time and position (temporal and spatial) distribution of the forces on the innermost surface of the arterial wall.

One of the main advantages of using CFD over experimental methods is that all blood flow properties (such as velocities, pressures, shear force) can be calculated throughout the simulation process. The other advantage is blood flow visualization which provides valuable metrics for quantitative analysis.

The processes of deriving the CFD model from the MRA or CT scan of patients include:

- Specifying the innermost part of the blood vessel (lumen) geometry of the patient;
- Reconstruction of 3D anatomic geometry from 3D digital imaging in medicine DICOM format using commercial imaging software;
- Extracting the specific 3D geometry in a numerical domain ('.step files' or '.stl files' formats);
- Meshing and simulation of the numerical domain on the CFD.

8.4.1 Segmentation and Reconstruction

Segmentation has a crucial role in the image analysis process. It is the process of partitioning a digital image into multiple segments of non-overlapping and constituent regions with homogeneous intensity or texture. Image segmentation through an automatic or semi-automatic process extracts the desired features of an object or other relevant information from a digital image [11].

Image segmentation is the removal of all parts of the image that are not required, leaving only the area of direct interest. The resultant 3D image is then easier to analyse. Blood vessel segmentation is needed to identify the vessel structures in the vicinity of organs, bones and other structures. Segmentation methods convert medical images to digital geometries which show the region of interest of the model [12].

The structure of in vivo organs cannot be measured easily (Fig. 8.3) so, by using reconstruction, relevant structures in medical images are captured (Fig. 8.4) and converted into digital 3D images (Fig. 8.5). Development of 3D artery reconstruction algorithms can be used in the assessment of disease such as the evaluation of the role of local hemodynamic forces on plaque progression [13].

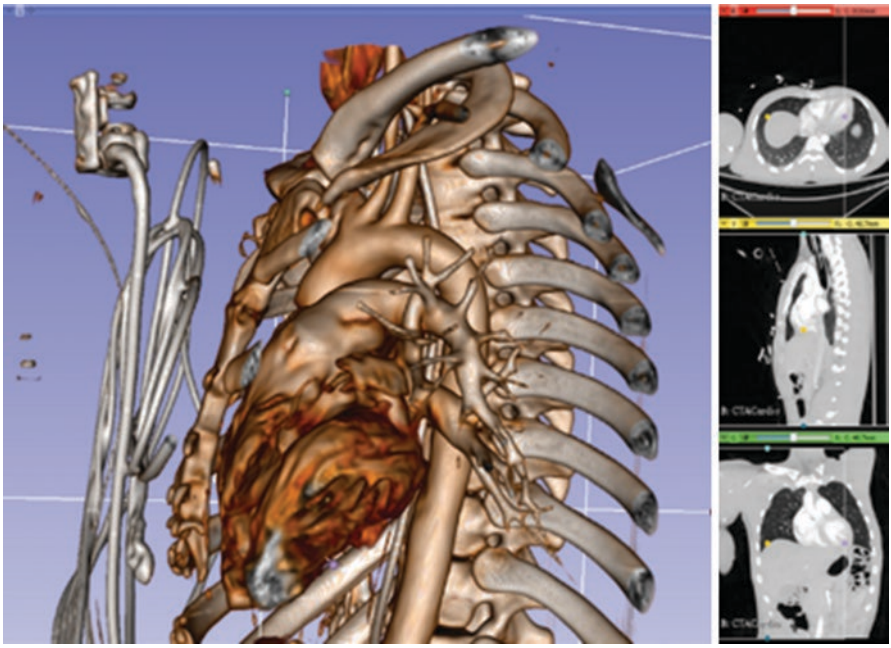
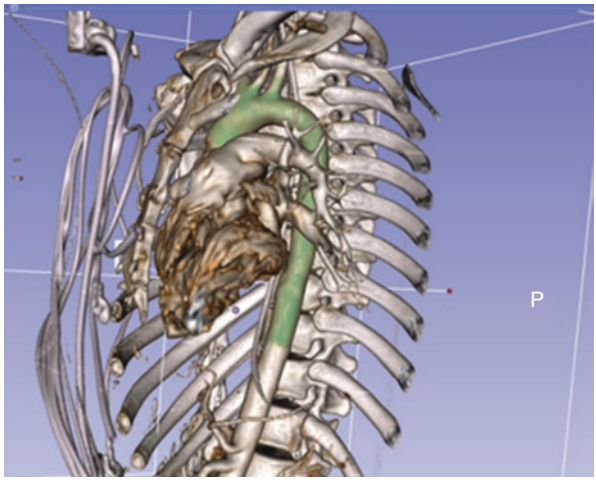


Fig. 8.3 3D image of chest (mrml format)

Fig. 8.4 The aorta is highlighted as separate from the rest of the chest elements



CTA and MRA imaging provide images in DICOM file format ('.dcm files'). 3D Data Loading and visualization of DICOM can covert to:

- Volume rendering of 3D images, in '.step files' format
- Surface rendering of 3D images, in '.stl files' format

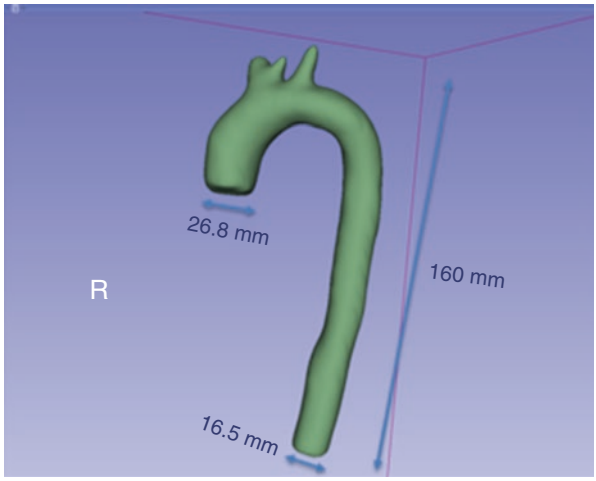


Fig. 8.5 3D digital image, post segmentation

8.4.2 Numerical Simulation

CFD is a computer simulation method for prediction of blood flow behaviour using hemodynamic parameters. The objective of CFD is to quantify flow characteristics at desired locations in a specific geometry through developing the governing equations (the Naiver–Stokes equations). The additional inputs to the geometry of CFD model are:

- Fluid properties (blood density and viscosity),
- Boundary conditions (rigid or flexible blood vessel),
- Initial conditions (pulsatile blood flow data),
- Discretisation information (mesh size/quality and time steps) and,
- Blood flow pattern (steady/unsteady and laminar/turbulent).

This information allows CFD to solve the Navier-Stokes (Eq. 8.1) and continuity (Eq. 8.2) equations to converge towards the final solution [14].

$$\overbrace{\rho\left(\underbrace{\frac{\partial v}{\partial t}}_{\substack{\text{Unsteady}\\ \text{acceleration}}} + \underbrace{\mathrm{v}.\nabla\mathrm{v}}_{\substack{\text{convective}\\ \text{acceleration}}}\right)}^{\text{Inertia (per volume)}} = \overbrace{\underbrace{-\nabla p}_{\substack{\text{Pressure}\\ \text{gradient}}} + \underbrace{\mu\nabla^2\mathrm{v}}_{\substack{\text{Viscosity}\\ \text{effect}}}}^{\text{Divergence of stress}} + \overbrace{\vec{f}}^{\substack{\text{Other}\\ \text{body}\\ \text{force}}}, \tag{8.1}$$

$$\underbrace{\frac{\partial \rho}{\partial t}}_{\substack{\text{change in}\\ \text{density}\\ \text{over time}}} + \underbrace{\nabla.(\rho\mathrm{v})}_{\substack{\text{Rate of}\\ \text{mass}\\ \text{flux}}} = 0, \tag{8.2}$$

where:

ρ: Fluid density
t: Time
v: Flow velocity
p: Pressure
μ: Dynamic viscosity
$\frac{\partial v}{\partial t}$: Change in velocity over time
∇: Gradient of vector parameter

CFD methods for a solution of fluid flow are classified into two distinct categories [15];

- Density based: suitable for high speed and compressible fluids,
- Pressure based: suitable for slow speed and incompressible fluids.

There are three different points of view in analysing problems in fluid mechanics [15];

1. Eulerian approach: Concerned with the fluid properties (pressure, velocity, density, etc.) in a specific point in space e.g. sitting on a riverbank and watching the water pass by a fixed location.
2. Lagrangian approach: Concerned with a specific particle of fluid as it moves through space, e.g. sitting in a boat and drifting down a river.
3. Arbitrary-Lagrangian-Eulerian approach: Concerned with a specific particle of fluid which can move, while the boundaries and interfaces move too, e.g. sitting in a moving vehicle on the riverbank and watching the water flow by.

In the context of blood vessels, the type of discretisation of the fluid domain can be described with either Arbitrary Lagrangian-Eulerian or Eulerian formulation, but the former will give more accurate results at the interface between the solid and the fluid [16].

8.4.3 Meshing

FEA software uses a Computer-Aided Design (CAD) model which represents the shape and structure of the simulating system as well as the material properties, and the applied forces and constraints (initial and boundary conditions). The process of dividing the CAD model into smaller domains called elements is referred to as meshing. The accuracy and robustness of the finite element computations are strongly related to the meshing size and quality (shape of the elements formed).

Starting with a bigger size and simple shape mesh and adding complexity is easier than starting with small size and complex ones. In CFD model analyses with extra-refined meshes, the computation time can take days and require supercomputers.

There are four common 3D-element (volume) shapes, as shown in Fig. 8.6;

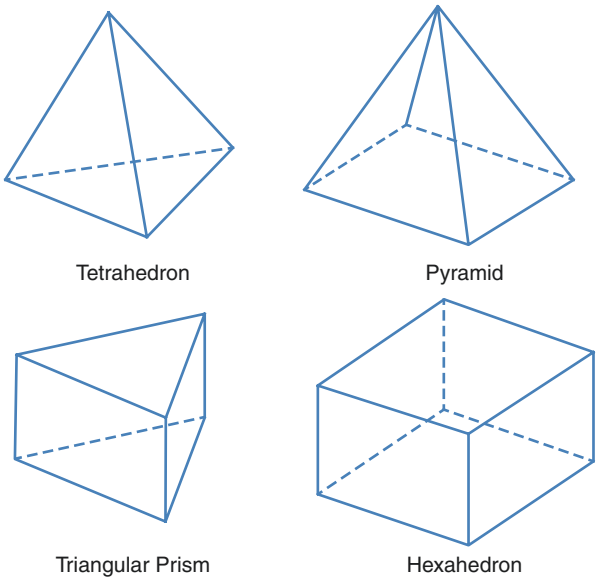


Fig. 8.6 Basic 3D-element shapes

1. Tetrahedron, with 4 faces and 6 edges. Tetrahedral meshes are relatively simple and can fit complex geometry better, but suffer from wall boundaries in CFD modelling. When modelling the interface between a wall and a fluid, the variations in fluid properties (velocity, temperature, density) will be much lower along the direction the fluid is travelling, than those in the cross-stream direction. Therefore, a thin brick- type mesh will be more suitable than other shapes.
2. Pyramid with 5 faces and 8 edges. The pyramid is used in transition areas between square and triangular—faced elements.
3. Hexahedron (brick), with 6 faces and 8 edges. For the same mesh quantity, hexahedral meshing has the highest accuracy and more computational efficiency with fluid flow direction.
4. Prism (wedge), with 5 faces and 9 edges. Prism meshing can efficiently resolve boundary layer problems.

The length of arteries is greater than their diameter, therefore the gradient (variation) of blood flow velocity in an artery is much greater in the radial than the longitudinal direction. An accurate resolution discretization with uniform mesh size of lumen geometry can impose excess computation. Therefore, each component (e.g. the blood flow (Fig. 8.7) and the arterial wall (Fig. 8.8)) needs to be discretised with a different resolution in different directions (anisotropic meshing) [17, 18]. The blood flow, as a non-Newtonian fluid, in a time-varying velocity profile imposes shear stress to the innermost aspect of the arterial wall. So, the near—wall regions must be the finest mesh possible to create a suitable boundary layer accurately [19].

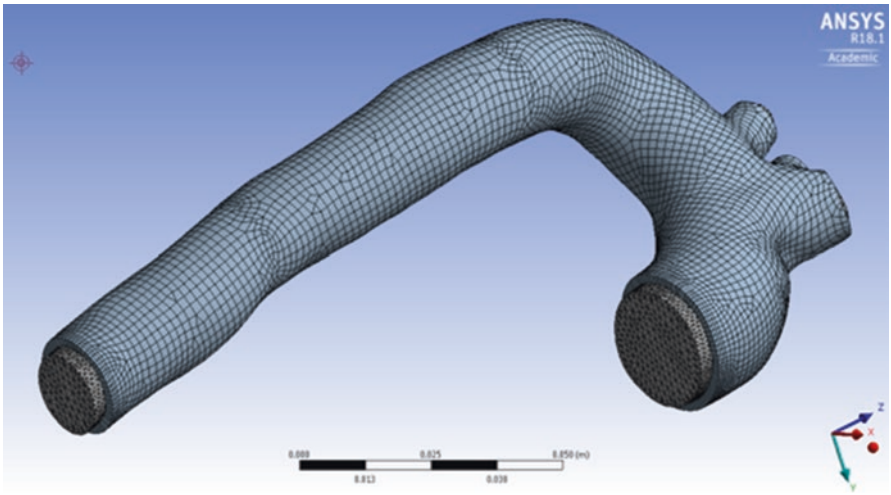


Fig. 8.7 Blood flow and artery vessel

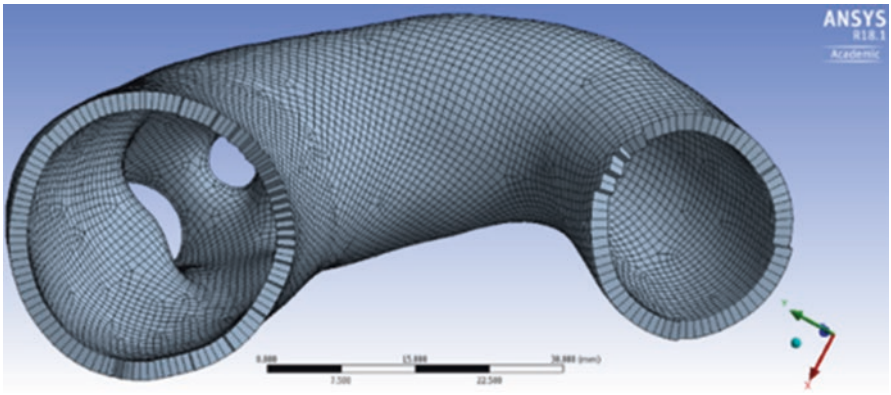


Fig. 8.8 Aorta shell meshing (STL format)

There are two basic ways to enhance the accuracy of the finite element model [20];

1. Increasing the number of elements in the model (Fig. 8.9).
2. Using a higher-order of the elements (Fig. 8.10).

The accuracy of the finite elements model is influenced by both spatial and temporal (mesh size and time step) refinement factors, so the more refinement produces a more accurate result. Spatial discretisation (meshing) is the process of subdividing the geometry of the model into a number of discrete volume elements (cells). Temporal discretisation (time-step) is a time-dependent solution of the FE model.

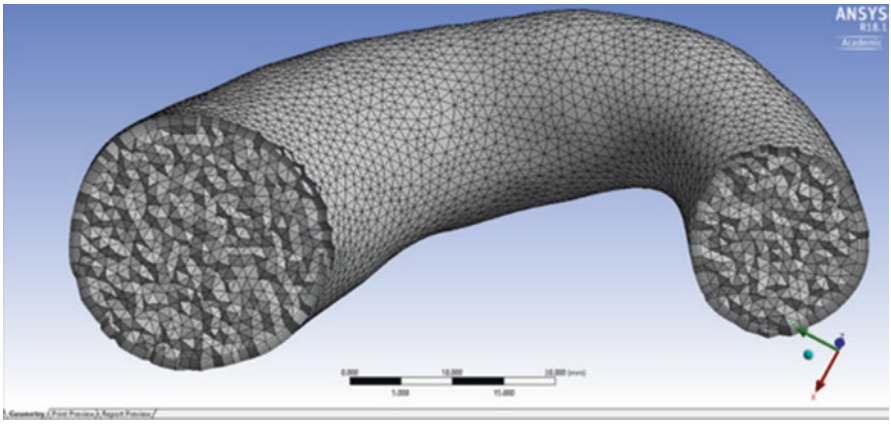


Fig. 8.9 Volume meshing (step format)

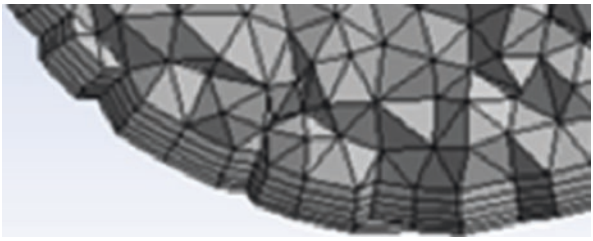


Fig. 8.10 Volume sub-layers meshing

8.4.4 Element-Based vs. Volume-Based Meshing

ANSYS-FLUENT and ANSYS-CFX are two independent solvers developed for performing CFD analysis. The main difference is in their discretization (meshing) methods, called finite difference and finite volume methods respectively. In the finite difference method, the number of control volumes are equal to the number of elements (as cell-centred or element-centred), whereas in the finite volume method, the control volume is assembled around the nodes (as vertex-centred or node-centred), so CFX solvers divide each element into sub-elements. In another words, in the finite difference method, the dependent values are stored at the node, but in the finite volume method, the dependent values are stored in the centre of the volume (Fig. 8.11).

Although, CFX uses only one solving approach for the governing equation of motion and FLUENT offers several solving approaches, Fluid-Solid Interaction (FSI) can perform better in CFX than FLUENT because there is a stronger connection between the volumes in CFX. In the case of a geometry with irregular and complex shape and structure, high quality meshes are produced in a CFX solver, saving a lot of pre-processing time. In the finite difference method, the

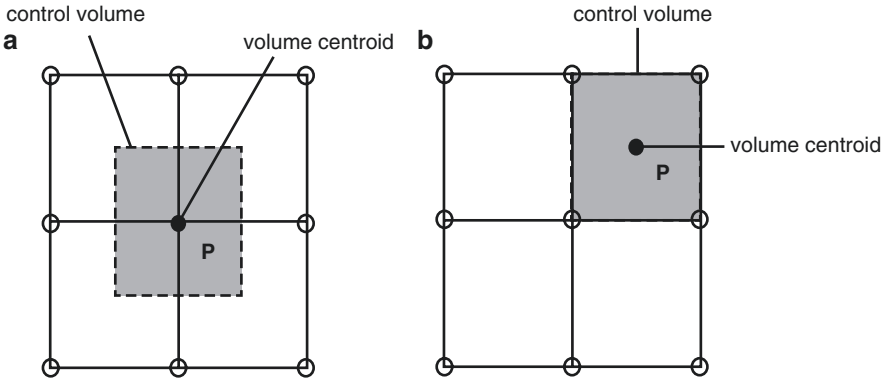


Fig. 8.11 Control volume of (**a**) cell-centred and (**b**) cell-vertex scheme

dependent values are stored at the nodes, but in finite volume method, the dependent values are stored in the centre of the finite volume.

8.4.5 Fluid: Solid Interaction

Modelling Fluid-Solid Interaction (FSI) as a movable or deformable structure, combines the laws of structural mechanics and fluid flow [21]. The interface between the solid wall and the fluid is a means of transferring force and heat from one to the other. FSI allows intersection of two separate meshes to be coupled and transfer heat and force between them. This interaction could be one-way or two-way (fully) coupled. In one-way coupled interaction, the solver simulates one media and then the other, whereas in the fully-coupled method, both media are solved in the same system simultaneously. One of the main advantages of the fully-coupled method is that it converges to the results in fewer iterations (in a shorter time), although each iteration needs more computing memory (Fig. 8.12).

The results of a CFD solution provide pressure data from blood flow at the interface, and the structural FEA solver uses the CFD results (blood pressure and forces) as an input to calculate the loads and deformations on the blood vessel. The deformed solid structure (blood vessel) makes a new FSI surface which in turn changes the boundary conditions of the blood flow for the next CFD calculation. This is a coupled approach where the interaction between fluid flow and a solid structure subject to moving boundaries are solved through multiple cycles. As the below figure shows, two-way coupling uses an iteration procedure in time-dependent calculations. FSI solution can help us calculate load transfer and get a better surface map, and consequently, a better estimate of the structural response [22].

The FSI modelling procedure is based on the generation of meshes. Through using a conformed mesh, FSI considers the interface characteristics as the physical boundary conditions. Therefore the interface becomes a part of the solution. Due to

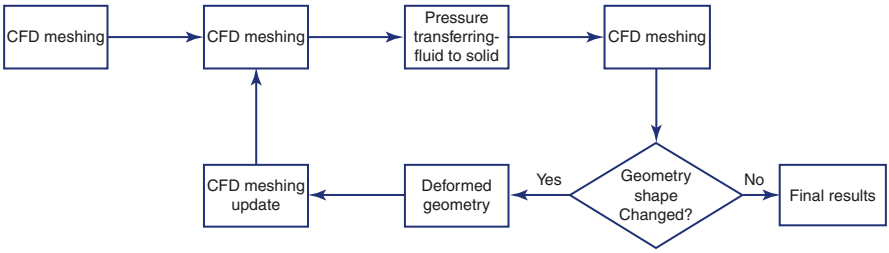


Fig. 8.12 Workflow of two-way FSI

the solid structure deformation, mesh updating is needed as a part of the solution in every iteration. FSI models need extensive computation and are therefore time consuming.

8.5 Material Properties

8.5.1 Blood Flow Properties

Viscosity is an important variable to describe fluid behaviour and its resistance to blood flow in the vasculature. Internal friction of the moving fluid generates fluid shear stress that determines fluid shear strain. If fluid shear stress is directly proportional to the fluid shear strain, the fluid is called Newtonian and if not, it is non-Newtonian. Blood is an incompressible suspension and it behaves as a non-Newtonian (shear-thinning) fluid. It is much easier to numerically model a Newtonian fluid than a non-Newtonian fluid, and for some calculations, the assumption of Newtonian behaviour is acceptable (in straight and fixed cross-section area vessels with an arterial diameter greater than 10 mm) [23–25].

Blood flow has a complex rheology, so a wide range of constitutive equations have been proposed to model its non-Newtonian behaviour and define the relationship between shear stress and shear strain rates. Numerical simulation of blood flow requires an appropriate constitutive model to reflect its shear thinning properties [26]. The most prevalent non-Newtonian models used for blood viscosity computation, with different degrees of accuracy, are the power law, Casson, Carreau and Carreau/Yasuda models [23].

The power-law model describes the shear thinning behaviour of blood that depends mainly on the haematocrit, which varies from one person to another. Equation 8.3 expresses the relationship between the two; the higher the haematocrit the greater the blood viscosity [27];

$$\mu = 1.4175 + 5.878H - 15.98H^2 + 31.964H^3, \tag{8.3}$$

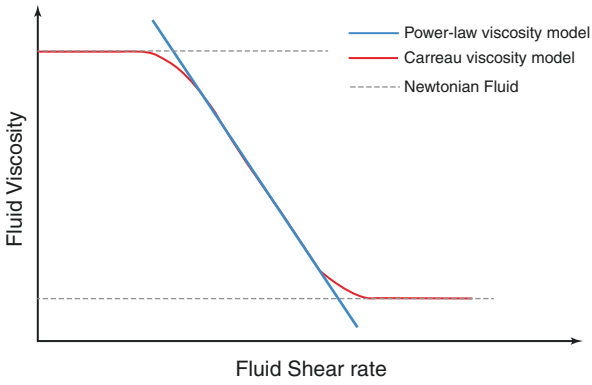


Fig. 8.13 Blood flow viscosity behaviour by Newtonian, power-law and Carreau models [28]

where 'μ' is the viscosity of whole blood in poise and H is the haematocrit (%) divided by 100.

The Casson model is specifically used for low shear stress blood flow in narrow arteries. The Carreau and Carreau-Yasuda models, as shown in Fig. 8.13, are similar to the power-law model, but they fit both Newtonian and non-Newtonian blood flow.

Although the instantaneous shear rate of blood flow varies from zero to approximately 1000 s^{-1} in a cardiac cycle, blood flow in a shear rate range greater than 100 s^{-1} is almost constant (thus it is behaving as a Newtonian fluid), with an approximate viscosity of 0.035 poise (Pa.s) [23, 28, 29] Blood viscosity also depends on factors such as smoking status and cholesterol levels, etc. [30].

8.5.2 Blood Vessel Properties

In addition to the geometry of the arterial wall, the mechanical properties of the artery should be considered. Hooke's law describes the relationship between the stress and strain rates for most homogenous materials (Eq. 8.4) within a certain range of stresses, under simple uniaxial loading conditions [31]. The stress versus strain curve of a multi-linear homogeneous material is shown in Fig. 8.14. The modulus of elasticity, E_1, is the slope of the linearly elastic range in the stress–strain curve. However, the linear range of plastic properties of the material can be shown by Young's modulus (e.g. E_2 and E_3).

$$\sigma = E \times \varepsilon, \tag{8.4}$$

where:

- E: Modulus of elasticity or Young's modulus (N/m^2),
- σ: Normal stress (N/m^2), and.
- ε: Strain (unitless).

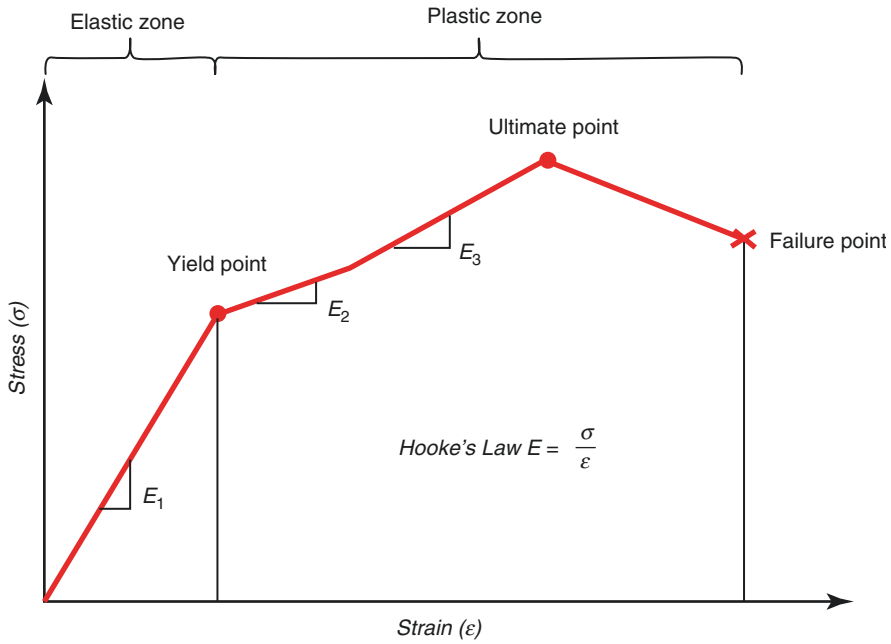


Fig. 8.14 Stress–strain curve for a homogenous material

Arteries are not linearly elastic and so not homogenous. The slope of the uniaxial loading curve varies with stress and strain, so, as strain rate increases in an artery, the artery becomes stiffer. Arteries are made of three different types of material, elastin, collagen and smooth muscle. Elastin is easily stretched while Collagen is not and resists arterial stretch. Moreover, the contraction and expansion of smooth muscle in the artery can resist strain. Collagen fibres have a wavy pattern in stress-free conditions. They only stretch when the artery has been already stretched and the collagen fibres straightened. There are three major zones in the stress-strain curve in the arterial wall including (Fig. 8.15):

- Zone I: the toe region,
- Zone II: the linear region, and
- Zone III: the yield and finally failure region.

Arterial walls have viscoelastic properties. The stress in viscoelastic material relates not only to load variation, but also on the rate of change of strain which is dependent on time, as shown in eq. 8.5 [32].

$$\sigma = E_1 \times \varepsilon + E_2 \times \frac{d\varepsilon}{dt}, \tag{8.5}$$

A large artery consists of three layers; the intima, media and adventitia [33], which are made from different constituent materials (Collagen, Elastin, and Smooth

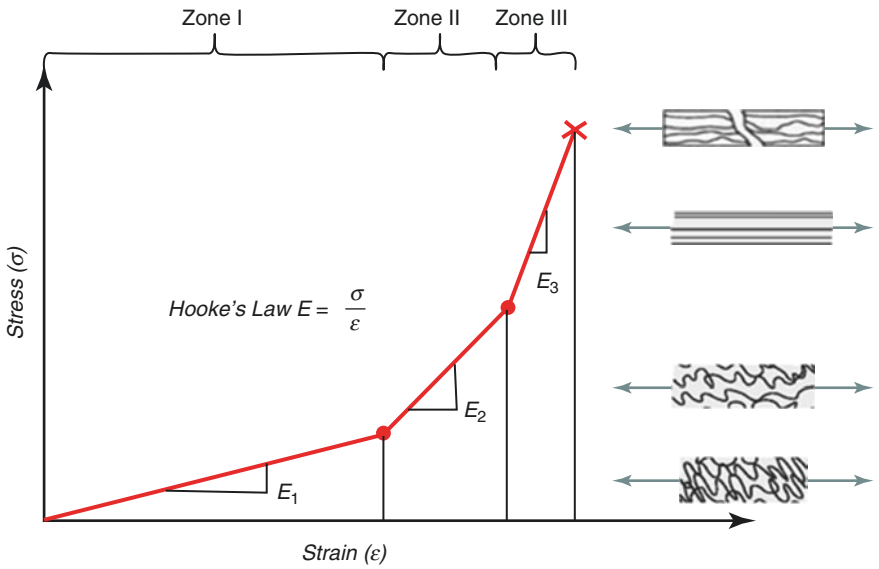


Fig. 8.15 Stress–strain curve for an arterial wall

Table 8.1 Mechanical properties of constituents of a large artery

Arterial tissue	E (MPa)
Collagen	0.3–10
Elastin	0.6
Smooth muscle	0.01
	0.01
	0.25

E modulus of elasticity, *MPa* megapascals

muscle) with different mechanical properties (as shown in Table 8.1). Hence, arteries are nonhomogeneous and anisotropic. The intima is made up of a monolayer endothelial lining, which is supported by loose connective tissue and allows the intima to move relative to the media. The media is made up of elastin fibres, smooth muscle cells and collagen fibres. Elastin fibres are able to stretch up to 2–3 times their original length without rupture. Collagen fibres are up to 5000 times stiffer than elastin. Smooth muscle cells contribute to arterial wall stiffness. The adventitia, is made of tough collagen fibres and connective tissue.

The average density of the artery is 1080 kg/m^3 and the Poisson's ratio 0.5 [34–37], and the average Modulus of elasticity is 1 MPa (Mega-pascal) [38, 39]. The ultimate tensile stresses in the adventitia is almost three times more

than the ultimate tensile stress in the media and intima. The mechanical parameters of the arterial wall play a crucial role in stress and strain analysis, as atherosclerosis can dramatically reduce the modulus of elasticity of the arterial wall [40].

8.5.3 Initial and Boundary Conditions

Every biomedical numerical simulation is defined under the limits of boundary conditions which describe the physiological conditions. The results of CFD modelling are valid, if boundary condition parameters are incorporated in the governing equations [5]. Some of the common initial and boundary conditions in CFD are the following;

- Inlet and outlet flow conditions;
- Loads and displacements in fluid solid interactions or contact nodes;
- Velocity and pressure conditions;
- Axisymmetric/symmetric boundary conditions;
- Periodic/cyclic initial conditions.

8.6 Pulsatile Flow in Arteries

A fluid with periodic flow variation is known as pulsatile flow (Womersley flow). The oscillatory nature of pulsatile blood flow imposes other forces, (apart from fluid driving forces in the case of steady flow), through endothelial cells to the arterial wall when viscoelastic (non-Newtonian) properties of blood have been taken into account [41]. Blood flow through curvatures and bifurcations of large and medium sized arteries can induce secondary flows (flow separation and recirculation). The secondary flows can be expected to have significant effects on arterial structure in the presence of atherosclerosis [42].

A numerical model can simulate pulsatile blood flow in an artery to investigate the effect of blood flow on the arterial wall to determine the resultant stresses. The numerical model develops a shear-thinning rheological behaviour and uses the time-dependent, three-dimensional, incompressible Navier-Stokes equation for non-Newtonian fluid. The main concern of numerical modelling is to create an interaction surface between periodically varying blood flow pressure and the elasto-plastic blood vessel. The stress analysis of a typical bifurcating artery, as shown in Fig. 8.16, under pulsatile blood flow pressure shows the distribution of maximum stresses at the innermost layer (intima) of an arterial wall. The shear stress contour plot shows the variation of the stress from 0.017–2.459 MPa (at the apex) which has been observed in the literature [43].

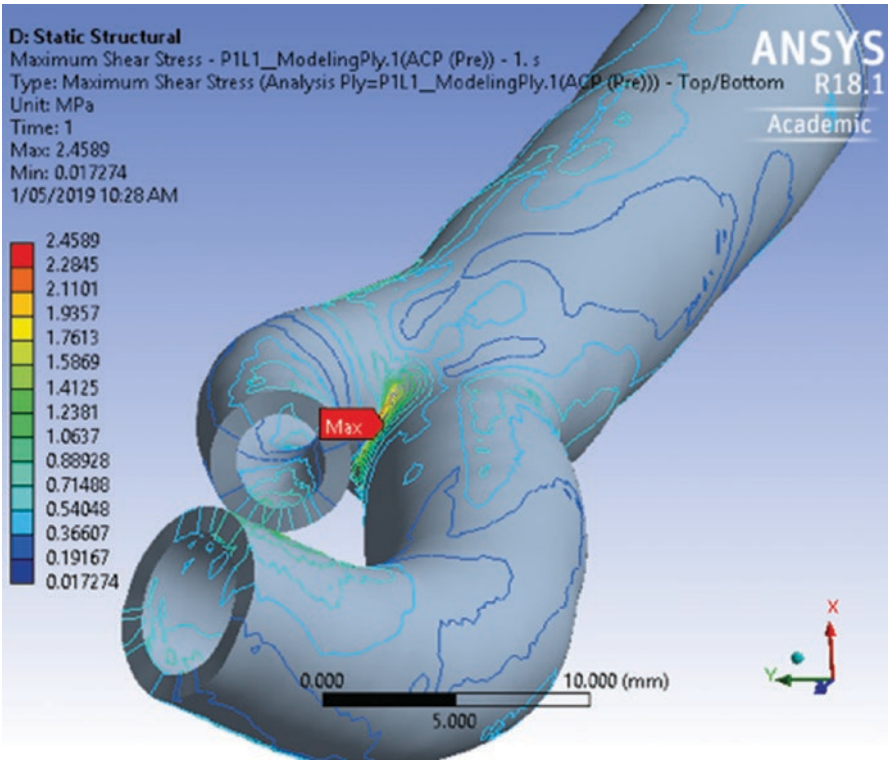


Fig. 8.16 Maximum stress contours at the inner surface of an arterial bifurcation

8.7 What Is Convergence?

CFD analysis deals with the governing Navier-Stokes equations which have inherent complexity and are highly non-linear in nature. As a result, CFD models cannot be solved easily. The convergence criteria in CFD analysis define how close to the exact solution is acceptable. In other words, the convergence criterion is the allowable error in the calculation. If the CFD model does not converge, then mesh refinement and changing the boundary conditions can be considered.

8.7.1 Result Analysis (Post-processing)

Nowadays, finite element software has been able to analyse and interpret complex structural and fluid flow systems using powerful computers. This capacity generates more finite element results, so the outcome of the design and analysis calculations

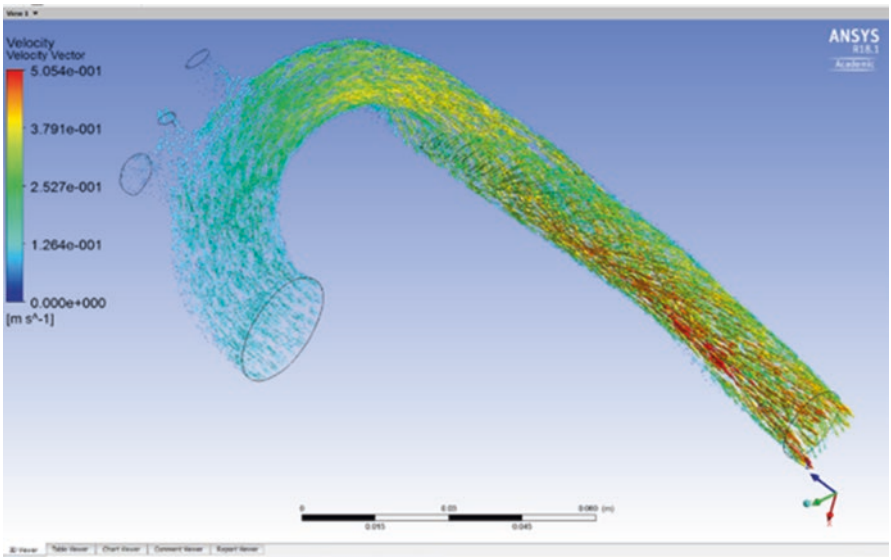


Fig. 8.17 Velocity flow vectors

can be an enormous file. Therefore, post-processing software helps the user in making design/analysis decisions by interpreting the FEA results by displaying them in graphical form (Figs. 8.17, 8.18, and 8.19) [44].

8.7.2 Validation and Verification

Verification and validation are the methods used to assess and estimate the error and uncertainty of CFD modelling and its results. Verification and validation generate confidence in the accuracy and reliability of CFD simulations [45, 46]. To verify and validate a CFD model, one or more of the following can be used:

1. Analytical methods;
2. Experimental methods;
3. Results from similar literature;
4. Benchmark simulation similar to the study.

8.8 Clinical Applications

CFD analysis allows medical researchers to use medical imaging of arteries to study haemodynamics, predict blood-flow behaviour and assess blood pressure distribution [47]. Computational simulation can obtain valuable information from in vivo measurements to quantify haemodynamic parameters of patient-specific anatomy

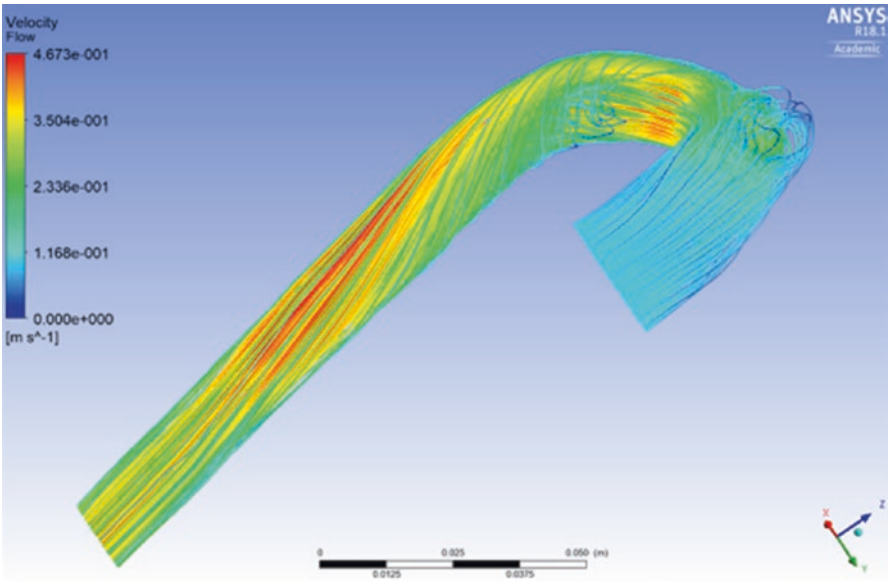


Fig. 8.18 Velocity flow pathway

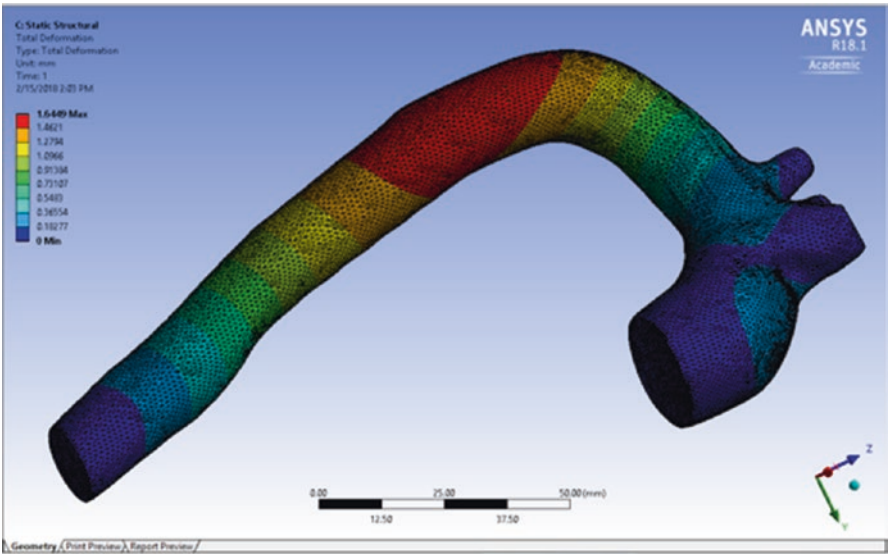


Fig. 8.19 Total deformation of Aorta

and pathology and consequently inform clinical decision-making. Furthermore, CFD techniques can be used to investigate pathological change in vessels but also to support preventative initiatives which lower clinical events. Finite element analysis utilises a multi-layer arterial wall and elastoplastic mechanical properties which are representative of in vivo situations and hard to reproduce experimentally.

8.8.1 Atherosclerosis

Blood flow velocity, oscillating pressure, and resultant wall shear stress (WSS) have been suggested as playing key roles in the development of early atherosclerosis [48]. Atherosclerosis affects specific arterial geography with certain biomechanical properties. The link between luminal haemodynamic and vascular geometries can be easily explored with CFD and explains why atherosclerotic plaque develops at bifurcations, branch origins and areas of external constraint such as the adductor canal.

CFD analysis of the stress contours and flow velocity profiles at the carotid artery bifurcation illustrate that arterial WSS is highest at the bifurcation apex, and lowest at the lateral side of the interior carotid artery origin [49]. From the flow point of view, the WSS depends on the magnitude and direction of the blood flow velocity vector at the arterial wall; i.e. WSS is highest in regions where blood flows parallel to the wall and lowest in regions where the flow is not parallel (secondary flow) [50]. Low WSS is traditionally associated with increase particle transit times and therefore plaque formation [3, 24].

8.8.2 Stenosis

Arterial stenosis from plaque burden results in reduced luminal flow and a pressure drop as well as turbulence and vortices flow post stenosis, which may cause intimal damage and dilatation. Compression of the subclavian artery as it crosses the first rib can cause stenosis if the artery is constrained by other neighbouring structures [51]. Blood flow is highest within the stenosis, becoming turbulent with lower pressure after the stenosis [52].

CFD is a reliable tool which can quantitatively investigate the haemodynamic characteristics of blood flow and consequently calculate the WSS distribution along the length of diseased artery and find the separation point post-stenosis for different degrees of narrowing. The turbulence precipitated by a stenosis results in forces on the wall of the artery beyond the stenosis at angles away from the direction of flow resulting in post-stenotic dilatation i.e. WSS forces work within the wall at the stenosis and dilating radial forces work on the wall beyond the stenosis.

8.8.3 Aneurysm

Aneurysm is a dilation of the arterial wall and the result of a complex biomechanical and biological predisposition which is created by pulsatile blood pressure creating radial forces on the wall of the artery. The interaction between arterial WSS and blood flow parameters play a critical role in the mechanisms of aneurysm

formation, growth, and rupture [53, 54]. The effect of arterial geometry plays a vital role in stress distribution and consequently rupture risk of the aneurysm [55]. Greater geometric tortuosity can increase arterial WSS [56].

CFD simulation can be employed to identify the relationship between haemodynamic changes resulting from dilatation and aneurysm growth, risk and location of rupture [55]. CFD modelling results indicate that the highest WSS occurs at the entrance or inlet of blood flow into the aneurysm where it is confronted with higher pulsatile blood flow forces and the lowest WSS is located at the widest part of the aneurysm wall [57]. In regions of low WSS, flow recirculation and thrombus deposition occur probably from prolonged interaction between the blood and the vascular endothelium leading to wall degeneration. An understanding of the interplay between wall shear stress and vascular remodelling will ultimately lead to a better understanding of AAA development, growth, and rupture potential [58].

8.8.4 Aortic Dissection

CFD and FEA are also useful in studying why and where are the most likely points in the aortic arch for spontaneous and traumatic dissections to occur. The subsequent behaviour of the false lumen might be predicted rather than only observed and management planned accordingly.

Thrombosis of the false lumen in aortic dissection is essential to reduce the long-term risk of aneurysmal change. Maintenance of flow and increase in diameter of the false lumen is due to the relatively high diastolic pressure in the false lumen due to higher outflow resistance and a flow gradient from false to true lumens during diastole. If this is reduced by treatment closing inflow to the false lumen, then the false lumen diastolic pressure falls and the lumen collapses. Thrombosis will occur when blood flow velocity and arterial WSS are low [59].

In complex dissection where the stent graft landing zone is critical, CFD can be used to predict the effects of various lengths of coverage on false lumen exclusion so that the treating surgeon can weigh up the risks of longer coverage and false lumen exclusion with the increased risk of paraplegia [60, 61].

8.8.5 Stents

Stent thrombosis and in-stent stenosis following arterial stenting is the result of complex interactions between the flowing blood, altered wall geometry, compliance mismatch, radial force and stent structure [62]. CFD modelling can be used to predict the effects of stenting in different vascular beds by providing detailed analysis of blood flow and the interaction forces and displacements between the blood flow and the stented artery. This can be used to influence stent design and improve treatment strategy.

8.9 CFD Benefits and Challenges

CFD is a quick and cost-effective means of studying blood flow conditions which enable us to investigate blood pressure variations and induced arterial wall stresses which cannot be achieved by bench top testing. Furthermore, CFD post-processing provides comprehensive visual images from vectors, contours, or animations which generate an insight into the interpretation of arterial diseases and treatment strategies.

Despite the benefits, some inherent limitations and challenges of CFD should be understood. CFD converts physical data to small numerical fragments to simplify complex problems which creates inaccuracies when compared to analytical equations [44]. Time consuming numerical calculations in complex CFD models may necessitate simplification of variables and parameters as well as modelling of only a few cases rather than large numbers, and this can introduce error [63, 64].

8.10 Conclusion

Clinical image-based CFD modelling and haemodynamic parameters, particularly those related to arterial WSS, can hold a prominent position in the patient-specific quantitative evaluation of arterial disease and treatment effects [64]. Using accurate properties of blood and arterial walls in modern CFD is enhancing our understanding compared to earlier techniques which assumed Newtonian properties and non-pulsatile vessels for example. This is serving to provide more accurate prediction of behaviour which can be informative in the clinical setting.

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Chapter 9
Physiological Haemostasis

Simon McRae

Key Learning Points

- The haemostatic response involves complex interactions between multiple proteins and cell types, and can be divided into primary and secondary haemostasis
- Primary haemostasis results in platelet plug formation and results from interactions between platelets and subendothelial adhesive proteins (von Willebrand factor and collagen)
- As part of the primary haemostatic response, platelets go through a sequence of events that involves initial platelet adhesion, resulting in intracellular signalling that triggers platelet shape change, activation with granule release, and finally aggregation.
- Secondary haemostasis results in sequential conversion of zymogens to active enzymes as part of the coagulation cascade, leading to the formation of a mesh-like network of cross-linked fibrin.
- The fibrinolytic system is responsible for the dissolution of thrombus composed of cross-linked fibrin, and plays a major role in helping maintain a patent vascular system

9.1 Introduction

Physiological haemostasis involves complex interactions between endothelial cells, platelets, and coagulation proteins, that result in a platelet plug and localised thrombus formation at the site of a break in vascular integrity. Numerous regulatory processes prevent widespread activation of coagulation, ensuring that blood remains fluid in the absence of vascular injury or other pathology. All components of the

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haemostatic process can be disturbed resulting in either a pro-thrombotic or bleeding tendency, and drugs that modify the haemostatic process are commonly used, particularly in patients with vascular disease. An understanding of normal haemostasis is therefore important for all clinicians that deal with this patient group.

9.2 Primary Haemostasis

Primary haemostasis is the initial response of the body to vascular injury, and involves interaction between platelets, adhesive proteins located in the subendothelial matrix (including collagen and von Willebrand factor), and circulating fibrinogen [1]. The end result of primary haemostasis is the formation of a stable platelet plug around which a fibrin network can then be built. This same process is responsible for the pathogenic thrombus formation in patients with arterial disease. Disorders of primary haemostasis tend to manifest as mucosal bleeding, including epistaxis, oral bleeding and menorrhagia, and often result in immediate difficulty with haemostasis in the post-operative setting.

9.2.1 Platelets

Platelets are small fragments of megakaryocyte cytoplasm that, in the resting state, are small discoid structures. The normal range for circulating platelet count in adults is between 150 and 400 × 10^9/L. Although anucleate, platelets are metabolically active, and interact with the local environment through the binding of surface glycoprotein receptors to specific ligands (for more detail on these interactions, see Chap. 10). Platelets go through a predictable cycle of response to vessel wall injury that involves initial platelet adhesion to the sub-endothelium, subsequent intracellular signalling that triggers platelet shape change and activation with granule release, and finally aggregation (Fig. 9.1) [2].

9.2.1.1 Platelet Adhesion and von Willebrand Factor

Endothelial injury results in the exposure of circulating blood to the subendothelial matrix that is rich in a number of adhesive proteins. von Willebrand factor (vWF) is a large adhesive glycoprotein produced by endothelial cells and megakaryocytes that play a central role in initial platelet adhesion [3]. The mature vWF molecule consists of disulphide-linked multimers of high molecular weight of up to 20,000,000 daltons [4]. When secreted into the plasma, these high molecular weight (HMW) vWF multimers are digested into smaller forms by the metalloprotease ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13). These smaller soluble forms bind less readily to platelet receptors, reducing the

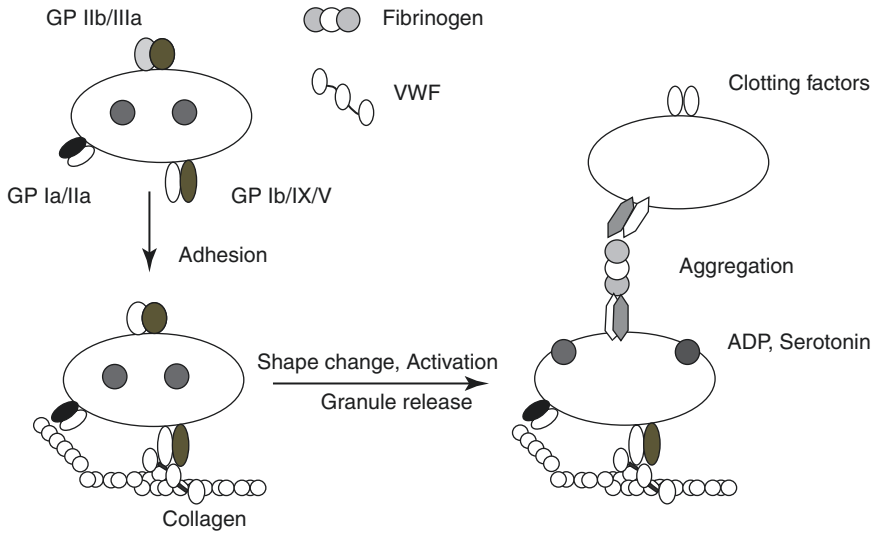

Fig. 9.1 Mechanism of platelet aggregation

chance of spontaneous platelet aggregation. However, vWF secreted into the subendothelial space binds to other molecules such as collagen, resulting in a conformational change that exposes the binding site for platelet glycoprotein (GP) receptor Ib [4]. Subendothelial vWF is therefore "primed" to interact with circulating platelets in the event of endothelial injury. Other important adhesive proteins include collagen type 1 and type 4, fibronectin, thrombospondin, laminin and vitronectin.

Initial platelet adhesion, particularly in high shear conditions, involves interaction between vWF and the GPIb/IX/V complex located on the platelet surface. This complex consists of four trans-membrane subunits GPIba, GPIbb, GPIX and GPV, with the N-terminal globular domain of GPIba responsible for the interaction with the A1-domain of vWF [1]. Binding of vWF to GP Ib is often reversible, and in animal models platelets can be seen to initially slide or translocate along the subendothelial surface due to cyclical attachment and then dissociation of the GP Ib/IX/V complex to vWF [2]. However, finally through further platelet receptor ligand interactions the platelet is stabilized on the subendothelial surface. The platelet glycoprotein Ia/IIa receptor (integrin $\alpha_2\beta_1$) binds collagen, an interaction that appears to be more important in low-shear conditions [5]. Glycoprotein VI, a platelet surface receptor that belongs to the immunoglobin superfamily, also directly binds collagen and further activates the GPIa/IIa receptor via intracellular signaling [6]. Other β_1 integrins also bind their respective subendothelial ligands ($\alpha_6\beta_1$—laminin; $\alpha_5\beta_1$—fibronectin), and there is increasing evidence that early binding of vWF to the glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) receptor contributes to the initial adhesion process [2]. Finally there is evidence that formation of platelet membrane tethers, that consist of smooth cylinders of lipid membrane pulled from the platelet surface under the influence of hemodynamic drag forces, contribute to platelet adhesion in high shear conditions [7].

In addition to being involved in initial platelet adhesion as described above, VWF also binds circulating factor VIII [8]. This significantly prolongs the half-life of the latter molecule. As a result, patients with reduced VWF levels will also have reduced FVIII levels due to the acceleration in clearance of FVIII.

VWF has also been shown to be involved in the regulation of blood vessel formation, with lack of VWF leading to enhanced angiogenesis in some vascular beds [9]. As a result patients with von-Willebrand disease have been documented to have increased rates of angiodysplasia that may result in intractable gastro-intestinal bleeding.

9.2.1.2 Platelet Activation and Shape Change

Following platelet adhesion, multiple pathways lead to platelet activation that results in platelet shape change, platelet granule release, and conformational change in the GP IIb/IIIa receptor that allows binding to fibrinogen and vWF, leading to platelet aggregation. Binding of vWF to the GP Ib receptor and collagen to the GP VI during the adhesion process triggers intracellular signaling via a pathway that involves activation of Src family kinases (Src), Syk and PI 3-kinase (PI3K). These events lead to the activation of phospholipase C-β (PLC), which hydrolyses membrane phospholipids to generate inositol (1,4,5) trisphosphate (IP3) [10]. The binding of IP3 to its receptors (IP3R) on the dense tubular system (DTS) then results in mobilisation of intra-platelet calcium stores, which has a number of consequences including;

1. *Thromboxane A2 (TXA2) generation*—the increase in intracellular calcium stimulates the production of arachadonic acid by PLC and phospholipase A2. Arachadonic acid is converted into TxA2 via the actions of the enzymes cyclooxygenase 1 (COX-1) and Tx synthase. TxA2 is released from the platelet and binds platelet receptors TPα and TPß. The effects of TxA2 in platelets are mediated primarily through TPα. Binding of TxA2 to this G-protein coupled receptor results in further PLC activation, leading to further intracellular calcium increase further reinforcing platelet activation [11]. Local diffusion of TxA2 also contributes to the recruitment to the site of injury and activation of further platelets. Aspirin or acetyl salicylic acid exerts its antiplatelet effect by blocking TXA2 synthesis, due to the irreversible acetylation of Serine-529 in COX-1. Because platelets are anucleate, no new COX can be generated, explaining why aspirin has a persistent functional effect that lasts the lifespan of the platelet (approximately 7 days).
2. *Granule release*—intracellular calcium mobilization also results in the release from the platelet of both the dense and alpha-granules. The dense granules contain high concentrations of the small molecules adenosine diphosphate (ADP) and serotonin, which further act to reinforce local platelet activation by binding to specific platelet surface membrane receptors upon release. ADP is a central player in sustained platelet activation. The receptors for ADP, the $P2Y_1$ and

$P2Y_{12}$ are seven transmembrane receptors that are coupled via heterotrimeric G-proteins to numerous intracellular effector molecules. $P2Y_1$ links to the G-protein Gq resulting in further activation of PLC and also protein kinase C activation. $P2Y_{12}$ is linked to the G-protein Gi that has an inhibitory effect on adenylate cyclase. ADP induced activation of the $P2Y_1$ receptor induces platelet shape change and rapid transient aggregation [12], whereas activation of the $P2Y_{12}$ receptor results in sustained irreversible aggregation [13]. The thienopyridine class of antiplatelet agents, ticlopidine, clopidogrel, prasugrel and ticagrelor all exert their antiplatelet effect by blocking the $P2Y_{12}$ receptor. The active metabolites of all agents have a free thiol moiety that forms a disulfide bridge with the extracellular cysteine residues Cys17 and Cys270 [14]. Released serotonin also binds to a G-protein coupled platelet surface receptor, the $5\text{-}HT_{2A}$ receptor. Binding is also associated with Gq-dependent activation of PLC, resulting in amplification of platelet activation, platelet shape change, and weak reversible platelet aggregation [15].

3. *Activation of the GP IIb/IIIa receptor*—in its resting state the GP IIb/IIIa receptor is unable to bind its ligands, namely fibrinogen and vWF. The above platelet signaling events through the activation of the small GTPase Rap1b and its interaction with a Rap1-GTP interacting adapter molecule (RIAM), lead to the binding of the proteins talin and kindlin to ß3 tail of GP IIb/IIIa receptor [16]. This leads to activation of the receptor and the resulting change in conformation allows the surface portion of the receptor to bind readily to fibrinogen and vWF. The binding of talin to the receptor tail also links it to the underlying actin cytoskeleton of the platelet, enhancing adhesive strength and platelet cohesion [17].
4. *Platelet shape change*—the normally discoid-shaped platelet with a smooth surface membrane undergoes dramatic shape change with stimulation, including extension of filopodia, and flattening or spreading on the subendothelial surface. The platelet cytoskeleton is primarily responsible for regulating the platelet's shape. Platelet activation leads to the rapid reorganization and polymerization of actin into filaments, resulting in the above conformational change [18].

Along with ADP, the serine protease thrombin plays an important role in sustaining platelet activation leading to irreversible platelet aggregation. Thrombin specific receptors, the protease-activated receptors (PARs), are located on the platelet surface. Two main PARs, PAR1 a high affinity receptor and PAR4, a low affinity receptor, are involved in thrombin mediated platelet activation [19]. Thrombin activates PARs by cleaving the N-terminal of the receptor, unmasking a hidden receptor-linked ligand. This ligand then interacts with the remainder of the receptor leading to G-protein coupled signaling that results in further platelet activation.

Finally platelet activation also results in the surface expression of a number of adhesion molecules, such as the glycoprotein P-selectin which is involved in interaction with both endothelial cells and also the recruitment of inflammatory cells to the area of injury, via binding of P-selectin to P-selectin glycoprotein ligand 1 (PSGL-1) located on the surface of leucocytes [20]. Platelets also secrete

chemokines such as RANTES/CCL5 and platelet factor 4 that also increase the local recruitment of inflammatory cells such as monocytes. This contributes to and can exacerbate the local inflammatory response that is often present in atherosclerotic plaque [21]. A more detailed outline of platelet activation is given in Chap. 10.

9.2.1.3 Platelet Aggregation

As the final part of the primary haemostatic response, platelets recruited to the site of vascular injury and activated by the above soluble agonists then undergo irreversible aggregation. This is mediated via the concurrent binding of either fibrinogen or vWF to the activated GP IIb/IIIa receptors on separate platelets, leading to their cross-linking and the formation of a platelet aggregate. In low flow vascular beds, binding of fibrinogen to the GP IIb/IIIa receptor appears to be the main process involved in platelet aggregation, whereas the interaction between GP IIb/IIIa and vWF is more important for aggregation in high shear vascular beds and pathological arterial thrombosis [7].

9.3 Interactions Between Primary and Secondary Haemostasis

While the primary and secondary haemostatic processes are often considered separately, they are intrinsically linked. As described above, the coagulation protease thrombin plays a central role in the activation of platelets. The activated platelet in turn provides the surface upon which the reaction complexes of the coagulation cascade form. In addition, as part of platelet activation the content of the negatively charged phospholipid phosphatidylserine on the outer surface of the platelet membrane increases from almost 0% up to 12%, providing a binding site for the proteins of the coagulation cascade [22]. Release of clotting factors, such as factor V, from platelet alpha granules, and the expression of other as yet still poorly defined platelet receptors for coagulation factors on the platelet surface provide additional methods in which activation of the coagulation cascade is localised to the site of platelet activation and vascular injury [23].

9.4 Secondary Haemostasis

Secondary haemostasis describes the process whereby exposure of tissue factor to the bloodstream leads to a series of enzymatic reactions that result in a sufficient burst of thrombin production to convert soluble fibrinogen into a stable network. This process is mediated by the formation of a series of reaction complexes, each consisting of an active enzyme and a co-factor, in which the presence of the latter results in an order of magnitude increase in the efficiency of the enzyme to bind to

and convert its target substrate, itself a pro-enzyme or zymogen, to its active form. Defects of secondary haemostasis, as typified by factor VIII deficiency or haemophilia A, may result in muscle, joint, intracerebral and soft tissue bleeding, and delayed bleeding post surgical or traumatic haemostatic challenge.

The coagulation factors involved in secondary haemostasis belong to the class of proteins known as serine proteases, so called because they have a serine residue which, along with histidine and aspartic acid, forms a catalytic triad at the centre of the active site of the enzyme [23]. Most of the reactions of secondary haemostasis take place on a phospholipid membrane surface, which is normally the surface of an activated platelet. Binding of the coagulation proteins to the phospholipid membrane surface requires the presence of calcium, and agents that chelate calcium such as EDTA or citrate can therefore be utilised to prevent activation of the coagulation cascade after blood collection.

The coagulation factors have a modular structure, and different factors share similar structural features. The coagulation factors II, VII, IX, X along with the natural inhibitors of coagulation, protein C and protein S, all undergo post-translational gamma-carboxylation of glutamate residues located at the amino-terminus. This modification is necessary for the efficient binding of these proteins to phospholipid surfaces. The carboxylation process is dependant on the presence of vitamin K, which is a co-factor for this process. Vitamin K deficiency or Vitamin K antagonists, such as warfarin that prevent the conversion of vitamin K to its reduced form by blocking the activity of the enzyme vitamin K epoxide-reductase, leading to a reduction in the activity of the coagulation factors, resulting in an anticoagulant effect.

9.5 The Coagulation Cascade

Early observations noted that exposure of blood or plasma to surfaces such as glass would also precipitate clot formation without the addition of further material (intrinsic activation of coagulation), and that this process could be accelerated by the addition of exogenous biological material such as macerated brain extract (extrinsic activation of coagulation). These observations led to the concept of “extrinsic” and “intrinsic” pathways of coagulation, and over time the coagulation factors involved in these separate pathways were identified (Fig. 9.2) [23, 24]. Tissue factor was identified as the “active” factor in the added tissue extract, and was demonstrated to activate factor VII in the first part of the extrinsic pathway. The intrinsic pathway, sometimes also called the contact activation pathway, was found to involve serial activation of the coagulation factors XII, XI and IX, with factor VIII acting as a co-factor for the latter. Both extrinsic and intrinsic pathways were found to then converge on the “common pathway” involving factor X, prothrombin (factor II), finally leading to the conversion of fibrinogen to fibrin by thrombin. The concept of the two separate pathways was reinforced by the fact that the most widely utilised laboratory assays of coagulation evaluated the extrinsic (the prothrombin time or PT assay) and intrinsic pathway (the activated partial thromboplastin time or aPTT) separately, with both assays impacted by common pathway defects.

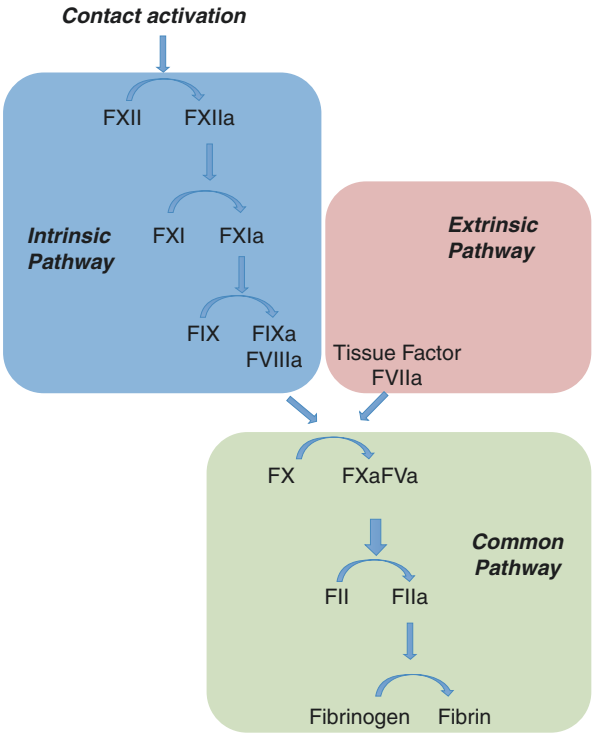

Fig. 9.2 The extrinsic and intrinsic pathways of coagulation

This concept that two separate independent pathways of sequential enzyme activation could lead to thrombus formation was for a long period of time a central tenet of understanding of the coagulation system and was known as the "waterfall" or "cascade" hypothesis of coagulation [23, 24]. It however became clear with time that the above model was unlikely to reflect physiological coagulation. The observation that inherited factor XII deficiency was not associated with a bleeding tendency raised questions regarding the physiological role of the intrinsic pathway [25]. It was also demonstrated that activated factor VII, or factor VIIa, had the ability to activate factor IX as well as factor X, and therefore that cross-communication between the pathways was likely [26]. With increasing knowledge of the role of the cell surface proteins in the coagulation process, and in particular the role of platelets, a cell-based model of haemostasis then emerged [27] This model divides the coagulation cascade into the separate steps of initiation, amplification, and then propagation (Fig. 9.3).

9.5.1 Initiation

Tissue factor (TF) is a transmembrane protein that is constitutively expressed on the surface of most non-vascular cells, including those located in the subendothelium. Exposure of cells expressing tissue factor to circulating blood is accepted as being

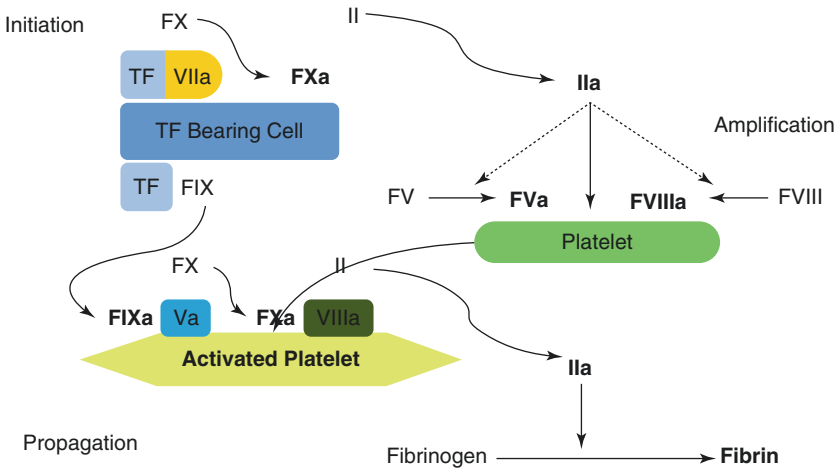

Fig. 9.3 Cell based model of haemostasis

the physiological trigger of coagulation. There is also evidence that tissue factor expression can be induced in the setting of inflammation on the surface of monocytes, and that microparticles derived from monocytes may also express TF in pathological states [28].

Upon exposure to circulating blood, TF can bind to both factor VII or factor VIIa, with approximately 1% of FVII circulating in the active form [29] FVII not already activated, is rapidly activated to FVIIa and the resulting TF/FVIIa enzymatic structure is known as the extrinsic tenase complex. Within the complex TF acts as a cofactor for VIIa, greatly potentiating the latter's capacity to convert factor X to factor Xa, and, to a lesser degree, factor IX to factor IXa.

The activated factor Xa formed by the extrinsic tenase complex then binds to the surface of the tissue factor-expressing cell and converts a small amount of prothrombin (factor II) to thrombin. This thrombin diffuses away, moving to the surface of nearby platelets leading to both platelet activation and the formation of FXIa, FVIIIa and FVa. These activated proteases then bind to the surface of activated platelets and are central to the amplification phase of coagulation as described below [30].

9.5.2 Amplification

The small amount of thrombin formed during the initiation stage of coagulation is insufficient to convert adequate amounts of fibrinogen to fibrin to form a stable thrombus that is resistant enough to fibrinolytic activity to allow healing to occur. It is however sufficient enough to be responsible for the subsequent amplification of the coagulation cascade. The thrombin produced results in;

1. Further local activation of platelets resulting in a suitable phospholipid surface on which the reactions of the coagulation cascade can proceed,
2. Activation of the co-factors factor V and factor VIII that then localize on the nearby surface of activated platelets,
3. Activation of factor XI that also binds locally to the platelet surface [31].

9.5.3 Propagation

Following the activation of the co-factors and their localization on the platelet surface, the stage is set for the formation of highly efficient enzymatic complexes that are responsible for the burst of thrombin generation that leads to clot formation. Factor IXa formed during the initiation step, binds to factor VIIIa on the platelet surface to form the intrinsic tenase complex. This then efficiently converts factor X to factor Xa, with the latter then binding to its co-factor, factor Va, to form the prothrombinase complex responsible for the effective conversion of prothrombin to thrombin. Factor XIa produced during amplification activates further factor IX, further reinforcing the haemostatic process [27].

The burst of thrombin generated during propagation then cleaves the fibrinopeptides a and b from soluble fibrinogen to form insoluble fibrin monomers. The transglutaminase Factor XIII, itself activated by thrombin, then forms bonds between separate fibrin monomers to form a firm network of cross-linked fibrin that is a requirement for stable thrombus formation [32].

9.5.4 Other Roles of the Contact Activation System

It has been increasingly recognized that there are complex interactions between the contact pathway of the coagulation system with complement and the inflammatory response [33]. FXII-mediated activation of prekallikrein to kallikrein leads to bradykinin production, after kallikrein cleaves high-molecular weight kininogen (HMWK). Kallikrein also cleaves several complement proteins including C3, C5 and factor B, leading to complement activation. Further evidence of the interaction between the two pathways is demonstrated by that fact the multi-ligand binding protein gC1qR can both activate the classical complement pathway by binding C1q, and the contact activation pathway by activating HMWK and FXII. There is emerging interest in manipulating the molecules of the contact activation pathway as a means of controlling the inflammatory response without increasing bleeding risk.

9.5.5 Natural Inhibitors of Coagulation

Normal coagulation is kept in check by several regulatory processes that cause thrombin production to plateau and then diminish, preventing localized activation of coagulation from becoming an inappropriately widespread activation of the clotting cascade.

The initiation phase of coagulation is regulated by tissue factor pathway inhibitor (TFPI), a protein produced by endothelial cells [34]. After a sufficient local concentration of FXa is generated in the initiation step of coagulation, TFPI is able to form an inhibitory quaternary complex with FXa, FVIIa, and tissue factor, effectively turning off the initiation phase of coagulation. Interestingly FV has been demonstrated to bind to TFPI and protect against premature clearance of the inhibitor, suggesting an anticoagulant potentiating role for the unactivated form of FV [35].

Central to regulation of the propagation phase of the coagulation cascade is the protein C anticoagulant pathway that involves protein C and protein S, both vitamin K dependent plasma glycoproteins synthesized in the liver [36, 37]. Thrombin itself initiates this inhibitory pathway after binding to thrombomodulin, a transmembrane protein located on the intact endothelial cell surface in all vascular beds particularly in the microcirculation. Binding of thrombin to thrombomodulin results in a change in substrate specificity that favours thrombin mediated cleavage of the vitamin K dependent protein C to its activated form activated protein C (APC) [38]. Binding of thrombin to thrombomodulin therefore results in its net enzymatic effect being switched from pro-coagulant to anti-coagulant. Another endothelial transmembrane protein, the endothelial protein C receptor (EPCR) binds protein C, helping to localize the protein at the endothelial surface potentiating activation by thrombomodulin bound thrombin. Once activated, APC diffuses away from EPCR and binds to the extrinsic tenase and prothrombinase complexes where it acts to inactivate factor VIIIa and factor Va respectively. Protein S acts as a co-factor for protein C in these reactions, as well as having some direct anticoagulant activity [39]. Protein S has recently been shown to help with localization of TFPI on phospholipid surfaces, increasing the efficiency of inhibition of FXa by TFPI. It appears clear that protein S exerts its anticoagulant effect by more than one mechanism. In plasma, PS circulates both free (40%) and bound to the C4b-binding protein (60%). It is the free form of PS that has cofactor activity [37].

Finally antithrombin (AT) is a single chain plasma glycoprotein that belongs to the serine protease inhibitor superfamily (serpins). It plays a central role in the inactivation of circulating activated clotting factors, forming a 1:1 complex that is cleared by the liver. It is the main physiological inhibitor of thrombin and also binds to factors Xa, IXa, XIa, and XIIa [40]. Thrombin inhibition by AT is potentiated more than 1000-fold by heparin, due to conformational change of the AT molecule upon heparin binding, and it is this mechanism that results in heparin's activity as an anticoagulant agent [41]. AT activity is also enhanced by heparan sulfates that are present on intact endothelial surfaces, one of many mechanisms that help to restrict the activation of coagulation to the site of vascular injury [35].

Inherited deficiency states of the main inhibitory proteins of coagulation, namely protein C, protein S and antithrombin, have all been described, and result in a significant pro-thrombotic tendency. Such deficiency states are relatively rare accounting, when combined, for less than 5% of individuals with venous thrombosis in a Caucasian population. A mutation in FV known as the FV Leiden mutation (Arg506Gln), which prevents proteolysis at one of the APC cleavage sites is far more common occurring in ~5% of Caucasians but results in a much milder prothrombotic condition.

9.6 Fibrinolysis

The fibrinolytic system is responsible for the dissolution of thrombus composed of cross-linked fibrin, and plays a major role in helping maintain a patent vascular system [42]. It is composed of a number of enzymes, most of which are serine proteases, that act in concert to convert insoluble fibrin to soluble fibrin degradation products (FDPs). The central protein of the fibrinolytic system is plasminogen, a single-chain glycoprotein consisting of 791 amino acids, which is converted to its active form plasmin by the cleavage of a single Arg561-Val562 peptide bond [43]. Tissue-type plasminogen activator (tPA) is the physiological activator of plasminogen on the thrombus surface. Activation of plasminogen to plasmin is potentiated in the presence of fibrin due to the fact that both plasminogen and tPA bind to lysine residues on the surface of fibrin, being brought into close proximity to each other, allowing plasminogen activation. Both tPA and another plasminogen activator, urokinase-type plasminogen activator, play a role in the activation of plasminogen that is bound to the endothelial cell surface.

Once activated, plasmin cleaves fibrin into soluble fibrin degradation products, of which D-dimer is one. D-dimer consists of two cross-linked fibrin D-domains and is not normally present in the absence of recent plasmin activity. It is therefore used as a laboratory marker of active thrombosis and is a sensitive test that can be used to rule out recent venous thromboembolism.

Like the coagulation cascade, the fibrinolytic system also has a number of inhibitory proteins that in normal circumstances prevent widespread activation of fibrinolysis. Plasminogen activator inhibitor-1 (PAI-1) is a 52-kd, single-chain glycoprotein that belongs to the serpin family, and is the main inhibitor of both tPA and uPA, doing so by forming a 1:1 complex that is cleared by the liver [44]. Circulating plasmin is quickly mopped up by α_2-plasmin that is present in the circulation at a high concentration. The most recently described inhibitor of fibrinolysis is thrombin-activatable fibrinolysis inhibitor (TAFI), a carboxypeptidase [45]. TAFI is activated by thrombin, a process that is markedly accelerated if thrombin is bound to thrombomodulin. The antifibrinolytic activity of TAFI is due the fact that it cleaves C-terminal lysine and arginine residues from fibrin. This significantly reduces the binding of plasminogen to fibrin, therefore decreasing the activation of plasminogen by tPA on the surface of the fibrin clot.

The fibrinolytic system is manipulated therapeutically by administration of either naturally occurring (streptokinase) or recombinant protein (r-tPA) plasminogen activator that exert the same effect as endogenous tPA, leading to activation of plasmin and resulting thrombus lysis.

9.7 Conclusions

Primary and secondary haemostasis both involve carefully balanced systems that if disturbed can lead to issues with either bleeding or pathological thrombosis. An improved understanding of the molecular processes involved has led to the

development of more targeted therapeutic options, such as the direct thrombin inhibitors and direct factor Xa inhibitors, with the aim of increasing the benefit and reducing the risks associated with anticoagulation. Increasing recognition of the interaction between the inflammatory and coagulation pathways may lead to novel therapeutic targets to control the inflammatory response. Continued advances in our understanding of the relationship between the structure and function of the proteins and receptors involved in haemostasis, along with improved technology, is likely to lead to further therapeutic advances in coming decades.

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Chapter 10
Hypercoagulable States

Simon J. McRae

Key Learning Points

- Routine thrombophilia testing in unselected patients with venous or arterial thrombosis is not recommended.
- Patients with provoked venous thrombosis should not undergo thrombophilia testing.
- Thrombophilia testing, particularly for type 1 thrombophilic conditions (antithrombin, protein C or protein S deficiency), may be considered in patients with unprovoked venous thrombosis in whom cessation of anticoagulation is being considered or who have a first degree female relative of child-bearing age.
- Unprovoked venous thrombosis at young age, a family history of venous thrombosis in first degree relatives, or recurrent thrombosis may indicate a greater likelihood of an underlying venous thrombosis.
- Testing for antiphospholipid antibody syndrome is recommended in patients with unprovoked venous thrombosis or unusual site arterial thrombosis. Patients with a confirmed diagnosis should receive anticoagulation with warfarin rather than a direct oral anticoagulant.
- Testing for an underlying myeloproliferative disorder and paroxysmal nocturnal haemoglobinuria should be performed in patients with unusual site thrombosis, particularly unprovoked abdominal vein thrombosis.

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10.1 Introduction

Abnormal thrombus formation is central to the acute pathophysiology of both arterial and venous disease. Formation of thrombus superimposed upon the surface of ruptured atherosclerotic plaque, producing vessel occlusion and resulting tissue ischemia, is a common mechanism leading to acute symptoms and presentation in patients with arterial disease. Deep vein thrombosis and its complication pulmonary embolism, are also important causes of morbidity and mortality and result from abnormal thrombus formation in the venous circulation. An understanding of conditions that may predispose to abnormal thrombus formation, including how the presence of these conditions may or may not impact on patient management, is therefore important for all clinicians involved in the management of vascular disease.

First used in 1937 [1] and then also in the first description of inherited antithrombin deficiency, the term "thrombophilia" can be defined as an increased tendency to develop thrombosis, which may be either acquired or inherited [3]. Thrombophilic conditions vary both in prevalence and in the magnitude of the associated increase in risk of thrombosis. The discovery during the 1990's of the high prevalence factor V Leiden and prothrombin gene point mutations that predispose to thrombosis [4, 5], meant that an underlying thrombophilic condition could be found in approximately 50% of unselected patients with venous thrombosis [6]. The belief that the presence of such a condition may influence prognosis and therefore help to guide patient management, led to a significant increase in laboratory testing for inherited thrombophilia [7]. It however has been demonstrated that testing for thrombophilia, particularly the more common inherited conditions, is unlikely to influence the management of the majority of patients in whom it is performed [8] and guidelines as a result have recommended against widespread testing in unselected patients [9–11].

The chapter describes individual inherited and acquired conditions that predispose to an increased risk of thrombosis. The potential clinical rationale for testing will be outlined, and current evidence and recommendations regarding the clinical utility of laboratory testing in specific clinical scenarios will be discussed.

10.2 Classification of Thrombophilia

Thrombophilic conditions can be broadly classified as being either inherited or acquired and will be described in these two broad categories.

10.2.1 Inherited Thrombophilia

In 2003 Crowther and colleagues proposed a classification of inherited thrombophilia into either type 1 conditions that involve a deficiency of one of the naturally occurring inhibitors of coagulation, and type 2 conditions that result in a gain of

function or an increase in the level of one of the procoagulant proteins [12]. The distinction is of clinical relevance as the majority of patients with a type 1 condition will develop a symptomatic episode of venous thrombosis during their lifetime, whereas the majority of individuals with a type 2 condition will not. Similarly the presence of a type 1 thrombophilia clearly increases the risk of recurrent venous thrombosis and therefore may influence decision making regarding the duration of anticoagulation [13], whereas type 2 conditions in isolation do not strongly influence recurrence risk and their absence or presence should not be used in isolation to determine duration of treatment [14].

10.2.1.1 Type 1 Conditions

Antithrombin Deficiency

Antithrombin (AT) is a single chain plasma glycoprotein belonging to the Serine Protease Inhibitor superfamily (serpins) [3]. It is a physiological inhibitor of thrombin and other activated coagulation factors (factors Xa, IXa, XIa). Heparin exerts its anticoagulant effect by binding to AT, resulting in a conformational change that increases the affinity of AT for thrombin more than 1000-fold. Familial AT deficiency, described in 1965, was the first identified inherited thrombophilia [2, 3]. Individuals with AT deficiency typically have AT levels ranging between 40 and 80% of normal, and estimates of the prevalence of the condition range from 0.02 to 0.15% of the general population [15]. Approximately 0.5–2% of unselected individuals with venous thromboembolism (VTE) will have AT deficiency [16] Estimates of the increase in risk of VTE associated with AT deficiency vary from 5 to 20-fold that of the general population, with pooled analysis suggesting an annual risk of venous thrombosis of approximately 1% in previously asymptomatic individuals with the deficiency state [17].

Protein C and Protein S Deficiency

Protein C (PC) and protein S (PS) are both vitamin K-dependent plasma glycoproteins synthesized in the liver [18]. When activated by thrombin, a process potentiated by the binding of thrombin to thrombomodulin on the intact endothelium, PC is converted to the active serine protease, activated protein C (APC). In combination with its cofactor, PS, APC inactivates both factor Va and VIIIa, and plays a central role in controlling the propagation phase of coagulation. In plasma, PS circulates both free (40%) and bound to the C4b-binding protein (60%). It is the free form of PS that has cofactor activity.

Inherited PC deficiency was first described as a cause of venous thrombosis in 1981 [19], whereas PS deficiency was initially described as a cause of venous thrombosis in 1984 [20]. PC and PS deficiency both have type I (quantitative deficiency) and type II (qualitative deficiency) subgroups, and in addition a type III PS

deficiency state with normal total circulating but reduced free levels can occur. The estimated prevalence of heterozygous PC deficiency in the general population is between 0.2 and 0.4% [21], and many of these individuals have no history of thrombosis. The community prevalence of PS deficiency is estimated at approximately 0.2% [22]. PC deficiency is found in 1–3%, and PS deficiency in 1–7% of unselected patients diagnosed with VTE. Estimates from case-control and family cohort studies of the increase in risk of VTE associated with PC deficiency range from 5.0 to 10-fold, and 8.5 to 30-fold for PS deficiency [9].

Homozygous PC deficiency is a rare condition that may present as neonatal purpura fulminans, which is an acute thrombotic disorder that manifests as extensive skin and soft tissue necrosis that can be fatal if not treated aggressively [23]. A protein C concentrate, Ceprotinin®, is available, and should be given in combination with anticoagulant therapy in such cases.

Care is needed when initiating warfarin in patients with confirmed protein C and S deficiency. This is due to the fact that both protein C and S are vitamin-K dependant proteins, and levels may reduce at warfarin initiation more quickly than procoagulant levels due to a shorter half-life. This may precipitate a pro-thrombotic state that can result in warfarin related skin necrosis [24]. Bridging therapy with an alternative anticoagulant, most commonly low-molecular-weight heparin, should be administered while warfarin is being initiated in this patient group.

10.2.1.2 Type 2 Conditions

Factor V Leiden

In 1993, Dahlback and colleagues noted that plasma taken from a family with a strong history of venous thrombosis was resistant to the anticoagulant effect of APC [25]. This phenotype became known as APC Resistance. A point mutation in the factor V gene (G1691A), resulting in an amino acid change (Arg^{506} to Gly) at the cleavage site involved in the inactivation of factor Va by activated protein C was identified as the cause in more than 90% of individuals, and became known as factor V Leiden (FVL) [4, 26]. The FVL mutation has a high community prevalence with 3–7% of Caucasians being heterozygous for the mutation, although a lower incidence is found in other ethnic groups [27]. It is the most commonly identified cause of inherited thrombophilia, being present in 12–20% of unselected patients with VTE [28]. The heterozygous state is a relatively low risk thrombophilia being associated with a 3 to 7-fold increase in risk of VTE [9] with one study finding greater than 90% of individuals remaining event free by the age of 65. Unlike individuals homozygous for natural anticoagulant deficiency states, homozygosity for FVL does not result in a catastrophic thrombotic state early in life, and it is estimated that 0.1% of the population are FVL homozygotes [9]. The risk of VTE, however, in homozygotes for FVL is greater than that in heterozygotes, with estimates of the magnitude of risk ranging from 25 to 80-fold that of the healthy controls [29].

The Prothrombin (G20210A) Gene Mutation

In 1996, Poort and colleagues described a common mutation (G20210) of the prothrombin gene, which has become known as the prothrombin gene mutation (PGM) [5]. Located in the 3′ untranslated region of the gene, the mutation is associated with increased mean plasma prothrombin levels due to increased efficiency of 3′ end processing of the gene, resulting in accumulation of the encoded mRNA [30]. The prevalence of the mutation in Caucasian populations is approximately 2%, and it is rare in Asian and African populations [31]. In unselected patients with venous thrombosis the mutation has been found in between 4.0 and 7.1% of individuals [9] and 18% of individuals with a strong family history of VTE. The PGM is a relatively weak risk factor for VTE, being associated with a 2 to 5-fold increase in risk [9].

FVL/PGM Compound Heterozygotes

Given the high community prevalence of both the FVL and PGM mutations it is not uncommon for individuals to be heterozygous for both conditions, with an expected prevalence of 1/1000 in Caucasian populations [32]. In a pooled analysis of case control studies, double heterozygotes were estimated to have a 20-fold increase in risk of VTE in comparison to healthy controls [32].

Other Inherited Conditions

Homozygosity for the C667T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, producing a thermolabile gene product with reduced function, is the commonest inherited cause of raised plasma homocysteine levels [33]. In prospective studies a 5 μmol/L (micromolar) increase in total plasma homocysteine levels has been shown to be associated with an approximate 1.3-fold increase in the risk of venous thrombosis [34] and patients with peripheral vascular disease have been shown to have a slight elevation of homocysteine levels in comparison to controls [35]. Conversely homozygosity for the C667T MTHFR mutation has been shown to have no association with venous thrombosis in folate-replete societies [34] and to have only a weak association with arterial disease (OR 1.2, 95% CI 1.0–1.4) [36]. A recent systematic review demonstrated that, in comparison to placebo, homocysteine-lowering interventions did not prevent heart attack or reduce death rates in participants at risk of, or living with, cardiovascular disease [37]. Performing testing for this mutation is therefore not recommended as part of routine clinical practice.

Elevated levels of the coagulation factors VIII, IX, XI and prothrombin (factor II) have all been shown to be associated with increased VTE risk. In the case of factor VIII, familial clustering of individuals with elevation of this factor has been demonstrated suggesting an underlying inherited cause, although a specific genetic defect

is yet to be identified [8]. Other common mutations within coagulation proteins that have been documented to increase the risk of venous thrombosis include the Plasminogen activator inhibitor 4G/5G mutation (OR 1.62) and the alpha-fibrinogen Thr312Ala point mutation (OR 1.4). However, there is no clear evidence that the presence of these mutations should alter patient management at present [38].

10.2.2 Acquired Thrombophilia

There are a number of important acquired conditions that predispose to venous or arterial thrombosis that can be defined by laboratory testing. External or environmental acquired risk factors such as recent surgery, hospitalization or cancer, while often playing a central role in the causation of venous thrombosis, will not be discussed further.

10.2.2.1 Antiphospholipid Antibodies

The term antiphospholipid antibody syndrome (APLAS) was first used in the 1980's to describe a non-inflammatory autoimmune condition characterized by the presence of antibodies directed against a variety of phospholipid membrane associated proteins, and a history of either arterial or venous thrombosis or adverse pregnancy outcomes [39]. Laboratory confirmation of the presence of antiphospholipid antibodies requires the demonstration of the presence of a lupus anticoagulant, characterized by prolongation of phospholipid dependant coagulation assays such as the APTT, or a positive immunoassay for anti-cardiolipin or anti-beta2-glycoprotein1 antibodies. False positive or negative test results for the presence of a lupus anticoagulant can be seen in the presence of a direct oral anticoagulant, and therefore ideally testing should be performed prior to such agents being commenced [40]. To classify a patient as having APLAS, antibody testing should be positive on at least two occasions 12 weeks apart [41]. The risk of an initial thrombotic event in patients with a positive test for antiphospholipid antibodies varies from no increase in blood donors in whom the often transient antibodies are an incidental finding, to an annual risk of thrombosis of 2–4% in patients with SLE who are antibody positive [39]. Thrombotic risk is highest in patients with a positive test result by all three separate assays (lupus anticoagulant, anti-cardiolipin or anti-beta2-glycoprotein1 antibodies all positive), with such patients classified as being "triple positive" [42].

Importantly, a recent randomised trial demonstrated warfarin to be superior to rivaroxaban in patients with triple positive APLAS (the TRAPS trial) [43]. A clear excess of recurrent thrombosis, predominantly stroke, was seen in patients receiving rivaroxaban. In addition patients with APLAS, particularly those with a positive test for a lupus anticoagulant, are at increased risk of recurrent thrombosis and therefore they will usually receive long-term anticoagulation after an

initial event [44]. Therefore confirmation of the presence of antiphospholipid antibodies is likely to influence clinical management.

10.2.2.2 Heparin Induced Thrombocytopenia

Heparin induced thrombocytopenia (HIT) is an immune-mediated adverse drug reaction to heparin. It results from the formation of antibodies, in the majority of patients directed against a complex of heparin and the positively charged molecule platelet factor 4 (PF4) [45]. These antibodies then bind to the heparin-PF4 complex bound to the platelet surface, leading to platelet activation most likely due to signaling via the platelet Fc receptors. Platelet and probable concurrent endothelial activation result in activation of the coagulation cascade and increased thrombin generation, manifesting clinically as increased risk of venous and arterial thrombosis. Without institution of alternative anticoagulation, patients with confirmed HIT have a daily incidence of new thrombotic complications of up to 6%, with the historical risk of death or amputation due to venous gangrene approaching 50% [46]. Early recognition of HIT is therefore important and monitoring of platelet counts between day 2 and 14 of exposure should be performed in all patients receiving heparin. A fall in platelet count to less than 150×10^9/L or all fall in total platelet count by greater than 50% should prompt laboratory investigation for HIT antibodies.

Heparin should be immediately ceased in patients suspected of having a diagnosis of HIT. Patients with a confirmed diagnosis of HIT antibodies should be started on a non-heparin alternative anticoagulant [47]. Due to a high rate of cross-reactivity with HIT antibodies, low-molecular-weight heparin should be avoided. Alternative anticoagulants include argatroban, bivalirudin, danaparoid, fondaparinux, or a direct oral anticoagulant (DOAC) [47]. Choice of drug will be determined by patient factors such as renal and hepatic function, and individual clinician familiarity with the alternative agents.

10.2.2.3 Myeloproliferative Disorders

The primary bone marrow disorders polycythaemia rubra vera (PRV), myelofibrosis and essential thrombocytosis (ET) make up the bcr-abl negative myeloproliferative disorders. In almost all patients with PRV, and a significant proportion with ET, a somatic acquired mutation known as the JAK2 V617F mutation will be detected [37]. Patients with PRV and ET in particular have been shown to be at an increased risk of both venous and arterial thrombosis. The pooled prevalence of all thrombosis among patients with myeloproliferative neoplasms (MPN) at initial diagnosis was 20.0%, with the prevalence of arterial thrombosis 16.2% and that of venous thrombosis of 6.2% [48].

Full blood examination is therefore recommended in all patients with arterial or venous thrombosis. Patients with acute thrombosis in the setting of an MPN may

also require cytoreductive therapy, including venesection to reduce the haematocrit to <0.45, as well as therapeutic anticoagulant therapy [49]. While the evidence is still limited, it appears that DOACs are efficacious and safe when used as anticoagulants in this setting [50]. Due to a high risk of recurrent thrombosis long-term anticoagulation is recommended in patients with MPN and clinically significant venous thrombosis [49].

It has been observed that a significant proportion of patients with unprovoked portal and mesenteric vein thrombosis will be found to have the JAK2 V617F mutation present, often without clear evidence of a myeloproliferative disease on the peripheral blood examination. The CALR mutation is present in a smaller proportion of patients [49, 51]. Testing for these mutations should therefore be performed in patients presenting with splanchnic vein thrombosis in the absence of an obvious alternative cause. As patients with a proven MPN and prior abdominal vein thrombosis have a high risk of recurrent events, long-term anticoagulation, is recommended in the absence of contra-indications [49].

10.2.2.4 Paroxysmal Nocturnal Haemoglobinuria

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare clonal bone marrow disorder, associated with a loss of glycosylphosphatidylinositol (GPI) anchor proteins on hematopoietic cells, that results in an increased susceptibility to complement-mediated haemolysis [52]. This results in an increase in the risk of thrombosis, and approximately 10% of patients with PNH will have thrombosis at presentation. Unusual site venous thrombosis particularly hepatic vein or cerebral venous sinus thrombosis is common. Recurrent venous thrombosis may occur in patients with PNH receiving standard anticoagulation, with the risk of recurrence appearing to be reduced by use of the complement (C5) inhibitor eculizumab [52]. Identification of this uncommon condition in patients presenting with unexplained unusual site venous thrombosis therefore has potential therapeutic implications.

10.3 Potential Reasons for Performing Thrombophilia Testing

Clinical utility is an important concept when considering laboratory investigations for any condition. The clinical utility of any investigation can be defined as the degree to which the clinical outcome of an individual patient is improved by the performance of that test. It is important that any clinician ordering thrombophilia testing is able to articulate clearly what implications the results of the test will have on the management of that individual patient. If the answer is no impact, then the test should not be performed. The evidence for potential indications for testing for an underlying thrombophilic condition are discussed below.

10.3.1 Patients with Venous Thrombosis and Their Relatives

10.3.1.1 Providing an Understanding of the Aetiology of a Thrombotic Event

As discussed above, a number of inherited conditions have been shown to be clearly associated with an increased risk of a first episode of venous thrombosis (Table 10.1) [8, 9]. It is understandable that patients with venous thrombosis may want to improve their understanding as to why an event occurred, and thrombophilia testing may help provide some explanation in individual cases. It however should be emphasized that venous thrombosis is a multifactorial disease with often many risk factors present at the time of an event, and therefore care should be taken in attributing an event entirely to an underlying thrombophilic condition. Guidelines have attempted to identify particular patient groups in which an underlying inherited thrombophilia is likely to be identified, and have suggested that younger patients (<50 years of age) with unprovoked venous thrombosis, or individuals with unusual site or recurrent venous thrombosis could be selected for testing [10]. As outlined below the impact on management of these test results remains unclear.

The cost-effectiveness of performing thrombophilia testing solely to understand the aetiology is questionable. As discussed below, it is also important that both the patient and clinician understand that testing for the common genetic mutations, the FVL and PGM mutations, is unlikely to change management, and that the results of a positive test for these conditions are not over-interpreted. Finally the potentially negative impact of testing including implications for insurance, and the risk of over-interpretation of results, should be taken into account before testing is performed. As a result it is not recommended that thrombophilia testing is routinely performed to understand the aetiology of an event.

10.3.1.2 Determining the Choice of Antithrombotic Agent for Initial Treatment of Thrombosis

The presence or absence of an inherited thrombophilia does not impact on choice of initial anticoagulant therapy. Testing for these conditions therefore should not be performed at the time of acute presentation, a recommendation

Table 10.1 Increase in risk of initial and recurrent venous thrombosis with inherited thrombophilia

	AT deficiency	Protein C deficiency	Protein S deficiency	FVL mutation[a]	PGM mutation[a]
Increase in risk of first episode VTE	5 to 20-fold	5 to 10-fold	5 to 30-fold	3 to 7-fold	2 to 3-fold
Increase in risk of recurrent VTE	2.0-fold (pooled data)			1.2 to 1.6 fold	1.4 fold

AT antithrombin syndrome, *FVL* Factor V Leiden, *PGM* prothrombin gene mutation, *VTE* venous thromboembolism

[a]Refers to heterozygote state

reinforced by the fact that adequate counselling regarding genetic testing is unlikely to occur in the emergency setting.

Acquired conditions that may alter the choice of initial anticoagulant choice include HIT (non-heparin anticoagulant required), APLAS (warfarin preferred over DOAC), the presence of a MPN (cytoreductive therapy also required), and PNH (addition of eculizumab may be considered). Testing for these conditions in acute presentations suggestive of their presence (e.g. significant unprovoked venous thrombosis for APLAS, unusual site thrombosis or recurrent thrombosis on anticoagulation for MPN and PNH) should be considered.

10.3.1.3 Determining the Risk of Recurrence and Therefore Optimal Duration of Anticoagulation

Patients with venous thrombosis are at risk of recurrent events, with approximately 30% of affected individuals subsequently experiencing a recurrent event within 5 years of ceasing anticoagulation [53]. A potential role for thrombophilia testing is therefore to identify those patients at greatest risk of recurrent thrombosis, in whom exposure to the increased risk of haemorrhage with long-term anticoagulation may be justified.

Patients with provoked venous thrombosis associated with transient major risk factors (surgery, limb fracture or other trauma, significant immobilisation) are at low risk of recurrent venous thrombosis, with an estimated rate of recurrence of 0.7% over a 2 year period after ceasing anticoagulation [54]. As the presence of a definable thrombophilia is unlikely to alter the risk/benefit of ceasing anticoagulation, thrombophilia testing is not recommended in this patient group [9–11].

Patients with unprovoked venous thrombosis have a substantially increased risk of recurrent thrombosis in comparison to patients in whom the event was associated with a definite provoking risk factor. A meta-analysis of this patient group confirmed a risk of recurrent thrombosis of approximately 10% in the first year after ceasing anticoagulation, with an incidence of 36% at 10 years [55]. These findings, combined with the lower incidence of major bleeding when patients receive anticoagulation with a DOAC, have led to guidelines recommending continued anticoagulation in patients with unprovoked venous thrombosis in the absence of contra-indications. As outlined below, this decision is unlikely to be modified by the absence of an underlying thrombophilia. Therefore, patients in whom a decision has been made on clinical grounds to continue anticoagulation should not have thrombophilia testing performed.

The question of thrombophilia testing therefore becomes focused on patients with unprovoked venous thrombosis in whom cessation of anticoagulation is being proposed, and whether estimates of recurrence risk will be sufficiently modified in this patient group by the results to lead to a change in decision making.

The high-incidence inherited thrombophilic conditions, the FVL and PGM mutations, do not significantly increase the risk of recurrent thrombosis. A recent meta-analysis found that patients heterozygous for the FVL mutation compared to patients

without the mutation had an approximate 1.6-fold increase in the risk of recurrent thrombosis [56]. When this was restricted to patients with an unprovoked event, this decreased to a 1.2-fold increase in risk that was no longer statistically significant. The same analysis found a borderline significant 1.4-fold increase in risk of recurrent venous thrombosis in patients heterozygous for the prothrombin gene mutation. This data suggests heterozygosity for the FVL or PGM should not be used by itself to determine duration of anticoagulation.

There is less data regarding the impact of antithrombin, protein C and protein S deficiency on the risk of recurrent venous thrombosis, and due to their lower incidence, data tends to be pooled for all three conditions. Data from prospective cohort studies of unselected patients with venous thrombosis has suggested an approximate twofold increase in the risk of recurrence in patients with deficiencies of these proteins in comparison to patients with normal levels [8]. A retrospective study of thrombophilic families found that individuals with AT, PC and PS deficiency had a cumulative incidence of recurrent thrombosis of 55% by 10 years after ceasing anticoagulation, in comparison to a figure of 25% in patients with FVL, PGM or elevated FVIII levels [57]. These data suggest that patients with confirmed AT, PC or PS deficiency may benefit from long-term anticoagulation. It is important to stress that the levels of these proteins may be spuriously low, for example in the case of recent extensive thrombosis, or, in the case of protein C and S, recent warfarin therapy. Therefore, repeat testing in the absence of confounding factors should be performed to confirm the diagnosis prior to therapeutic decisions being made. While data is lacking on clinical factors that can be used to reliably identify patients with venous thrombosis that will have a deficiency of one of the natural inhibitors of coagulation, it would appear reasonable to focus testing on patients with unprovoked events, younger age (<50 years of age), unusual site of thrombosis, or a strong family history (>1 first degree relative) of venous thrombosis. If testing is to be performed it is suggested that it be performed at either the time cessation of anticoagulation is being considered, or one month after cessation.

As previously mentioned, patients with APLAS have been demonstrated to have an increased risk of recurrent thrombosis, with estimates of risk ranging from 10 to 60% per annum [44]. In addition, patients with antiphospholipid antibody syndrome have been demonstrated to have an increased risk of death after ceasing anticoagulation, contributed to by the fact that this patient group is at increased risk of not only recurrent venous thrombosis but also arterial complications. Therefore, long-term anticoagulation is generally recommended for patients who meet the diagnostic criteria for this condition.

10.3.1.4 Determining the Need for Primary Prophylaxis in Asymptomatic Family Members

Another possible role for thrombophilia testing is determined if the baseline risk of venous thrombosis is sufficient to warrant primary prophylaxis with anticoagulation. Given the lack of evidence supporting a role for anti-platelet therapy in the

Table 10.2 Risk of venous thrombosis in asymptomatic family members with inherited thrombophilia

	AT deficiency	Protein C deficiency	Protein S deficiency	FVL mutation[a]	PGM mutation[a]
Overall risk (risk/year)	1.5–2.0%	1.0–1.5%	1.5–2.0%	0.5%	0.3–0.4%
Oral contraception (risk/year exposure)	4–5% (pooled data)			0.3–2.0%	0.2–2.0%
Pregnancy (risk/pregnancy 95% CI)	16.6% (0.0–45.1)	7.8% (0.0–33.8)	4.8% (0.0–20.0)	1.1% (0.3–0.9)	0.9% (0.2–2.0)
Antepartum risk	7.3% (1.8–15.6)	3.2% (0.6–8.2)	0.9% (0.0–3.7)	0.4% (0.1–0.9)	0.0% (0.0–0.2)
Postpartum risk	11.1% (3.7–21.0)	5.4% (0.9–13.8)	4.2% (0.7–9.4)	2.0% (0.9–3.7)	0.9% (0.2–2.0)

[a]Refers to heterozygote state

primary prevention of venous thromboembolism, at present this would require an estimate of risk that was sufficiently high to justify exposure to the 1–2% annual risk of major haemorrhage associated with ongoing oral anticoagulant therapy.

As shown in Table 10.2, the annual risk of venous thrombosis in previously asymptomatic patients varies from approximately 0.3% with the PGM to up to 2% in patients with AT or protein S deficiency [8, 9, 57]. This is against a background rate of approximately 0.1% per annum in the general population, with incidence increasing with age. It is generally accepted that given the risk associated with oral anticoagulation, that primary prophylaxis is therefore not justified in patients with any of the known inherited thrombophilias. It has been shown that between 50 and 60% of episodes of venous thrombosis in previously asymptomatic family members with thrombophilia will occur in the context of an additional environmental risk factor such as surgery. While not clearly demonstrated in clinical trials, it is possible that more aggressive thromboprophylaxis may be justified particularly in patients with type 1 thrombophilic conditions [9]. Again, if testing is performed for this indication, care must be taken to avoid over-interpretation of the test result by both patient and other clinicians.

10.3.1.5 Making Decisions Regarding the Use of the Oral Contraceptive Pill

Knowledge of whether a previously asymptomatic individual is a carrier of a known inherited thrombophilia may influence decision-making regarding exposure to the pro-thrombotic effects of oral contraception. Estimates of the annual risk of thrombosis with the use of a combined oestrogen/progesterone oral contraceptive (OCP) in previously asymptomatic relatives identified due to a family history of thrombosis [8, 58] are shown in Table 10.2. Generally women of child bearing age have a low annual risk of thrombosis of approximately 1–2/10,000/year. The increase in annual risk of venous thrombosis with OCP use is higher in previously asymptomatic individuals with type 1 thrombophilic states, and most clinicians would accept

that the magnitude of risk justifies avoidance of the combined OCP and use of other contraceptive measures, including progesterone only pills or intrauterine devices, that do not increase the risk of thrombosis. Estimates of the risk with OCP use in family members heterozygous for FVL and PGM vary, and decisions may be influenced by patient perception of the benefit obtained from OCP use, and the presence of other risk factors for venous thrombosis such as obesity.

It is worth emphasising that negative testing for an underlying thrombophilia may provide false reassurance in this setting. Family members testing negative for the thrombophilia identified in the proband have still been shown to have a risk of venous thrombosis significantly higher than the remainder of the community possibly due to unidentified inherited factors [58].

10.3.1.6 Determining the Need for Thromboprophylaxis During Pregnancy

The risk of venous thrombosis during pregnancy in women with no prior history of thrombosis associated with the presence of common inherited thrombophilic conditions, derived from a meta-analysis of available studies, is shown in Table 10.2 [59]. Two-thirds to three-quarters of pregnancy related episodes of venous thrombosis will occur during the post-partum period. Estimates for type 1 conditions are derived from family studies and therefore cannot be extrapolated to women diagnosed incidentally. The case for prophylactic anticoagulation during pregnancy can be made most strongly for women with type 1 conditions, particularly for antithrombin deficiency and, to a lesser extent, protein C deficiency. Other risks factor such as obesity, other medical conditions and the strength of family history of thrombosis are likely to influence decision making. As a minimum, post-partum prophylaxis should be administered for 6–8 weeks. In FVL and PGM heterozygotes ante-partum prophylaxis is generally not recommended in women with no prior history of events. Post-partum prophylaxis should be considered, again particularly in women with additional risk factors.

10.4 Patients with Arterial Thrombosis

The association between inherited thrombophilic conditions and arterial disease has not been clearly demonstrated. Case reports and small studies have linked antithrombin, protein C and protein S deficiency to arterial disease, however the data are inconclusive [8]. Larger studies have evaluated the link between the FVL and PGM mutations with both coronary artery disease, myocardial infarction and stroke. Generally the findings have been of either no link or a weak association with odds ratios of <1.5 [8, 9], with some data suggesting a stronger association with myocardial infarction in younger patients with the additional risk factor of smoking. There is also no conclusive evidence supporting an association of thrombophilia with

peripheral arterial disease. Based on the lack of a clear association of inherited thrombophilia with arterial disease, and no data supporting that a change in management based on the knowledge of the presence of a thrombophilic conditions improves patients outcome, it is recommended that testing for inherited thrombophilia should not be performed in patients with arterial disease outside clinical studies.

As stated above, the association of antiphospholipid antibodies with an increased risk of arterial disease is more definitive. It is generally recommended that patients with APLAS and arterial disease should be treated with warfarin rather than antiplatelet agents, although the evidence supporting this approach remains minimal [44]. Myeloproliferative disorders can also be associated with arterial disorders, and should be considered in patients with unexplained arterial thrombotic events in the absence of traditional risk factors.

The clinical utility of measuring homocysteine levels in patients with arterial disease at present remains unclear. While a number of trials have shown benefit of B-vitamin supplementation on surrogate end-points of arterial disease, a meta-analysis found no reduction in clinical end-points in patients with mild hyperhomocysteinemia and either cardiovascular disease or stroke with supplementation therapy [60]. This should be distinguished from patients with severe hyperhomocysteinemia who constitute a small proportion of patients with thrombosis (~0.2%), who may present with premature arterial disease [61].

10.5 Potential Detrimental Effects of Thrombophilia Testing

A small number of studies have examined the potential psychological impact on patients of performing thrombophilia testing [62]. While the general conclusion was that the impact was low, it was clear that many patients were unclear that they had been tested, and the knowledge of having a thrombophilia did cause significant distress in some individuals. Other potential drawbacks to testing for inherited thrombophilia may include difficulty with obtaining or changes to the cost of life-insurance, and questionable cost-effectiveness [8].

Perhaps due to the uncertainty about the clinical implications of the finding of the presence of a low risk thrombophilic condition, studies have found that patients tested for these conditions show a low degree of satisfaction with no impact on quality of life [63].

10.6 Conclusion

It can be concluded that, despite the ability to detect an underlying thrombophilia in up to 50% of patients with venous thrombosis, it is doubtful that performing laboratory testing for thrombophilias has a positive effect on patient outcome in the

majority of patients. The strongest case for testing for inherited thrombophilia can be made for type 1 conditions, although these conditions will be detected in only approximately 5% of patients with venous thrombosis. The evidence that testing for FVL and the PGM abnormalities improves patient outcome is limited.

Widespread testing for inherited thrombophilia in unselected patients is recommended against, with a stronger case for testing able to be made for patients with previously-asymptomatic female first-degree relatives of child-bearing age particularly with type 1 conditions. Prior to any testing being performed, the clinician involved in test-ordering should counsel the patient regarding the implications of both a positive and negative test result, and how this will change patient management. If it is unclear how the test result will change treatment for the individual or relatives, then testing should not be performed. Clinicians have been shown to adhere poorly to guidelines that recommend against widespread thrombophilia testing, and continuing education is required in this area [63].

Testing for acquired thrombophilic conditions, including APLAS, MPN and HIT is more likely to impact clinical decision making, and should be performed in patients with suggestive clinical features.

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Chapter 11
Platelets in the Pathogenesis of Vascular Disease and Their Role as a Therapeutic Target

James McFadyen and Karlheinz Peter

Key Learning Points

- Platelets are central mediators of haemostasis and pathological thrombosis
- Platelets possess important pro-inflammatory functions and promote the development of atherosclerosis
- Platelet activation leads to the activation of the major platelet adhesion receptor, GPIIb/IIIa, which facilitates platelet aggregation (Fig. 11.1)
- Clinically available anti-platelet drugs act to inhibit this process and thus platelet aggregation
- Current anti-platelet approaches also inhibit pathways important for physiological haemostasis and therefore are associated with the risk of bleeding
- New therapeutics which inhibit thrombosis, but not haemostasis, are in development

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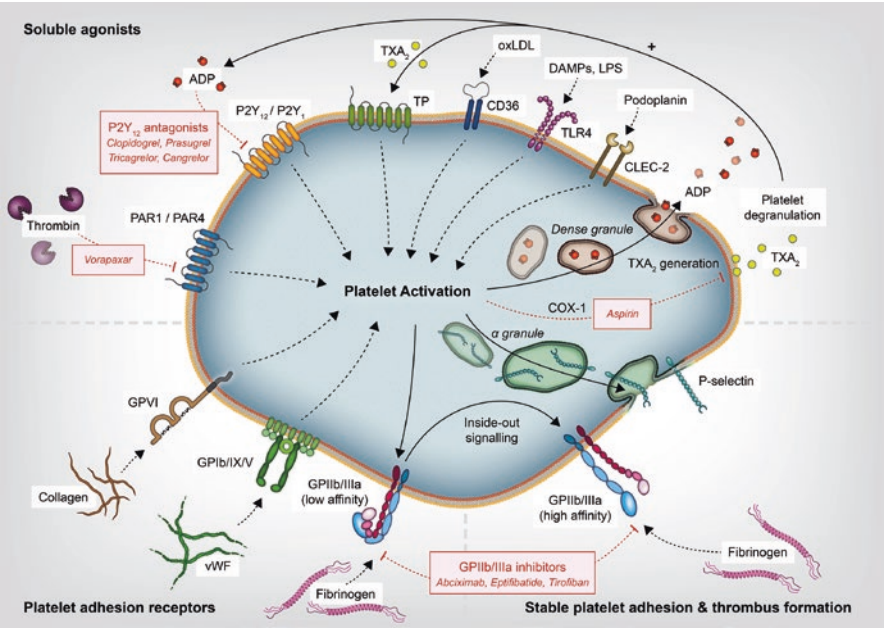

Fig. 11.1 Platelet structure and activation pathways. Platelets express specialised adhesion receptors (GPIIb/IIIa, GPIb-IX-V and GPVI) and G-protein coupled receptors (GPCRs) that bind to their specific ligands as indicated. The major platelet adhesion receptor, GPIIb/IIIa, exists in a low affinity conformation on the resting platelet. The activation of platelets by soluble agonists, or platelet-ligand binding results in platelet degranulation (secretion), shape change and the activation of GPIIb/IIIa (inside-out signalling), allowing platelets to form high affinity interactions with adhesive proteins, such as fibrinogen and vWF, thus promoting stable platelet aggregation and thrombus formation. Platelet granules, such as alpha and dense granules, contain important proinflammatory and prothrombotic mediators that act in a paracrine and autocrine fashion to reinforce platelet activation. Platelets express a range of other receptors such as CD36, TLR4 and CLEC-2, which all play a role in the proinflammatory and prothrombotic role of platelets. Highlighted are the current antithrombotic therapies, which inhibit either soluble agonist induced activation or platelet adhesion receptor function

11.1 Introduction

Platelets are anucleate cells that are derived from bone marrow megakaryocytes and are the smallest blood cells in the circulation, with an average diameter of 2–5 μm in humans. Platelets are the second most abundant cell type in the blood stream with nearly one trillion in the circulation at any one time. Once released into the circulation, platelets have a lifespan of 7–10 days. The vascular endothelium synthesises and secretes nitric oxide (NO), the eicosanoid prostacyclin and the ectonucleotidase, CD39, to help maintain platelets in a quiescent, non-reactive state. However, upon encountering damaged endothelium or exposed subendothelial layers, such as atherosclerotic plaque rupture, platelets have the ability to adhere, activate and aggregate with great alacrity. Indeed, the accumulation of platelets in the context of

atherosclerotic plaque rupture is the precipitating event leading to vessel occlusion ultimately causing myocardial infarction or ischaemic stroke—two of the leading causes of mortality and morbidity globally. Consequently, the use of anti-platelet drugs is globally one of the most widely applied pharmaceutical therapies.

This chapter will discuss the platelet receptors, soluble agonists and respective G protein coupled receptors that mediate platelet adhesion, activation and aggregation. The recently discovered proinflammatory functions of platelets and how they participate in the pathogenesis of atherosclerosis will be outlined. Finally, an overview of current and future anti-platelet therapies will be provided in addition to an outline of the commonly employed platelet function tests.

11.2 Platelet Structure and Function

11.2.1 Platelet Adhesion Receptors

In order to carry out their specialized role, platelets have a distinct structure (Fig. 11.1). The resting platelet has a characteristic 'discoid' morphology and expresses a large number of adhesion receptors on the cell surface to regulate platelet-extracellular matrix, and platelet-platelet interactions. Further, adhesion receptors allow the transmission of extracellular signals by activating intracellular signalling pathways, which in turn can modulate platelet adhesion and activation responses. The most abundant platelet adhesion receptor is Glycoprotein (GP) IIb/IIIa (also known as integrin $\alpha_{IIb}\beta_3$ or CD41/CD61) with approximately 80,000 copies per platelet [1]. The major ligand of GPIIb/IIIa is fibrin(ogen) and therefore this receptor plays an essential role in mediating stable platelet adhesion via interaction with immobilised fibrin(ogen) and also platelet aggregation, via soluble fibrin(ogen) which acts to crosslink adjacent platelets [2]. Whilst GPIIb/IIIa exists in a low affinity state on the resting platelet, upon platelet activation it undergoes a conformational change (so-called integrin inside-out signalling) such that GPIIb/IIIa exposes the ligand (fibrin(ogen)) binding pocket and thus adopts a high affinity conformation towards fibrin(ogen) [3]. The importance of GPIIb/IIIa in mediating platelet adhesion and aggregation is underscored by the bleeding phenotype seen in patients with Glanzmanns thrombasthenia, who exhibit a deficiency of GPIIb/IIIa expression or whose GPIIb/IIIa is functionally impaired.

11.2.2 Glycoprotein IIb/IIIa (GPIIb/IIIa) Structure

GPIIb/IIIa has a large extracellular portion formed by one α and β subunit with a single transmembrane spanning region and short cytoplasmic tail (Fig. 11.1) The α subunit, β propeller domain and β_3 subunit domain come together to form the

globular, ligand binding head [3]. The other domains of the α and β subunit, comprise two flexible 'stalks'. As such, the integrin has a bent conformation in the resting state and upon activation extends in a process associated with reorganisation of the ligand-binding domain [3].

The major ligands of GPIIb/IIIa are fibrinogen, von Willebrand factor (vWF) and fibronectin, which all bind to the extracellular globular head [4]. A common feature of all GPIIb/IIIa ligands is the presence of arginine-glycine-aspartic acid (RGD) motifs, which are recognised by the ligand binding site of GPIIb/IIIa [5]. The RGD sequence is contained within the α chain of fibrinogen. However, fibrinogen, the major GPIIb/IIIa ligand binds to the integrin via the C terminus of the fibrinogen γ chain, which lacks an RGD motif [5]. Whether RGD sequences or the α chain of fibrinogen bind to similar or distinct regions of the integrin remains unresolved. GPIIb/IIIa has a low affinity for its ligands in the resting conformation [6]. However, upon platelet stimulation, the integrin extends and opens the globular head region, thus allowing more efficient ligand binding between the propeller domain and βA domain [7]. (Figs. 11.1 and 11.2) Ligand binding itself may also induce conformational changes in GPIIb/IIIa.

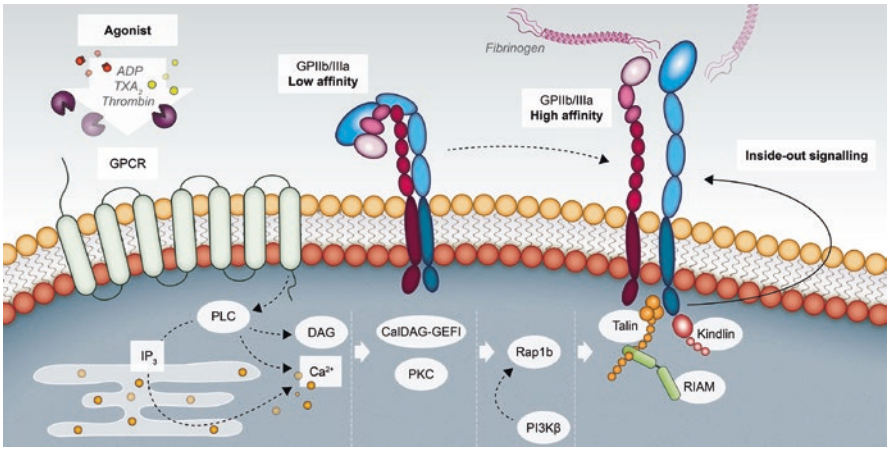

Fig. 11.2 GPIIb/IIIa inside out signalling. A schematic representation of GPIIb/IIIa inside-out signalling events leading to the activation of GPIIb/IIIa. Activation of platelets by G-protein coupled receptors (GPCR), leads to the activation of phospholipase C (PLC) leading to the mobilisation of intracellular calcium and activation of protein kinase C (PKC). These signals result in the activation of the Guanine nucleotide exchange factor CalDAG-GEF1, ultimately leading to activation of the small GTPase Rap1b. Rap1b mediates GPIIb/IIIa activation by forming a complex with RIAM, which is thought to activate and localise talin to the plasma membrane. Binding of talin to the integrin disrupts the salt bridge between the cytoplasmic tails of GPIIb/IIIa, thus allowing long range conformational changes of the extracellular integrin domains. Kindlins, a family of FERM domain containing proteins, bind to integrin β-cytoplasmic tails and appear to play an essential role in integrin inside out signalling. PI3Kβ, activated downstream of G_i linked signals, plays an important role in Rap1b activation and therefore sustains GPIIb/IIIa activation

11.2.3 Glycoprotein GPIIb/IIIIa Activation

Although the cytoplasmic tails of GPIIb/IIIa are short, they play a fundamental role in regulating GPIIb/IIIa signalling and adhesive function [8]. The ability of signalling events at the cytoplasmic portion of the integrin to induce long range conformational changes of the extracellular domains such that GPIIb/IIIa switches from a low affinity to high, is referred to as "inside-out signalling". Whilst the cytoplasmic tails have no intrinsic signalling activity, this function is carried out by the over 20 proteins that have been identified to bind to the cytoplasmic tail of GPIIb/IIIa to facilitate bidirectional signals [9]. Importantly, these proteins also link GPIIb/IIIa to the actin cytoskeleton, thus allowing the transmission of biochemical and biophysical (mechanical) signals [10].

11.2.4 GPIIb/IIIa Inside-Out Signaling

The activation of GPIIb/IIIa by inside-out signalling can be initiated by the stimulation of G protein coupled receptors on platelets after stimulation by soluble agonists such as ADP and thrombin or by signals generated by platelet adhesion receptors such as GPIb-IX-V or GPVI after binding to their respective ligands [11, 12] (Fig. 11.2). A common feature after agonist stimulation is the activation of phospholipase C (PLC)—liberating 1, 4, 5-inositol triphosphate (IP_3) and diacylglycerol (DAG), and subsequent release of intracellular calcium stores and activation of protein kinase C (PKC) and CalDAG-GEFI [13, 14]. PKC and CalDAG-GEFI are critical messengers that activate the small GTP binding protein Rap1b that is essential for activation of GPIIb/IIIa [13, 14]. The importance of these signalling processes is underscored by the observation that CalDAG-GEFI deficient platelets demonstrate impaired GPIIb/IIIa activation, prolonged bleeding times and protection from thrombosis [13]. Likewise, CalDEF-GEFI deficiency leads to impaired aggregation responses to ADP and TxA_2 [13]. However, some of these platelet function defects seen in CalDEF-GEFI platelets can be overcome by more potent platelet agonists such as collagen or thrombin [13]. These findings have led to the notion that CalDEF-GEFI mediates the rapid but reversible activation of Rap1b whilst PKC is required for sustained Rap1b and therefore sustained GPIIb/IIIa activation.

The effector molecule of Rap1b is the small GTPase RIAM (Rap1-GTP-interacting molecule), which binds to Talin and localises it to the plasma membrane [15]. Talin is a cytoplasmic partner of GPIIb/IIIa that serves as an essential mediator of GPIIb/IIIa activation [16]. Talin exists in an autoinhibited basal state, however, upon PKC mediated-RIAM activation, it becomes activated and serves to activate GPIIb/IIIa [17]. In the resting, low affinity state, the transmembrane domains of the α_{IIb} and β_3 subunits are closely associated and reinforced by a salt bridge between these subunits which act to lock GPIIb/IIIa in the low affinity conformation [18]. Activated Talin binds to the cytoplasmic domains of the α_{IIb} and β_3 subunits and

facilitates disruption of the salt bridge and the close association of the subunits [16]. This allows the extension of the β_3 subunit and activation of GPIIb/IIIa to an extended, high affinity conformation.

Another family of proteins that bind to the cytoplasmic domains of the β_3 subunits are the Kindlins [19]. The Kindlins appear to play an important role in modulating Talin-mediated integrin activation, however the precise mechanisms underpinning this function remain to be elucidated [19]. The fundamental roles of RIAM, Talin-1 and Kindlin-3 are highlighted by the finding that mice with deficiencies of these proteins exhibit defects in GPIIb/IIIa activation [15, 19].

11.2.5 Glycoprotein IIb/IIIa Outside-In Signalling

The binding of fibrinogen to GPIIb/IIIa results in integrin clustering and the formation of nascent multiprotein signalling complexes, followed by the formation of larger, actin based signalling complexes [20, 21]. Whilst GPIIb/IIIa lacks intrinsic signalling activity, the cytoplasmic tail of the β_3 subunit contains evolutionary conserved NPXY motifs (arginine, prolene, X = any amino acid, tyrosine) that serve as a recognition site for phosphotyrosine binding (PTB) proteins, which play integral roles in propagating outside-in signals. Indeed, many of the over 20 identified cytoplasmic tail binding partners of GPIIb/IIIa are protein kinases or phosphatases [9]. As such, GPIIb/IIIa can transmit signals and contractile mechanical forces from the extracellular to the intracellular environment. This is perhaps best reflected by GPIIb/IIIa ligation-dependent morphological changes that are associated with dynamic alterations of the actin cytoskeleton [22]. These changes are mediated by the effector molecules of the Rho GTPases such as cdc42, Rac1 and RhoA and result in the extension of filopodia, lamellipodia and full platelet spreading. Thus, GPIIb/IIIa outside-in signalling plays a central role in sustained platelet activation, full platelet spreading, granule secretion and fibrin clot retraction [23].

Integrin outside-in signalling is initiated by ligand binding and clustering of GPIIb/IIIa [20]. Src kinase, which is constitutively bound to the β_3 cytoplasmic tail, is then activated and recruits Syk [21]. Src and Syk may then phosphorylate multiple substrates, including the adapter proteins SLP-76 and ADAP, and PLCγ2 that promote GPIIb/IIIa association with the actin cytoskeleton and platelet activation [24, 25]. Multiple other proteins that mediate outside-in signalling are recruited to these nascent signalling complexes, including PI3 kinase (PI3K), Rac and VASP (vasodilator-stimulated phosphoprotein) [11]. Whilst all the components of the signalling complex are yet to be fully elucidated, the β_3 tail, PLCγ and α-actinin appear to be three central proteins mediating the transition of the nascent signalling complex to an actin-based complex [26].

The activation and phosphorylation of Src kinases downstream of GPIIb/IIIa ligation also induces the phosphorylation of focal adhesion kinase (FAK) [27]. FAK is a ubiquitously expressed tyrosine kinase, which is phosphorylated and recruited into the formation of the nascent focal adhesion complex [28]. In addition to

phosphorylated FAK, the nascent adhesion complex comprises of paxilin, talin, vinculin and actin [28, 29]. The assembled signalling complexes provide a means to link the intracellular cytoskeleton to the extracellular GPIIb/IIIa. One of the major downstream effectors of FAK is phosphorylated c-CBL, which associates and activates PI 3-kinase p110β (PI3Kβ) by recruiting the p85 regulatory subunit [30]. The activation of PI3Kβ results in the stimulation of the small GTPase Rap1b, which is critical for integrin dependent platelet adhesion and spreading on fibrinogen [30].

11.2.6 Glycoprotein Ib-IX-V Complex

The Glycoprotein Ib-IX-V complex (GPIb-IX-V) is the second most abundant platelet adhesion receptor with approximately 25,000 copies expressed on the platelet surface [31]. GPIb-IX-V is expressed exclusively on megakaryocytes and platelets and plays a critical role in haemostasis and thrombosis. This is highlighted by the bleeding diathesis seen in patients with Bernard Soullier Syndrome, which is caused by a congenital deficiency in GPIb-IX-V expression or function [32]. The GPIb-IX-V complex is composed of four different subunits—GPIbα, GPIbβ, GPIX and GPV [33]. Each subunit is a type 1 transmembrane protein and contains a leucine rich repeat (LRR) domain in the extracellular domain [34]. The GPIb-IX complex is highly stable, and contains the GPIbα, GPIbβ and GPIX subunits in a ratio of 1:2:1. The interactions of the subunits are critically important in stabilising the individual domains and preventing their unwanted, premature proteolytic degradation. The importance of the interactions between the GPIbα, GPIbβ and GPIX subunits is highlighted by the fact that a mutation in one of these three subunits can eliminate the GPIb-IX expression in platelets. In contrast to the tight association of the GPIb-IX complex, it is thought the GPV subunit is only weakly associated via the transmembrane domain and is not essential for the normal expression of the GPIb-IX complex.

The extracellular N terminal region of the GPIbα subunit is the major ligand binding domain of the GPIb-IX-V complex containing binding sites for ligands such as vWF, thrombin, high molecular weight kininogen (HMWK), Factor XI and XII, Mac-1 and P-selectin [35–37]. As a consequence, the GPIb-IX-V complex plays an important role in mediating the adhesion of platelets to the subendothelial matrix, the endothelium, leukocytes and facilitating the assembly of coagulation factors on activated platelets [33].

The primary function of the GPIb-IX-V complex is to serve as an adhesion receptor for vWF under high shear [38]. Indeed, recent evidence suggests that the effects of shear stress on the GPIb-IX-V-vWF interaction may be biphasic, with increasing force enhancing the bond strength (catch bonds) but above a force threshold, this interaction may weaken (slip bond) [39]. In contrast to integrin GPIIb/IIIa, the interaction between GPIb-IX-V and vWF does not support stable platelet adhesion or the formation of stable platelet aggregates in isolation. Rather, the interaction between A1 domain of vWF and GPIb-IX-V has a fast on-off rate and as such

facilitates platelet rolling or translocation under high shear, thus facilitating firm platelet adhesion mediated by the interactions of GPIIb/IIIa and/or GPVI with their respective ligands [40]. Indeed, at high or pathological shear rates, GPIb-IX-V is the only receptor that can mediate platelet adhesion and is therefore a prerequisite for the capture of platelets under high shear conditions [41].

11.2.7 Glycoprotein VI

Glycoprotein VI (GPVI) represents a member of the immunoglobulin (Ig) superfamily of receptors and serves as the major receptor for collagen on platelets [42]. GPVI is expressed exclusively on platelets and megakaryocytes. GPVI is composed of two extracellular immunoglobulin domains with a relatively short cytoplasmic domain containing a basic amino acid rich region that can bind calmodulin and a proline rich motif that can bind the Src homology 3 (SH3) domain of the SFKs, Fyn and Lyn [43]. GPVI is complexed with the Fc receptor (FcR) γ chain via interactions between the transmembrane and cytoplasmic domains [44]. Each FcRγ chain can participate in signalling events via its tyrosine based activation motif (ITAM) [45]. The majority of GPVI exists as a monomer on the surface of resting platelets which has a low affinity for collagen [46]. Platelet activation leads to the dimerisation of GPVI, which has a unique conformation and enhanced affinity for collagen [46].

The major ligand of GPVI is collagen where collagen binding to GPVI produces robust intracellular signals that play important roles in mediating thrombosis and haemostasis. This is underscored by the recent description of patients with GPVI deficiency associated with a mild bleeding diathesis [47]. In response to vascular injury and exposure of subendothelial collagen, platelets tether to vWF bound collagen, and then adhere and activate via GPVI interactions with exposed collagen. The interaction of GPVI and collagen generate intracellular signalling events that facilitate the activation of integrin $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$, resulting in additional GPVI clustering thus supporting stable platelet adhesion and activation [12, 48]. Whilst integrin $\alpha_2\beta_1$ can also bind collagen, at arteriolar shear rates, GPVI plays the predominant role in mediating stable platelet adhesion and aggregation [12].

11.2.8 Other Platelet Receptors

Recently, platelets have been demonstrated to express C-type lectin-like receptor (CLEC-2), which binds the transmembrane glycoprotein, podoplanin. Podoplanin is abundantly expressed in lymphatic endothelial cells, with the binding of podoplanin to platelet CLEC-2 triggering platelet activation. This interaction appears to play an important role in mediating the development of lymphatic vessels, the integrity of the blood-lymphatic junction and maintaining vascular integrity in the context of inflammation [49].

Platelets also express the 'scavenger' receptor CD36 and a number of pattern recognition receptors such as the toll like receptors (TLRs). CD36 has been demonstrated to play an important role in mediating platelet activation in response to oxidised low-density lipoprotein (oxLDL) and microparticles, whilst platelet TLR4 causes platelet activation in response to the danger associated molecular patterns (DAMPs) such as lipopolysaccharide (LPS) and high mobility group box 1 (HMGB1) [50, 51].

11.2.9 Platelet Granules

Platelets contain granules, which contain an array of proteins and other factors that reinforce the haemostatic process but are also pro-inflammatory and thereby contribute substantially to the inflammatory role of platelets. Alpha granules are the most abundant and contain platelet membrane proteins such as GPIIb/IIIa, glycoprotein (GP) Ib-IX-V in addition to the platelet adhesion proteins P-selectin, fibrinogen, vWF and coagulation Factor V [52, 53]. They also contain a vast array of chemokines, cytokine and growth factors such as Chemokine Ligand 4 (CXCL4) (PF4) and CXCL7 and the growth factors vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) [54]. In contrast, dense granules contain small molecules such as adenosine diphosphate (ADP), adenosine triphosphate (ATP), calcium, serotonin and polyphosphate.

11.2.10 Platelet Membrane

The plasma membrane of resting platelets is similar to all eukaryotic cells in that the phospholipids are distributed asymmetrically [55]. The outer leaflet of the membrane is composed almost entirely of choline phospholipids whilst the inner leaflet contains all of the phosphatidylserine (PS) and the phosphoinositides [56]. The asymmetrical distribution of phospholipids plays an important role in mediating platelet activation and platelet procoagulant function [57, 58]. After platelet stimulation, the phosphoinositide, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), is hydrolysed to inositol triphosphate (IP_3) and diacylglycerol (DAG) by the action of phospholipase C (PLC) [59]. Both IP_3 and DAG play essential roles in mediating downstream effects that underpin platelet activation [60]. IP_3 acts as a critical regulator of the cytosolic calcium concentration by acting as a secondary messenger that facilitates the release of calcium from the endoplasmic reticulum [61]. DAG facilitates the translocation of protein kinase C (PKC) from the cytosol to plasma membrane [62]. Further, metabolism of phospholipid or DAG on the inner leaflet by phospholipase A_2 (PLA2) plays a fundamental role in arachidonic acid liberation and subsequent thromboxane A_2 (TxA_2) synthesis [63].

The platelet membrane also plays a central role in mediating coagulation *in vivo* by the provision of a phosphatidylserine (PS)-positive surface, which provides the

requisite negatively charged surface for the assembly of complexes of coagulation factors [64]. After potent agonist activation, platelets can express PS on their outer surface [65]. The importance of platelet PS exposure to haemostasis is highlighted clinically by Scott syndrome, which is associated with impaired platelet PS exposure, manifesting as a severe bleeding diathesis [66].

11.3 Mediators of Platelet Activation

11.3.1 Soluble Agonists and Their G Protein-Coupled Receptors (GPCRs)

The release and generation of soluble agonists, such as ADP, thrombin and TxA_2 from platelets and damaged cells play a critical role in the haemostatic and thrombotic response. These agonists activate platelets via the interaction with G protein coupled receptors (Fig. 11.1).

11.3.1.1 Adenosine Diphosphate (ADP) and the P2Y Receptors

ADP is stored by platelets in dense granules at high concentrations and is released upon platelet activation and degranulation where it activates platelets via autocrine and paracrine signalling. Platelets have two ADP receptors—$P2Y_1$ and $P2Y_{12}$—with both being important for normal platelet responses to ADP [67]. Indeed, $P2Y_1$ deficient mouse platelets demonstrate impaired platelet aggregation and shape change in response to ADP [68]. Whilst $P2Y_{12}$ deficient mouse platelets show impaired platelet aggregation, they do have a normal shape change in response to ADP [69]. The importance of the P2Y receptors in facilitating paracrine platelet signalling is demonstrated by the fact that platelet activation is impaired in response to low dose TxA_2 and thrombin in the absence of ADP receptors [70].

The $P2Y_1$ receptor is coupled to the G_q subunit, which upon activation, leads to the hydrolysis of $PI(4,5)P_2$ by PLCβ and therefore intracellular calcium mobilisation and PKC activation—both of which are important for integrin activation and granule secretion [71, 72]. $P2Y_1$ G_q mediated calcium increase has been confirmed to be critical for ADP-induced platelet shape change via RhoA activation [72]. The $P2Y_{12}$ receptor is linked to G_i, and mediates PI3K activation, thus playing a central role in the activation of the small GTPase, Rap1b which is an essential mediator of sustained integrin activation [73]. Further, G_i signalling inhibits the production of cyclic AMP (cAMP) thus relieving the platelet inhibitory effect of cAMP dependent protein kinase [74]. The importance of $P2Y_1$ and $P2Y_{12}$ in platelet haemostatic function is highlighted by the severe bleeding phenotype in $P2Y_1$ and $P2Y_{12}$ deficient mice [75]. The $P2Y_{12}$ receptor is irreversibly inhibited by the thienopyridine, clopdiogrel.

11.3.1.2 Thromboxane A_2 (TxA_2) and the Thromboxane Receptor (TP)

TxA_2 is generated in platelets by the conversion of arachidonic acid to endoperoxidases by cyclo-oxygenase and their subsequent metabolism to TxA_2 by thromboxane synthase [76]. The effect of TxA_2 as a paracrine messenger to amplify platelet activation is spatially constrained due to its short half-life. The receptor for TxA_2, TP, is coupled to G_q and G_{13} and as such, plays a role in platelet degranulation, shape change, integrin activation and thus aggregation [72, 77]. The role of TxA_2 and the TP receptor in platelet function is demonstrated by TP deficient mice, which demonstrate a bleeding phenotype and the inability to form stable thrombi *in vivo* [78]. Clinically, the anti-platelet drug aspirin inhibits cyclo-oxygenase and thus TxA_2 generation.

11.3.1.3 Thrombin and the Protease Activated Receptors (PAR)

Thrombin is the central effector protease of the coagulation cascade in addition to being the most potent platelet agonist [72]. Thrombin is generated on the surface of activated platelets and endothelial cells and can activate platelets by interactions with thrombin receptors on the platelet surface. Human platelets express two thrombin receptors, the protease activated receptors PAR1 and PAR4, which are linked to G_q and G_{13} proteins [79, 80]. PAR1 is the major thrombin receptor on human platelets as it can mediate platelet activation at low thrombin concentrations whereas PAR4 has a much lower affinity for thrombin and thus only activates platelets in the presence of high thrombin concentrations [79]. The activation of PAR1 by thrombin is rather unique in that thrombin cleaves an N-terminal extracellular domain of PARs, which creates a tethered ligand that activates the receptor [79]. PAR1 and PAR4 are coupled to G_q and G_{13} intracellular signals [81]. PAR1 and PAR4 ligation triggers potent G_q signalling and therefore results in the activation of PLCγ and resultant activation of PKC and calcium mobilisation, required for integrin activation and platelet degranulation [82]. The activation of G_{13} results in Rho and Rho kinase activation, which via inhibition of myosin light chain phosphatase, leads to actin contraction and rapid shape change and degranulation [83]. Whilst there has been some debate as to whether PAR receptors directly couple to G_i, it appears that thrombin does not directly induce G_i mediated signals. Rather, G_i signalling is induced by thrombin indirectly via released ADP and subsequent $P2Y_{12}$ signalling [84].

11.4 Platelet Thrombus Formation

11.4.1 Platelet Adhesion

The inciting event underlying pathological thrombus formation is typically the erosion or rupture of an atherosclerotic plaque [85]. This leads to the exposure of a number of proteins and vascular matrix components that act as adhesive ligands for

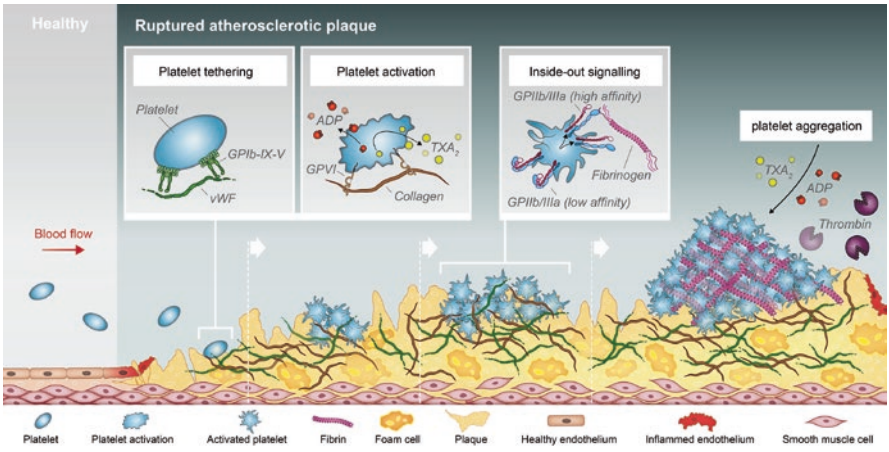

Fig. 11.3 The prothrombotic function of platelets. The inciting event leading to the formation of a pathological arterial thrombus is the rupture of an atherosclerotic plaque. This leads to the exposure of a number of highly reactive subendothelial matrix proteins such as vWF and collagen. Under arterial shear, platelets tether to the site of arterial injury via interactions of platelet GPIb-vWF. These interactions facilitate the engagement of other receptor-ligand interactions such as GPVI-collagen that lead to platelet activation, ultimately leading to activation of GPIIb/IIIa (inside-out signalling). Activation of GPIIb/IIIa allows stable platelet-platelet interactions (platelet aggregation) and the formation of a platelet thrombus. The release of soluble agonists within the confines of the nascent platelet thrombus amplifies platelet activation and thrombin generated catalyses fibrinogen to fibrin thus forming a mesh that stabilises the platelet rich thrombus

platelets and also platelet activators. Indeed, the subendothelial matrix is significantly altered with the development of an atherosclerotic plaque such that collagen, fibrinogen/fibrin and tissue factor are all highly expressed. Upon plaque rupture, the initial capture and adhesion of platelets to the vessel wall under arterial shear is mediated by platelet GPIb-vWF interactions. Here, vWF, derived from both the injured endothelium and circulating plasma pool, binds via its A3 domain to collagen thereby allowing the capture of platelets via interaction between GPIb and the vWF A1 domain [86]. The GPIb-vWF interaction is characterised by a fast ‘on’ and ‘off’ rate and therefore whilst it can support the initial tethering platelets, other interactions are required to mediate stable platelet adhesion. In this regard, by slowing down translocating platelets, GPIb-vWF interactions facilitate the binding of GPVI to collagen and GPIIb/IIIa to fibrinogen, which then mediate stable platelet adhesion [86] (Fig. 11.3).

11.4.2 Platelet Activation and Aggregation

Platelets are either activated by soluble agonists or by receptor-ligand interactions induced by platelet adhesion, both generating downstream signalling pathways ultimately leading to autocrine and paracrine platelet activation/recruitment. Upon

activation, the platelet undergoes shape change, in a process linked to re-organisation of the cytoskeleton, which facilitates the generation of multiple, stable adhesion contacts. In concert with shape change, platelets generate TxA_2 and degranulate, thereby releasing important soluble agonists such as ADP, which act in an autocrine and paracrine fashion to amplify GPIIb/IIIa activation and stabilise the platelet thrombus, whilst granule-derived fibrinogen and vWF maintain a reactive surface for further platelet recruitment to the growing thrombus [86]. The activated integrin GPIIb/IIIa binds to its major ligand in the circulation, fibrinogen, which mediates platelet-platelet interactions and therefore the formation of platelet aggregates [3] (Fig. 11.3). Together, these processes produce a platelet rich thrombus at sites of vascular injury necessary for efficient haemostasis. Conversely, the formation of platelet thrombi at sites of atherosclerotic plaque rupture, or exaggerated platelet thrombus formation, can lead to pathological thrombosis such as acute myocardial infarction and ischaemic stroke.

The generation of thrombin at sites of vascular injury plays a central role in the stabilisation of the platelet thrombus. Thrombin generation at the site of plaque rupture is initiated by the exposure of tissue factor, which then forms a catalytic complex with Factor VIIa, initiating the 'extrinsic' pathway of blood coagulation [87]. Activated platelets and endothelial cells, at sites of vascular injury, express the negatively charged phospholipid, phosphatidylserine (PS), which is required to allow the assembly of the tenase and prothrombinase complexes [88, 89]. The generated thrombin not only potently stimulates platelets, but also cleaves fibrinogen to form a fibrin mesh that anchors, crosslinks and thus stabilises the platelet thrombus [90]. Platelets bind to fibrin via GPIIb/IIIa and therefore contractile forces generated by the platelet cytoskeleton can be transmitted via the integrin to mediate clot retraction as a means to consolidate and stabilise the thrombus [91, 92].

11.4.3 Platelet Adhesion to Inflamed Endothelium

The early phases of atherosclerosis are associated with endothelial inflammation, which disrupts normal endothelial function and is associated with platelet and leucocyte adhesion. Whilst the endothelium serves as a barrier between platelets and the highly reactive subendothelial matrix, under pathological conditions, the endothelium expresses a number of adhesive ligands and receptors for platelets, allowing for platelet adhesion and activation on the endothelium [93, 94]. Indeed, the inflamed endothelium expresses the selectins, P-selectin and E-selectin, in addition to vWF secreted from endothelial Weibel-Palade bodies. P-selectin and vWF are the receptors for platelet PSGL-1 and GPIb, respectively, with these interactions mediating platelet tethering and rolling. Akin to thrombus formation, the stable adhesion of platelets to the inflamed endothelium is largely mediated by GPIIb/IIIa. Here, the expression of intercellular adhesion molecule-1 (ICAM-1) is upregulated in the context of inflammation and can bind fibrinogen/fibrin which then acts as a bridge to engage platelet GPIIb/IIIa [95, 96] (Fig. 11.4).

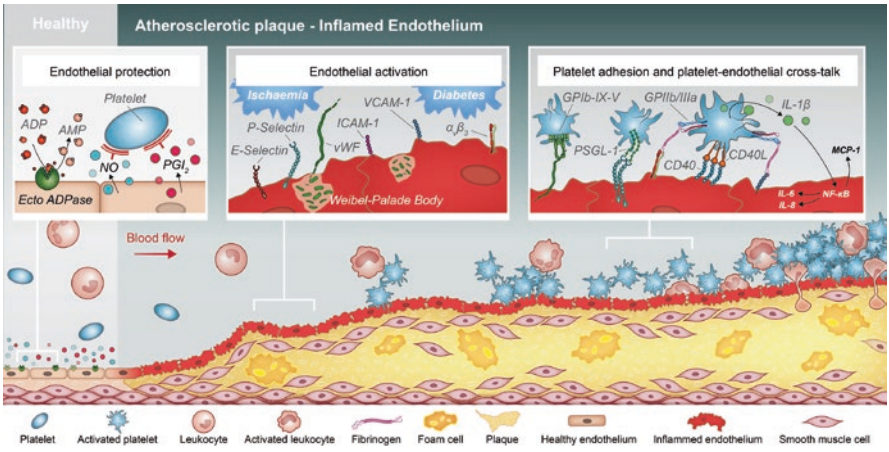

Fig. 11.4 The role of platelets in atherosclerosis. The healthy endothelium liberates ectoADPase, NO and PGI_2 which prevent unwanted platelet adhesion to the endothelium. However, under pro-inflammatory conditions, such as endothelial ischaemia or diabetes, the inflamed endothelium releases vWF, and upregulates the expression of pro-adhesive molecules such as P-selectin and ICAM-1. This permits the tethering of platelets to the inflamed endothelium via interactions between platelet GPIbα and endothelial bound P-selectin and vWF. Stable platelet adhesion is contingent upon GPIIb/IIIa, which binds to fibrinogen immobilized by endothelial ICAM-1 and $\alpha_v\beta_3$. The activation of endothelial bound platelets leads to the release of platelet derived proinflammatory mediators such as IL-1β, which incites further endothelial inflammation. These interactions ultimately lead to the enhanced recruitment, adhesion and transmigration of leukocytes to areas of endothelial inflammation—all important aspects of atherogenesis

11.5 The Proinflammatory Role of Platelets

11.5.1 Platelets as Immune and Inflammatory Mediators

Fundamental to the inflammatory role of platelets is the expression of immune receptors and capacity to store and produce a large range of proinflammatory molecules. Platelet granules contain over 300 different proteins, including a vast array of chemokines and other proinflammatory molecules [97]. Chemokines play an important biological role in their ability to recruit and activate leucocytes [98]. Platelet α granules contain the CXC chemokines PF4 (CXCL4), β-thromboglobulin and epithelial-derived neutrophil-activating peptide 78 (ENA-78 or CXCL5) [99]. PF4 and β-thromboglobulin are amongst the most abundant proteins contained in α granules and serve to enhance leucocyte chemotaxis and activation [99]. Non-chemokine, proinflammatory molecules contained within platelet α granules include CD40L, TREM-1 and TGF [97]. As a consequence, platelet activation has been demonstrated to play a central role in the pathogenesis of a broad range of thrombo-inflammatory and immune conditions including atherosclerosis [100–102].

11.5.2 Platelets Induce Endothelial Activation

The binding of platelets to the endothelium facilitates endothelial cell activation and thus aids the endothelium in taking on a proinflammatory function [94] (Fig. 11.4). Endothelial bound platelets become activated and liberate numerous proinflammatory cytokines [94]. Foremost amongst these are IL-1β and PF4. IL-1β plays a central role in facilitating the platelet dependent proinflammatory endothelial phenotype by activating the NFκB pathway, upregulating ICAM-1 and $\alpha_v\beta_3$ expression and inducing the release of IL-6, MCP-1 and GM-CSF [103]. PF4 serves to enhance E-selectin expression by activating the NFκB pathway [104]. CD40 ligand is a member of the tumour necrosis factor (TNF) superfamily of molecules that binds to its specific receptor on endothelial cells, CD40 [105]. Binding of CD40 ligand to endothelial CD40 results in the production of inflammatory cytokines such as IL-8 and MCP-1, the generation of reactive oxygen species, enhanced expression of matrix metalloproteinases and expression of adhesion molecules such as ICAM-1, VCAM-1 and E-selectin [105].

11.5.3 The Role of Platelets in Leukocyte Recruitment and Activation

In addition to promoting endothelial activation, activated platelets bound to the endothelium serve to facilitate leucocyte recruitment and transmigration to sites of endothelial injury [106]. Endothelial-adherent platelets enhance the recruitment of leukocytes by expressing P-selectin, which serves as the ligand for the constitutively expressed leukocyte receptor PSGL-1 and thereby enabling leukocyte tethering and rolling even at high shear rates [107]. The interaction between P-selectin and PSGL-1 enhances the expression of the leucocyte integrin Mac-1 ($\alpha_M\beta_2$, CD11b/CD18), which binds to platelet GPIb and junctional adhesion molecule (JAM-c) to mediate stable adhesion [36, 108]. Furthermore, additional interactions between platelets and leucocytes can occur to facilitate the stable platelet-leukocyte adhesion. Foremost amongst these include the binding of fibrinogen to leucocyte Mac-1 which can serve as a bridge to the platelet integrin GPIIb/IIIa and the binding of platelet CD40 ligand to leucocyte CD40 [94]. Activated platelets release chemokines such as platelet factor 4 (PF4) and RANTES (regulated on activation, normal T cell expressed and secreted), which serve to enhance the recruitment of leucocytes to areas with adherent platelets [109, 110]. Further, the release of platelet chemokines also stimulates the production of the proinflammatory cytokines such as TNFα, IL-1β, IL-8 and monocyte chemoattractant protein-1 (MCP-1) from monocytes [111]. The importance of platelets in facilitating leukocyte recruitment is highlighted by numerous studies, which demonstrate that either platelet depletion or inhibition significantly reduces the level of neutrophil recruitment to inflamed organs [112]. In keeping with the cross talk between platelets and endothelial cells, leukocyte binding to platelets facilitates their adhesive capacity and proinflammatory phenotype [113].

11.6 The Contribution of Platelets to Atherosclerosis

Platelets contribute to the development and progression of atherosclerosis from the early stages of endothelial inflammation through to plaque rupture and subsequent atherothrombosis [94]. Platelets influence atherogenesis via multiple mechanisms, by enhancing endothelial inflammation, by facilitating leukocyte adhesion and transmigration, and by the release of a plethora of inflammatory factors (Fig. 11.4). Platelets adhere to the perturbed endothelium early on in the development of atherosclerosis, which coincides with proinflammatory gene expression in endothelial cells. Interestingly, the presence of platelets occurs before leukocytes, suggesting they play an important role in leukocyte recruitment in the context of atherosclerosis [100]. Accordingly, in experimental models, the inhibition or depletion of platelets attenuates atherosclerosis. Thus, it is interesting to speculate that some of the protective effects of anti-platelet therapy in humans in secondary prevention of cardiovascular disease may be due in part to these effects, however this is yet to be established. Moreover, subclinical plaque rupture is a frequent event with 9% of autopsies on patients not dying from myocardial infarction demonstrating ruptured fibrous caps (22% in patients with cardiovascular risk factors), suggesting that rather than every plaque rupture precipitating an ischemic event, it is likely that the thrombotic response to plaque disruption is dynamic with thrombosis and thrombolysis occurring simultaneously in patients with acute coronary syndrome [114]. Consequently, a rupture prone plaque may suffer periodic disruptions in its fibrous cap resulting in ongoing interactions with activated platelets. Thus, in addition to their role in acute plaque rupture, at any given time, activated platelets may be associated with unstable plaques presumably in a number and frequency proportional to the degree of plaque instability. The detection of such activated platelets potentially may allow identification of unstable plaques prior to rupture [115].

11.7 Current Anti-platelet Therapies

Anti-platelet drugs are the cornerstone of therapy in patients with acute coronary syndromes (ACS), stable coronary artery disease, and those undergoing coronary, carotid and peripheral artery revascularisation procedures [116–118].

11.7.1 Aspirin

Aspirin (salicylic acid) blocks the production of TxA_2 via the irreversible inhibition of cyclooxygenase-1 (COX-1), thereby inhibiting the generation of TxA_2 and its effect on platelet activation and aggregation (Fig. 11.1). Aspirin acetylates a key serine residue at the COX-1 catalytic centre, irreversibly corrupting its enzymatic

function. Platelets lack the machinery to resynthesize COX-1 and thus aspirin leads to irreversible platelet inhibition for the lifespan of the platelet (7–10 days), despite aspirin's relatively short plasma half-life of 15 min.

Aspirin has a well-established clinical benefit in vascular disease, particularly myocardial infarction and stroke, in both the acute and chronic setting. Aspirin is the first-line anti-platelet therapy in ACS, reducing the relative risk of mortality by 23% after 5 weeks of aspirin therapy [119, 120]. In addition, aspirin provides major benefits in secondary prevention, reducing the relative risk of MI by 25% [121]. However, the benefits are less well established in primary prevention as the benefits of a reduced rate of myocardial infarction are tempered by an increased rate of haemorrhagic stroke. Indeed, a recent, large scale clinical trial (ASPREE study) has demonstrated that the use of aspirin for the primary prevention of cardiovascular disease in elderly patients is not associated with clinical benefit [122].

The optimal dose of aspirin had been established as 75–150 mg daily, in order to achieve its full platelet inhibitory effect. Higher doses (up to 325 mg daily) demonstrate increased gastrointestinal side effects with no additional anti-platelet effect. Significantly, approximately one-third of patients receiving aspirin therapy demonstrate 'treatment failure' manifested by recurrent thrombosis. This has led to the notion of aspirin resistance, which may be associated with evidence of biochemical resistance or heightened platelet reactivity on platelet function testing. Clinical scenarios associated with a heightened inflammatory response such as diabetes, post cardiac surgery and acute coronary syndromes have all been associated with heightened platelet reactivity. Moreover, polymorphisms of the COX alleles and conditions associated with elevated platelet turnover (such as immune thrombocytopenia and essential thrombocytosis) can also lead to the reduced anti-platelet effect of aspirin. However, to date, a uniform definition of aspirin resistance and the management has yet to be clearly defined.

11.7.2 $P2Y_{12}$ Receptor Antagonists

The $P2Y_{12}$ receptor has an important role in mediating sustained activation of the major platelet adhesion receptor, GPIIb/IIIa, in response to ADP stimulation [123] (Fig. 11.1). $P2Y_{12}$ receptor antagonists inhibit the amplification of platelet activation induced by ADP, resulting in potent antithrombotic effects [123]. The $P2Y_{12}$ inhibitors comprise two classes of drugs: the thienopyridines (clopidogrel, prasugrel, and ticlopidine) and the nucleoside/nucleotide derivatives (cangrelor and ticagrelor).

11.7.2.1 Clopidogrel

Clopidogrel is a thienopyridine, and therefore is a prodrug that requires metabolism by the cytochrome P450 (CYP450) system into the active metabolite that irreversibly inhibits the $P2Y_{12}$ receptor [116, 117]. Consequently, the concurrent

use of other drugs that inhibit the CYP450 metabolism of clopidogrel may diminish its anti-platelet effects. This is typified by the proton pump inhibitors (PPIs), which are also substrates of the CYP450 pathway, and have been associated with heightened platelet reactivity in those taking clopidogrel concurrently. However, to date, no randomised controlled trials have addressed the issue of whether there is an increased rate of adverse cardiovascular outcomes in patients co-administered clopidogrel and a PPI. Notwithstanding, the FDA and European Medicines Agency (EMA) currently recommend that PPIs, other than omeprazole and esomeprazole, be used in patients prescribed clopidogrel. However, up to 10% of patients experience recurrent ischaemic events despite receiving dual antiplatelet therapy (DAPT) with aspirin and clopidogrel [124]. In addition, 30–40% of patients receiving clopidogrel continue to exhibit elevated platelet reactivity. These challenges led to the development of novel, potentially more potent $P2Y_{12}$ antagonists [123].

11.7.2.2 Prasugrel

Prasugrel is a third generation thienopyridine that, similar to clopidogrel, is a prodrug that is converted into its active metabolite by the CYP450 system that irreversibly inhibits the P2Y12 receptor. However, prasugrel has greater *in vivo* bioavailability, is associated with less inter-individual variability and thus is associated with more potent anti-platelet effects compared to clopidogrel [116, 117]. Moreover, no drug-drug interactions that influence prasugrel metabolism have been described.

11.7.2.3 Ticagrelor

In contrast to the thienopyridines, Ticagrelor is a nucleoside/nucleotide anatagonist that is a reversible, direct acting antagonist of the P2Y12 receptor. Thus, unlike clopidogrel and prasugrel, ticagrelor does not require metabolism by the CYP450 system to achieve its anti-platelet effects. As a consequence, ticagrelor is associated with a faster, more potent and predictable anti-platelet effect compared to clopidogrel [116, 117]. Ticagrelor can cause dyspnoea more often than other P2Y12 inhibitors, which is often resolved by its replacement by either prasugrel or clopidogrel.

11.7.2.4 Cangrelor

Cangrelor is a direct, reversible $P2Y_{12}$ antagonist that has been approved by the FDA and the European Medicines Agency (EMA) [125]. Cangrelor is given intravenously and has a rapid onset of action and ultra-short half-life (3–6 min) thus allowing the rapid recovery of platelet function [123]. Therefore, cangrelor is a therapeutic option in clinical scenarios where $P2Y_{12}$ inhibition with a rapid onset

and offset of action is desired, such as for patients with ACS, who are $P2Y_{12}$ antagonist naïve and need urgent PCI or those who require DAPT bridging before surgery [126].

11.7.3 Dual Anti-platelet Therapy

With the high rates of recurrent ischaemic events, despite aspirin therapy, in addition to the widespread application of percutaenous coronary intervention (PCI), the use of dual anti-platelet therapy (DAPT) has become one of the most widely and intensively adopted therapies in cardiovascular medicine. DAPT generally comprises aspirin in addition to a P2Y12 antagonist. In this regard, there is strong evidence for the use of DAPT to prevent recurrent ischaemic events in patients with ACS and/or post PCI where DAPT prevents stent thrombosis. Whilst the optimal duration of DAPT remains a matter of much debate this is often based on the perceived risk of recurrent ischaemia versus the bleeding risk [116, 117].

11.7.4 PAR-1 (Protease Activated Receptor-1) Antagonists

Combining thrombin blockade with DAPT had been widely regarded as a potential anti-thrombotic strategy [127, 128]. On the basis of the TRA 2P–TIMI 50 trial, the PAR-1 antagonist vorapaxar was approved by the FDA for the reduction of ischaemic events in patients with a history of MI or peripheral vascular disease [129, 130]. However, the use of vorapaxar in addition to standard anti-platelet agents, has not been demonstrated to be of benefit in the context of ACS [131]. Importantly, in two large randomized trials (TRA 2P–TIMI 50 and TRACER trials), the rates of bleeding, in particular intracranial haemorrhage, were substantially increased by the addition of vorapaxar to standard anti-platelet therapy. Consequently, the concerns about safety have limited the use of vorapaxar in clinical practice.

11.7.5 GPIIb/IIIa Inhibitors

GPIIb/IIIa inhibitors are ligand-mimetic molecules that prevent fibrinogen from binding to platelets, thereby directly inhibiting their aggregation (Fig. 11.1). Three agents are currently in use: abciximab, a humanized Fab fragment of a mouse monoclonal antibody; eptifibatide, a cyclic heptapeptide with a lysine-glycine-aspartic acid (KGD) motif mimicking the fibrinogen binding sequence within GPIIb/IIIa; and tirofiban, a non-peptidic small molecule also mimicking the fibrinogen binding site [132]. First marketed in the mid-1990s, these drugs have been widely used in patients with ACS and those undergoing PCI. However, the early

clinical trials assessing GPIIb/IIIa inhibitors were conducted before the routine use of $P2Y_{12}$ antagonists. Therefore, the clinical benefit derived from GPIIb/IIIa inhibitors seems to be restricted to certain high-risk subgroups, such as patients with MI undergoing PCI without pretreatment with a $P2Y_{12}$ antagonist [133]. Importantly, whilst the GPIIb/IIIa inhibitors are potent anti-thrombotic drugs, they are associated with bleeding complications in up to 50% of patients [134].

11.7.6 Phosphodiesterase Inhibitors

Cilostazol and dipyridamole are phosphodiesterase inhibitors that inhibit platelet aggregation. Cilostazol is a phosphodiesterase III inhibitor that inhibits the degradation of cAMP, which in turn leads to an increase in Protein Kinase A (PKA) that acts to impair platelet aggregation. Cilostazol also causes vasodilation since PKA impairs smooth muscle contraction. In this regard, cilostazol has demonstrated benefit in patients with peripheral arterial disease and intermittent claudication. Dipyridamole inhibits the uptake of adenosine into platelets and endothelial cells, thus resulting in the accumulation of cAMP, which mediates its platelet inhibitory effects and vasodilatory properties. The use of dipyridamole has been most widely studied for the prevention of ischaemic stroke in combination with aspirin.

11.7.7 Current Therapeutic Landscape

Whilst a detailed description of the current therapeutic guidelines regarding anti-platelet therapy are beyond the scope of this chapter, the current recommendations concerning anti-platelet therapy are summarized in Table 11.1. The recommended duration of DAPT is often individualised and is particularly contingent upon the bleeding risk. In addition, we refer the reader to the most recent guidelines published by the European Cardiac Society and American College of Cardiology [116, 117].

Table 11.1 Recommended anti-platelet regimes in patients with cardiovascular disease (*BMS* bare metal stent, *DES* drug-eluting stent)

Indication	Treatment recommendation
Acute coronary syndrome	PCI or non-PCI: lifelong ASA plus P2Y12 antagonist at least 12 months
Stable ischaemic heart disease	BMS: Aspirin lifelong plus P2Y12 antagonist at least 1 month DES: Aspirin lifelong plus P2Y12 antagonist at least 6 months
Stroke	Aspirin or clopidogrel
PAD	Aspirin or clopidogrel DAPT if history of previous lower extremity revascularisation

11.8 Perioperative Management of Anti-platelet Therapy

The continuation of DAPT peri-operatively is associated with a reduction in the rate of thrombotic complications. However, importantly, the use of DAPT throughout the peri-operative period is associated with a significant increase in the risk of bleeding complications and rate of blood transfusion. Therefore, the relative risks of thrombotic complications and bleeding risk need to be assessed on a patient by patient basis, taking into account the cardiovascular risk profile, bleeding risk of the procedure, and the pharmacokinetic profile of the anti-platelet drug(s). The cessation of P2Y12 antagonists is often 7–10 days (with a minimum of 5 days) prior to any planned procedure given this roughly equates to the lifespan of a platelet. For patients who undergo surgery on anti-platelet therapy, bleeding is associated with an increased morbidity and mortality. The management of anti-platelet associated bleeding is complicated by the fact that no specific reversal agent exists for any of the anti-platelet drugs. The management of major haemorrhage in this setting is often managed empirically with the use of anti-fibrinolytics, such as tranexamic acid, platelet transfusions or desmopressin. Interestingly, the role of platelet transfusions in the context of anti-platelet related bleeding has recently been called into question with a randomized controlled trial demonstrating that patients with anti-platelet associated intracerebral bleeding administered platelets had adverse clinical outcomes compared to standard care [135]. The use of pro-haemostatic agents such as prothrombin complex concentrate or recombinant Factor VIIa have been described, however the use of these agents can be associated with a risk of major thrombotic complications.

11.9 Novel Anti-platelet Drugs in Development

The major limitation of all currently used anti-thrombotic approaches is the inherent risk of bleeding associated with their use. This is particularly the case in elderly patients and those with co-morbid conditions, such as renal failure. Given these patients are usually at high risk of recurrent ischaemic events, they often do not receive optimal anti-thrombotic therapy due to the risks of bleeding. These issues, coupled with recent progress in our understanding of potentially important differences between the haemostatic and thrombotic response have led to the development of several new classes of drugs in early phase development that potentially hold the promise to differentially target pathological thrombosis without impeding haemostasis [118].

Inhibitors of GPVI, PAR-4 and protein disulfide isomerase (PDI) are currently being evaluated in early phase clinical trials on the basis that these targets display marked anti-thrombotic effects with no significant impact on bleeding in animal models [118]. Likewise, conformation specific inhibitors of GPIIb/IIIa, and PI3Kβ inhibitors also display significant anti-thrombotic effects in pre-clinical models. However,

how these agents ultimately translate in human studies and where their utility in the context of our existing anti-platelet drugs remains to be established [136].

11.10 Platelet Function Testing

The widespread use of anti-platelet drugs for the treatment and prevention of arterial thrombosis has led to great interest in the potential of 'tailored' anti-platelet therapy. As such, a multitude of platelet function tests have been utilised as a potential means of monitoring anti-platelet therapy. However, to date, no studies have convincingly demonstrated that the tailoring of anti-platelet therapy, in patients taking aspirin and clopidogrel, in response to platelet function testing is associated with clinical benefit. Despite this, platelet function testing remains widely performed. Table 11.2 summarises the currently employed platelet function tests.

Table 11.2 Summary of common platelet function tests

Platelet function test	Method	Limitations
Light transmission aggregometry	Platelet agonists added to platelet rich plasma with changes in the optical transmission of light measured as a read out of platelet aggregation	Lack of standardisation results in significant inter-laboratory variability No normal values Time consuming
VerifyNow assay	Point of care test detecting changes in light transmission, after the addition of a platelet agonist, as a marker of platelet aggregation. Different assays available for the evaluation of platelet inhibition in response to aspirin, P2Y12 inhibitors and GPIIb/IIIa inhibitors	Expensive Lack of clinical data demonstrating improved clinical outcomes
PFA-100	Measures time to aperture closure by platelet aggregates formed under shear	Not sensitive to the effects of aspirin or P2Y12 inhibitors
VASP phosphorylation assay	Flow cytometry based assay that indirectly measures the degree of P2Y12 receptor inhibition	Insensitive to low levels of P2Y12 inhibition Requires specialised equipment and skilled personnel
Thromboelastography (TEG)/rotational thromboelastography (ROTEM)	Measures viscoelastic properties of whole blood clot formation	Questionable specificity and sensitivity to the effects of aspirin and P2Y12 inhibitors
Flow cytometry	Measures the binding of fluorescence labelled activation-specific antibodies to platelets. Allows the simultaneous assessment of GPIIb/IIIa activation, platelet degranulation and platelet-leukocyte aggregate formation	Expensive Requires expertise Access mainly limited to research settings

Whether the results of platelet function testing for patients taking anti-platelet therapy can assist in the optimal timing of anti-platelet cessation prior to surgery has yet to be established.

11.11 Conclusion

Given the fundamental role of platelets in cardiovascular medicine combined with the importance of anti-platelet therapies and the burgeoning field of novel therapeutics, the field of platelet biology represents a complex yet exciting field of medicine.

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Chapter 12
Abdominal Aortic Aneurysm Pathology and Progress Towards a Medical Therapy

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Key Learning Points

- Important risk factors for AAA include male gender, advanced age, prior or current smoking and a positive family history. Diabetes appears to be negatively associated with AAA diagnosis and growth. However, the exact reasons for this are unclear
- Elective surgery is the only means to treat AAA but is associated with significant peri-operative morbidity and mortality, and concerns regarding the durability of repair;

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- Elective surgery does not improve survival in patients with small (<55 mm) AAAs. Patients with small AAAs are managed conservatively through repeated imaging which confers no therapeutic benefit and is associated with decreased health-related quality of life;
- A medical therapy which effectively slows the growth of small AAAs may improve patient care and a large body of work to identify promising drug leads has been conducted;
- To date, no randomized controlled trial has delivered an effective medical therapy for small AAAs. This may relate to difficulties in translating findings from commonly used laboratory models to the patient and to weaknesses in the design of previous trials.

12.1 Introduction

An aneurysm can be defined as an abnormal, focal dilatation within an artery which causes vessel diameter to exceed 1.5 times the expected size, and in some cases has a natural history of progressive enlargement and eventual rupture [1, 2]. The infrarenal aorta is the most common site of aneurysm formation in humans. An infrarenal aortic diameter of ≥30 mm is usually used to diagnose an abdominal aortic aneurysm (AAA), although other definitions have been suggested, for example those based on normalizing aortic diameter to body surface area [2–5]. AAA is usually asymptomatic, but can be readily diagnosed through the use of imaging such as ultrasound or computed tomography.

There are, however, no currently available medications which effectively slow AAA growth and open surgical or endovascular aneurysm repair (EVAR) are the only treatments for AAA [4]. Four large randomised controlled trials and subsequent meta-analyses have demonstrated that elective repair of asymptomatic AAAs with diameters smaller than 55 mm (regardless of method used) does not improve patient survival [6–10]. Importantly, most asymptomatic AAAs detected through population screening, or incidental imaging are smaller than 55 mm and current guidelines recommend that such patients should be treated conservatively with cardiovascular risk management, and regular imaging assessments to monitor AAA growth [11]. Surgery is only recommended when AAA diameter exceeds 54 mm in men, 50 mm in women, or if the AAA becomes symptomatic [11]. Conservative management of small asymptomatic AAA has been associated with reduced health-related quality of life [12, 13]. Moreover the majority of AAAs managed in this way eventually expand to a size where surgical repair is required [8, 14]. For example, approximately 65% of patients with AAAs measuring 40–55 mm within the conservative arm of the United Kingdom (UK) Small Aneurysm Trial had undergone surgical repair within 5 years of recruitment [6]. Due to the absence of an effective treatment for small asymptomatic AAAs there is significant interest in identifying non-surgical therapies capable of slowing the growth of small AAAs, and this is reflected by an increase in the number of drug trials conducted over the

last decade. The aim of the current chapter is to summarise how results from epidemiological studies and laboratory studies have contributed to current understanding of AAA pathophysiology. In addition this chapter includes a discussion of current and past clinical trials examining potential medical therapies to limit small AAA growth.

12.2 AAA Epidemiology

Findings from a recent study suggest that the global death rates attributable to AAA rose by 12% in the 20 years between 1990 and 2010 to 2.8/100,000 [15]. The highest rates of death attributable to AAA were observed in higher income countries, with Australasia, Western Europe and North America having the highest mortality rates of 8.38/100,000, 7.68/100,000 and 6.11/100,000 respectively [15, 16]. In contrast, reports from screening studies and epidemiological studies in a number of developed countries suggest that AAA prevalence is declining. The national screening programmes run in the UK and Sweden have reported that the prevalence of AAA is markedly lower than anticipated (observed prevalence of approximately 2.0% in 65 year old men, compared to 5–7% found in earlier studies) [17–20]. Moreover, reductions in the rates of hospitalisation and death attributable to AAA have been reported for a range of countries including Australia, New Zealand, England and Wales [20–22]. Reasons for the falling AAA prevalence remain incompletely understood, although several independent studies have linked this to a decrease in smoking rates [22, 23]. Whatever the reasons, the potential negative impact of declining AAA prevalence on the financial viability of screening programmes has been raised [20]. Some (not all) clinical trials have shown that ultrasound screening programmes in men aged ≥65 years reduces AAA-related mortality by limiting deaths due to AAA rupture [24, 25]. A meta-analysis has suggested that screening focusing solely on older men with a history of smoking (making up 69% of the assessed population), would account for 89% of the reduction in AAA-mortality expected from a screening programme including all men aged 64–75 years [24]. The US Preventative Services taskforce has suggested that screening be restricted to individuals considered to be at high risk (persons with a history of smoking, and/or family history of AAA), in an attempt to improve cost-effectiveness [26]. In contrast, AAA screening in the UK and Sweden is offered to all males in the year of their 65th birthday, and it has been suggested that this may not be financially viable in the light of falling AAA incidence [27]. Final findings from an Australian clinical trial demonstrated that a AAA screening program of all men aged >65 years is unlikely to be effective within Australia [28], but there is more support for such a program in New Zealand [29]. Of note, Māori people are hospitalised for AAA repair at a significantly earlier age than those of European ancestry (difference of 8 years of age at presentation between these two populations), and Māori women have increased risk of developing AAA than their European counterparts (relative risk 1.56 [95% confidence intervals 1.37–1.79]) [30]. Thus, any AAA screening

program in New Zealand would need to be tailored in order to appropriately meet the needs of specific high-risk groups.

12.2.1 Risk Factors Identified from Epidemiological Studies

The precise initiating factors for AAA development remain unclear. However, epidemiological studies have consistently associated male sex, old age, Anglo-European race, tobacco smoking, family history and prior diagnosis of atherosclerosis-associated cardiovascular disease with increased risks of being diagnosed with an AAA [12, 31]. Smoking has been shown to be the strongest modifiable risk factor for AAA. Approximately 20% of AAA patients report having a family member who has also received an AAA diagnosis [12], highlighting the importance of inherited factors in AAA pathogenesis (detailed below). Individuals with a first-degree relative affected by AAA are at an approximately two-fold higher risk of developing AAA, when compared to those with no family history of AAA [32, 33].

12.3 AAA Pathogenesis

Current understanding of AAA pathogenesis is built upon evidence provided by epidemiological studies, clinical data and samples collected from AAA patients, and pre-clinical models that mimic features of the human disease. Collectively, these data suggest that AAA is a multifactorial disease which is caused by a combination of environmental, genetic, molecular and biological factors. A range of animal models for AAA have been described, however those in mice have been most widely reported, most likely due to their well-characterised genomes and the relatively low cost of small compared to large animal research [34–36] (Table 12.1). AAA is most commonly induced in rodents through subcutaneous delivery of angiotensin-II (dyslipidaemic strains are more susceptible to AAA induction), infusion into the aortic lumen of elastase, adventitial application of calcium phosphate or chloride to the infra-renal aorta, or transplant of decellularised aortic xenografts [34, 35]. Several modifications to these established models aiming to incorporate known clinical risk factors including smoking, dyslipidaemia and hypertension have been suggested as a means to more closely mimic the human disease [34, 37]. Animal models have been used to investigate the pathophysiology of AAA and to test potential therapeutic interventions. This chapter includes a discussion of the key mechanisms implicated in AAA pathogenesis, incorporating findings from the study of patients and rodent models.

Table 12.1 Examples and characteristics of commonly utilised rodent models in AAA research (modified from [36, 38])

Model	Similarities to human AAA	Key differences to human AAA	Pros and cons
Angiotensin II infusion	• Marked aortic inflammation, angiogenesis and proteolysis; • Aortic rupture commonly reported; • Males more susceptible to AAA formation;	• AAA develops predominantly within the supra-renal aorta; • Aortic dilation arises secondary to aortic dissection and intra-mural hematoma;	• Subcutaneous implant of angiotensin-II releasing pump is simple and relatively non-invasive; • Dyslipidaemic mouse strains often develop comorbid atherosclerosis, similar to patients; – Significant inter- and intra-strain heterogeneity in response to angiotensin-II; – Models aortic dissection rather than AAA;
Angiotensin II infusion and BAPN feeding[a]	• Indications of significantly higher inflammatory signalling than standard angiotensin-II model; • AAA rupture occurs in ~80% animals.	• Limited extracellular matrix degeneration, loss of vascular smooth muscle cells and aortic inflammation;	• Subcutaneous implant of angiotensin-II releasing pump is simple and relatively non-invasive; • Reportedly higher incidence of aortic dilatation than traditional angiotensin-II model; – Model may not be suitable to study efficacy of potential drug therapies;
Elastase perfusion (luminal)	• Transmural inflammation elastic fibre destruction and angiogenesis; • Males more susceptible to AAA formation;	• Limited intraluminal thrombosis; • AAA rupture uncommon;	• Does not require transgenic strains; • Can be performed in any rodent species; • Generally good inter-animal reproducibility; – Technically challenging surgical procedure; – Limited evidence of longer term progressive aortic expansion;

(continued)

Table 12.1 (continued)

Model	Similarities to human AAA	Key differences to human AAA	Pros and cons
Calcium chloride or phosphate (adventitial)	• Aortic calcification, inflammation, angiogenesis and proteolysis;	• No intraluminal thrombus formation; • No AAA rupture;	• Does not require transgenic strains; • Can be performed in any rodent species; • Generally good inter-animal reproducibility; – Severity of aortic dilatation limited; – Limited evidence of longer term progressive aortic expansion;
Xenograft	• Transmural inflammation, intraluminal thrombus formation and elastic fibre destruction; • Aortic rupture reported after modification of the model;	• Implanted xenografts are decellularised, thus AAA formation involves extracellular matrix alone.	• Does not require transgenic strains; – Highly challenging surgical procedure; – Complete loss of cells within the transplanted aorta not representative of human AAA;
Elastase (adventitial) and BAPN feeding[a]	• Medial elastin fragmentation, medial thinning, influx of T cells to the aorta and matrix metallo-proteases; • Marked intraluminal thrombus formation; • Progressive AAA dilatation over time (reported up to 100 days post-surgery); • AAA rupture reported;	• No evidence AAA propensity is greater in older mice;	• Does not require transgenic strains; • Appears to be suitable to study longer term effects of drugs on AAA growth; – Currently not widely studied.

[a]Note, these are newly described models and require further validation and characterisation (detailed in [60, 76])
BAPN: 3-amino proprionitrile

12.3.1 Tissue Samples

Examination of aortic biopsies recovered from patients demonstrates that AAA leads to pathological changes to all layers of the aortic wall (Fig. 12.1 and discussed in detail in [4]). This is in contrast to atherothrombosis which appears to involve less

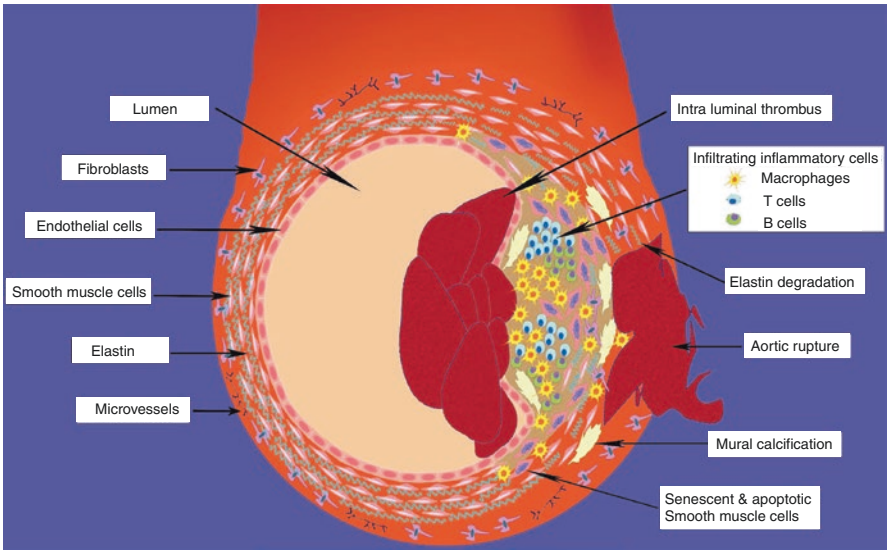

Fig. 12.1 Schematic diagram illustrating AAA pathophysiology. Schematic cross section of the aorta that shows normal wall architecture on the left side comprising a mono-layer of endothelial cells, organised layers of vascular smooth muscle cells and elastin filaments within the tunica media, and fibroblasts within the tunica adventitia. This is contrasted by the right-hand side of the figure showing pathological changes typically found in AAA samples. These include intraluminal thrombus, and aortic wall inflammatory cells including macrophages, T-cells and B-cells, and vascular smooth muscle cell senescence and apoptosis

marked changes in the media and adventitia [4]. AAA samples recovered from patients and animal models demonstrate marked inflammation, which involves cells involved in the innate (notably mast cells, macrophages, neutrophils and dendritic cells), and adaptive (B and T lymphocyte) immune response [37]. Chronic inflammation has been implicated in the destruction of the aortic extracellular matrix owing to the secretion of matrix degrading enzymes (particularly matrix metalloproteinases), oxygen-derived free radicals and pro-inflammatory cytokines from activated immune cells. This in turn is believed to induce an inflammatory phenotype in the vascular smooth muscle cells within the aortic wall with subsequent apoptosis [38]. Microarray and bioinformatic analyses have demonstrated that the gene signatures of AAA biopsies recovered from both patients and angiotensin-II infused mice are significantly enriched for pro-inflammatory molecules, inflammatory cell markers and proteinases [39–42]. Interventional studies in rodent models have also provided data supporting a pathological role for inflammation in AAA development and progression [37]. For example, depletion of B, T or mast cells has been reported to limit AAA severity in some, but not all commonly employed mouse models. In contrast, mice deficient in T-regulatory cells develop more severe AAAs than controls, whereas increasing T-regulatory cell numbers is reported to reduce AAA pathology [37]. Most AAAs have areas of calcification, although the role of calcification in AAA pathogenesis is controversial [4].

The degree to which atherosclerosis contributes to AAA pathogenesis remains controversial. Atherosclerosis is a common co-morbidity in AAA patients, and traditional theories have suggested that AAA is simply an end-stage manifestation of atherosclerosis arising from dysregulated positive remodelling in response to arterial stenosis, or loss of vascular smooth muscle cells from the tunica media as a consequence of intimal thickening [37]. Other observational evidence suggests that atherosclerosis and AAA may in fact be distinct diseases. Diabetes, a major risk factor for atherosclerotic disease appears to be inversely associated with AAA diagnosis and AAA growth [43, 44]. More recently, genetic studies have identified specific risk alleles for AAA, some of which do not appear to be a risk factor for atherosclerosis-associated cardiovascular disease [32, 45, 46]. Furthermore, recent meta-analyses have suggested that AAA growth rates may be slower in patients with concurrent lower limb occlusive disease [47–49]. The mechanisms underpinning this association remain unclear, with some studies suggesting that localised haemodynamic perturbations resulting from distal arterial occlusion may slow AAA growth [50]. The UK Small Aneurysm Trial investigators reported that lower ankle-brachial pressure indices were associated with slower AAA growth, although this relationship was not independent of potential confounders [51]. Other studies have reported that athero-occlusive disease within the carotid and coronary arteries may be associated with slower AAA growth, albeit to a lesser extent than lower-limb atherothrombosis and with considerable inter-study heterogeneity [48, 52].

Most AAA patients have a large non-occlusive thrombus within the aneurysmal sac. Owing to close contact with the arterial bloodstream, the AAA intra-luminal thrombus is continually remodelled and its size is closely correlated with AAA sac size [4]. Early investigations suggested that the thrombus may play a protective role by shielding the aortic wall from high-pressure blood flow and reducing AAA wall stresses [4, 53]. This is refuted by data suggesting that the thrombus contributes to aortic hypoxia, and acts as a secondary site of accumulation of activated platelets and leukocytes which release wall degrading proteases and cytokines, thereby contributing to AAA pathogenesis [54, 55]. Studies have reported that mice receiving aspirin, clopidogrel or clotting factor Xa and IIa inhibitors develop less severe AAAs than controls in response to angiotensin-II [34, 56–58]. Interpretation of these findings is however complicated by the fact that the angiotensin-II infused model more closely mimics human aortic dissection than AAA. Until recently, few animal models have convincingly replicated the intra-luminal thrombosis seen in AAA patients (although thrombosis has been reported in the xenograft and elastase models), which has made it difficult to elucidate the extent to which this process contributes to AAA pathogenesis [35, 37]. This limitation may have been overcome through a new method of inducing AAAs, involving peri-adventitial elastase application, followed by ongoing oral administration of 3-aminoproprionitrile (BAPN) to inhibit collagen cross-linking. Reported data suggest that mice treated in this way develop extremely large infra-renal AAAs with marked intraluminal thrombus formation [59].

12.3.2 Blood Samples

Analysis of blood samples have suggested that the pathological processes occurring within the AAA wall are reflected in the systemic circulation of patients and experimental animals. A large body of evidence suggests that serum concentrations of a range of pro-inflammatory cytokines, extracellular matrix degrading proteases and extracellular matrix components are significantly higher in AAA patients than controls [60–63]. Similarly, chronic turnover of the intra-luminal thrombus is reflected by significantly elevated concentrations of fibrinogen, D-dimer and thrombin-antithrombin III complex in venous blood samples collected from AAA patients compared to non-aneurysmal controls [64]. Other studies have highlighted differences in the circulating concentrations of non-protein molecules including small non-coding RNAs and novel lipids in AAA patients compared to non-aneurysmal controls [2, 65]. Collectively, these observations have led to the hypothesis that detection of differentially expressed molecules may provide a blood test for AAA. However, to date, none of the suggested markers have shown sufficient specificity and sensitivity for clinical use [60–62]. Clinically the availability of a blood marker which identified small AAAs most likely to progress would be particularly valuable. While a large number of markers associated with AAA growth have been identified, very few have been consistently reported in more than one study (D-dimer is one example [63]).

12.3.3 Insight Provided by Genetic Studies

There is evidence for a strong genetic predisposition for AAA [4, 37]. AAA heritability is estimated to be >0.7 (i.e. genetic components may explain over 70% of the risk of developing AAA) [12, 32]. Despite this, the genetic loci driving AAA susceptibility are poorly characterised and genome-wide association studies (GWAS), have suggested multiple single nucleotide polymorphisms (SNPs) which may influence the risk of developing AAA. Jones and colleagues recently conducted a meta-analysis, combining data from six independent GWAS, providing a total population of 4972 AAA cases and 99,858 controls [32]. Meta-analysis and subsequent assessment in an independent validation cohort confirmed the association of five previously reported SNPs with AAA diagnosis, and identified a further four novel risk loci which were suggested to be specific to AAA (Table 12.2). AAA-associated SNPs were predicted to influence a range of molecular processes including inflammation, lipid metabolism, gene transcription and protease activity, however network analyses suggested a central role for matrix metalloproteinase-9 in driving these effects [12, 32]. It should, however, be noted that the loci identified in this and other analyses have relatively small effect sizes, with each SNP suggested to individually influence AAA risk by no more than ~20% [32]. Thus, it seems unlikely that these SNPs alone would fully explain the high degree of heritability seen for AAA.

Table 12.2 Single nucleotide polymorphisms (SNPs) showing significant associations with AAA presence following meta-analysis of genome wide association study data (adapted from [12, 33])

SNP	Chromosome	Closest gene(s)	Predicted biological function	Minor allele	Major allele	Minor allele frequency	Odds ratio (95% CI)
rs602633	1	PSRC1 CELSR2 SORT1	Mitosis (PSRC1), plasma membrane associated protein (CELSR2), and lipid metabolism (SORT1)	T	G[a]	0.199	0.88 (0.84–0.92)
rs4129267	1	IL6R	Inflammation	T	C[a]	0.370	0.88 (0.85–0.91)
rs10795061[b]	1	SMYD2	Gene regulation	T[a]	C	0.337	1.13 (1.09–1.17)
rs10757274	9	CDKN2B-S1/ ANRIL	Unknown	A	G[a]	0.462	0.81 (0.78–0.83)
rs10985349	9	DAB2IP	Tumour suppressor	T[a]	C	0.195	1.17 (1.12–1.23)
rs9316871[b]	13	LINC00540	Unknown	A	G[a]	0.201	0.87 (0.84–0.91)
rs6511720	19	LDLR	Lipid metabolism	T	G[a]	0.096	0.80 (0.76–0.85)
rs3827066[b]	20	PCIF1, ZNF335, MMP9	Gene regulation (PCIF1 and ZNF335), and protease activity (MMP9)	T[a]	C	0.179	1.22 (1.17–1.28)
rs2836411[b]	21	ERG	Gene regulation	T[a]	C	0.369	1.11 (1.07–1.15)

[a]Denotes effect allele—shown odds ratios refer the risk of having an AAA for carriers of the effect allele, compared to those with the non-effect allele. *95% CI* 95% confidence intervals

[b]SNPs suggested to be specific to AAA

SNP Single nucleotide polymorphism, *PSRC1* Proline and serine rich coiled-coil 1, *CELSR2* Cadherin EGF LAG seven-pass G-type receptor 2, *SORT1* Sortilin 1, *IL6R* Interleukin 6 receptor, *SMYD2* SET and MYND domain-containing 2, *CDKN2B-S1/ANRIL* CDKN2B antisense RNA1, also known as ANRIL, *DAB2IP* DAB2 interacting protein, *LINC00540* Long intergenic non-protein coding RNA 540, *LDLR* low-density lipoprotein receptor, *PCIF1* Pancreatic and duodenal homeobox 1 C-terminal inhibiting factor 1, *ZNF335* Zinc finger protein 335, *MMP9* Matrix metalloproteinase 9, *ERG* v-ets avian erythroblastosis virus E26 oncogene homologue, *GWAS* genome-wide association studies

Additional research has suggested that epigenetic modifications may also contribute to AAA risk. Unlike SNPs which are a direct alteration of the encoding nucleotide sequence, epigenetic factors alter gene function through chemical modification or post-transcriptional silencing, which are not reflected by changes to the DNA code [2, 33]. These include micro-RNAs (which are a class of small non-coding RNA which have been shown to regulate gene expression at the post-transcriptional level), histone modifications (post-translational modifications to histone proteins which can impact gene expression by altering chromatin structure), and DNA methylation and hydroxylation (which can affect promoter function and impact on transcription). Studies in animal models and patients have suggested the importance of multiple epigenetic changes in AAA pathogenesis (discussed in [2, 33, 66]). Drugs capable of reversing epigenetic changes have been developed, but are primarily being assessed for their ability to treat cancer. Despite this, the potential cardiovascular benefits for these agents has been suggested, although much of the literature has focused on atherosclerotic disease, with little consideration of AAA [67].

12.4 Factors Contributing to AAA Rupture

AAA rupture is thought to occur when the arterial wall becomes too weak to withstand the mechanical pressures exerted by the arterial blood flow [68]. To date, the most accepted indicator of rupture risk is AAA diameter. A recent meta-analysis has suggested the annual risk of rupture is 3.5% for AAAs measuring 55–60 mm, 4.1% for 61–70 mm AAAs, and 6.3% for those above 70 mm [69]. It is, however, well documented that some small AAAs can rupture and some large AAAs remain stable suggesting that infra-renal aortic diameter alone does not fully explain a patient's rupture risk. There is interest in utilising imaging approaches to characterise the biomechanical forces exerted upon the AAA wall as a means to provide a more specific indication of AAA rupture risk. Several recent studies have demonstrated that calculated wall shear stress is significantly higher in ruptured AAAs when compared to intact AAA controls [68]. Known risk factors for AAA rupture, including complex arterial geometry, current smoking and small body habitus, are also reported to unfavourably influence the biomechanical parameters of the AAA by increasing wall stress, or reducing aortic wall strength [70, 71]. It should be noted, however, that experimental biomechanical models are underpinned by assumptions, regarding aortic wall thickness and variations in blood pressure, which limit their current clinical utility, and focus on more patient-specific models is warranted to improve translational potential [72, 73].

Meta-analysis of individual patient data has highlighted that current smoking (compared to those who have quit, or never smoked), female sex and increased mean arterial blood pressure and pulse pressure are associated with significantly higher risk of AAA rupture after adjusting for AAA diameter (adjusted hazards ratios [95% confidence intervals] 2.02 [1.33–3.06]; 3.76 [2.58–5.47]; 1.32 [1.11–1.56] and 1.11 [1.02–1.22] respectively; Hazards ratios for blood pressure parameters relate to an increase of 10 mmHg) [44]. The same analysis identified a significant inverse relationship between body mass index (BMI) and AAA rupture

(hazards ratio [95% confidence interval] 0.93 [0.88–0.99]/kg/m^2 after adjusting for AAA diameter). The authors did not demonstrate any relationship between commonly prescribed cardiovascular drugs and reduced rupture incidence [44], suggesting that better understanding of the cellular processes underpinning AAA rupture is needed to identify potential therapeutic targets.

Identifying the molecular pathways leading towards rupture might provide a basis for the development of drugs capable of limiting AAA rupture. It is rarely ethically appropriate to use AAA rupture as an outcome measure for clinical studies, as most patients undergo corrective surgery once their AAA approaches 55 mm (50 mm in women) or becomes symptomatic to minimise the risk of rupture [44]. Study of AAA rupture in animal models may have relevance for drug development. Aortic rupture is a common outcome in the angiotensin II rodent model, and has also been reported in the xenograft and elastase models [35, 37]. Moran and colleagues, for example, previously reported that mice receiving a kinin B2 receptor agonist showed significantly higher rupture rates in response to angiotensin-II infusion, compared to controls [38]. This effect was abrogated following neutrophil depletion, suggesting a pathological role for neutrophils activated through the kinin B2 receptor in accelerating aortic wall destruction [38]. More recently, Fashandi and colleagues have described a model which was reported to increase the rate of AAA ruptures and may permit research specifically investigating AAA rupture, however, further validation of this is required [74].

12.5 Discovering Effective Medications for AAA; Current Progress in Clinical Trials

As detailed in the sections above, epidemiological and biochemical findings implicate multiple factors including hypertension, inflammation, extracellular matrix remodelling and thrombosis in AAA pathogenesis and complications. A logical hypothesis is therefore that an effective therapy will inhibit one or more of these processes, and this is reflected in the design of previous and current clinical trials seeking to identify novel drugs to treat small AAAs. Often, the selection of a drug to test was informed by findings from epidemiological association studies, and/or results from basic science experiments utilising human tissues, cell lines or animal models of AAA. To date, relatively few trials have been conducted in this field, possibly owing to practical challenges in study design such as slow AAA growth rate resulting in small effect sizes, difficulties in reproducibly measuring AAA size and loss of patients due to requirements for surgical repair [16, 75]. Many of the published trials have assessed potential off-label benefits of already approved medications (so-called ‘drug repurposing’), which, if successful can help bypass long and expensive routes to translation associated with *de novo* drug development. On the other hand, this may further complicate trial design as many potential participants may need to be excluded if they are prescribed similar medications as part of their standard care, thereby limiting feasible study sample sizes. Moreover, inter-study heterogeneity in the outcome measures used and the methods used to assess them can complicate direct

comparison, subsequent meta-analysis and overall generalisation [76]. The remainder of this chapter focuses on the reported outcomes from completed randomised controlled trials, the design of ongoing trials, and the insight these have provided into AAA pathophysiology. Examples of clinical trials with reported outcomes and those currently in progress are provided in Tables 12.3 and 12.4 respectively.

Table 12.3 Examples of previous trials assessing medications for AAA

Trial title and relevant references	Interventions	Duration	No. participants	Effect on AAA growth	Other outcomes and observations
Trials assessing anti-hypertensive agents					
Propanolol for small AAAs [84]	Propanolol (80–120 mg bid) vs placebo	30 months	548	No difference between treatment groups (**primary outcome measure**)	High rates of withdrawal in both medication and placebo groups (39% vs 21%, respectively)
Propanolol (Viborg study) [85]	Propanolol (40 mg bid) vs placebo	24 months	54	No difference between treatment groups (**primary outcome measure**) growth	Trial stopped owing to high drop-out rate (60%) in the propranolol group
Propanolol (Cambridge study) [86]	Propanolol (40 mg/day) vs placebo	Not specified	477	No difference between treatment groups (**primary outcome measure**)	Poor adherence to medication in group receiving propranolol
The AARDVARK trial [89]	Perindopril arginine (10 mg/day) vs amlodipine (5 mg/day) vs placebo	24 months	227	No difference between treatment groups (**primary outcome measure**)	No differences in requirement for surgical AAA repair between groups.
Trials assessing anti-inflammatory agents					
The PHAST study [96]	Doxycycline (100 mg/day) vs placebo	18 months	286	More rapid AAA growth in patients receiving doxycycline (**primary outcome measure**)	Trial stopped following interim analysis (75% of data collected), showing futility of medication.

(continued)

Table 12.3 (continued)

Trial title and relevant references	Interventions	Duration	No. participants	Effect on AAA growth	Other outcomes and observations
The AORTA trial [99]	Pemirolast (mast cell inhibitor at 10, 25 or 40 mg bid) vs placebo	12 months	326	No difference between treatment groups (**primary outcome measure**)	No difference in adverse event rates between groups. No difference in circulating inflammatory biomarker profiles between treatment arms.
Trials assessing anti-hyperlipidaemic agents					
Statin use in AAA repair [55]	Atorvastatin (80 mg/day) vs placebo	4 weeks	40	Not assessed	No significant inter-group differences in the expression of matrix metalloprotease-9 (**primary outcome measure**), other matrix metalloproteases or their endogenous inhibitors in aortic wall biopsies taken during open surgical repair.
The FAME-2 trial [102]	Fenofibrate (145 mg/day) vs placebo	24 weeks	140	No difference between treatment groups	No difference in serum concentrations of osteopontin or kallistatin between the groups (**primary outcome measures**)

12.5.1 Trials Assessing Anti-hypertensive Medications

Early research suggested that AAA severity was significantly less in experimental animals receiving the beta-blocker propranolol compared to controls, associated with an increase in the cross-linkage of collagen and elastin fibres within the aortic extracellular matrix [77–80]. These observations were supported by retrospective clinical data which suggested that AAA growth was slower in patients prescribed propranolol, compared to those who were not [81]. Several randomised controlled trials have investigated the difference in growth rate of small AAAs in patients allocated propranolol and those receiving placebo [82–85]. These studies independently demonstrated no benefit of the drug on AAA growth [76], and reported high rates of adverse events which greatly reduced patient adherence with the trial medication (see Table 12.3). This was particularly noted in one trial which was terminated due

Table 12.4 Examples of current clinical trials assessing potential medications for AAA

Trial and related references[a]	Phase	Interventions	Duration	Target sample size	Primary outcome measure	Recruitment status[b]
Trials assessing anti-hypertensive agents						
ACTRN12611000931976: The TEDY study [117]	4	Telmisartan (40 mg/day) vs placebo	24 months	300	AAA growth as assessed by infra-renal aortic volume on CTA.	Active, not recruiting
NCT01904981: The BASE trial	4	Atenolol (50 mg/day) vs valsartan (80 mg/day)	Not specified	400	AAA growth (imaging modality not specified)	Unknown
NCT01425242: The PISA study	N/A	Aliskerin (150 mg/day) vs amlodipine (5 mg/day)	12 months	Not Reported (actual recruitment: 3)	Change in AAA wall inflammation (assessed by FDG-uptake assessed via PET-CT)	Terminated (insufficient patient recruitment).
Trials assessing anti-inflammatory agents						
NCT02225756: The ACA4 trial	2	Cyclosporine A (2 treatment groups at non-specified doses) vs placebo	Not specified (indicated as a 'short course')	360	AAA growth as assessed via CTA	Unknown
NCT01756833: The N-TA^3CT study	2	Doxycycline (100 mg bid) vs placebo	24 months	258	AAA growth assessed by maximum transverse diameter via CTA.	Active, not recruiting
NCT02007252: ACZ885 for the treatment of AAA	2	Canakinumab (50 mg/month) vs placebo[c]	12 months	Not Reported (actual recruitment: 65)	AAA growth assessed via ultrasound	Terminated (lack of efficacy following interim analysis)
Trials assessing anti-platelet agents						
NCT02070653: The TicAAA study	2	Ticagrelor (90 mg/bid) vs identical placebo	12 months	Not Reported (actual recruitment: 145)	AAA growth as assessed by infra-renal aortic volume on MRI.	Completed[d]

(continued)

Table 12.4 (continued)

Trial and related references[a]	Phase	Interventions	Duration	Target sample size	Primary outcome measure	Recruitment status[b]
Trials assessing anti-glycaemic agents						
NCT03507413: The MetAAA study	2	Metformin (2000 mg/day) vs placebo	12 months	170	AAA growth as assessed via CTA	Not yet recruiting
Trials assessing anti-lipidaemic agents						
ACTRN12612001226897: The FAME trial [104]	4	Fenofibrate (145 mg/day) vs placebo	At least 2 weeks prior to open AAA repair	42	Aortic wall macrophage number (biopsies collected at open surgery); Serum and aortic osteopontin concentrations	Active, not recruiting

[a]Registration number of trial

[b]Based on information presented on the clinical trials database as of October 2018

[c]Based on a search for interventional trials for small abdominal aortic aneurysm. Trials assessing ruptured AAA or peri-operative medications are not included here (see https://clinicaltrials.gov/ct2/results?cond=abdominal+aortic+aneurysm&Search=Apply&age_v=&gndr=&type=Intr&rslt=, and http://www.anzctr.org.au/TrialSearch.aspx?searchTxt=abdominal+aortic+aneurysms&isBasic=True accessed October 2018)

[d]Included as current trials as outcome data have not yet been reported

to slow recruitment and high dropout rates [83]. Collectively this suggests that propranolol is unlikely to be an effective or practical therapeutic for AAA.

A significant body of evidence from studies conducted in experimental animals and patient populations suggests a pathological role for the renin-angiotensin system in AAA [37, 85, 86]. Angiotensin converting enzyme (ACE) has been suggested as a potential therapeutic target for AAA. Bicknell and colleagues recently reported the outcomes of the AARDVARK study, a 3-armed randomised controlled trial which monitored AAA growth over 2 years in groups of patients randomised to receive either perindopril arginine (10 mg/day), amlodipine (5 m/day) or placebo [87]. The three-way design was utilised to test the hypothesis that ACE inhibition may confer therapeutic benefits to AAA independently of reductions in blood pressure, evidenced by significant reductions in AAA growth in patients receiving perindopril compared to amlodipine. The AARDVARK trial was planned as a pilot study, reflected by relatively small sample sizes in each treatment arm (Table 12.3). However, presented power calculations suggested that the study was adequately powered to detect a 20% difference in AAA growth between the perindopril and amlodipine groups. Study authors reported good patient retention (attrition rate of 4%), and adherence to medication as evidenced by pill counting (>80% for all treatment groups at each timepoint assessed). Systolic blood pressure in patients receiving placebo remained stable during follow-up, but dropped significantly from baseline in those receiving perindopril or amlodipine for 12 months (mean [standard deviation] difference from baseline −9.5 [13.1] and −6.7 [12] mmHg, respectively, both $p < 0.001$). Despite this, no difference in AAA growth rates was observed between any of the groups. Sensitivity analyses accounting for factors known to influence AAA growth did not change these results [87].

12.5.2 Trials Assessing Anti-inflammatory Agents

Two anti-inflammatory agents have been tested as potential AAA therapeutics. The tetracycline drug, doxycycline, has attracted interest based on a reported ability to suppress inflammation and proteolysis, with potential to preserve the aortic extracellular matrix [85, 88]. Studies utilising a range of pre-clinical models (predominantly angiotensin-II, and elastase-infused mice) have independently reported that animals receiving doxycycline develop less severe AAAs than controls [89, 90]. Infra-renal aortic biopsies collected from patients randomised to receive doxycycline for 2 weeks prior to open AAA repair showed significantly lower concentrations of CD8+ T-cells, neutrophils and pro-inflammatory markers, than those allocated to placebo [91]. These encouraging observations were further supported by a series of pilot clinical studies suggesting that the drug was generally well tolerated and adhered to, and that patients allocated to doxycycline exhibited slower AAA growth rates [92, 93]. The Pharmacological Aneurysm Stabilisation Trial (PHAST) was the first large-scale study to directly assess the potential benefits of doxycycline and randomised 286 patients with AAAs measuring 35–50 mm to

receive active drug (doxycycline 100 mg/day, n = 144) or placebo (n = 142) for 18 months [94]. The study was, however, terminated prematurely following an efficacy interim analysis (conducted after collecting ~75% of anticipated data), which demonstrated no benefit for the drug. Of interest, AAA growth rates were statistically significantly higher in patients receiving doxycycline compared to controls, although the difference between groups was not considered to be clinically relevant. The reasons behind this unexpected result remain unclear, and further investigation is ongoing [94].

Mast cells emerged as a potential therapeutic target owing to their presence within aortic biopsies recovered from AAA patients, the demonstrated ability for mast cell secretions to degrade the aortic extracellular matrix (chymase), and observations that genetic deletion of mast cells, or mast-cell chymase protected against AAA formation in rodent models [95, 96]. Building from this, the recent Anti-inflammatory ORal Treatment of AAA (AORTA) trial recruited patients with medium-sized AAAs (infra-renal aortic diameter 39–49 mm), to determine the therapeutic potential of the mast cell inhibitor pemirolast [97]. The AORTA trial adopted a multi-arm design in which patients were allocated to placebo (n = 84), or one of three pemirolast regimes which aimed to identify a dose which effectively slowed AAA growth over a 12 month follow-up period. The primary outcome for this study was the change in AAA diameter from baseline as assessed by standardised ultrasound imaging. The investigators reported that there was no statistically significant difference in medication compliance, adverse events or drop-out rates between the groups suggesting that the drug was well tolerated. Despite this, there was no significant difference in AAA growth rates between the groups in both intention to treat, and per protocol analyses [97].

12.5.3 Trials Assessing Dyslipidaemic Drugs

The recently published FenofibrAte in the ManagemEnt of abdominal aortic aneurysm-2 (FAME-2) trial assessed the potential for the peroxisome proliferator activated receptor-α ligand fenofibrate to favourably modify AAA pathology following observations within a mouse model that fenofibrate limited AAA severity [98, 99]. Mice receiving fenofibrate had significantly lower aortic concentrations of a number of pro-inflammatory proteins including osteopontin than controls, suggesting that fenofibrate was able to blunt aortic inflammation and extracellular matrix remodelling [98–100]. Building from this, the FAME-2 study was a double-blind placebo controlled randomised trial to determine whether patients receiving 145 mg/day fenofibrate for 6 months would exhibit similar reductions in AAA pathology [100]. Primary outcome measures for FAME-2 were changes in serum osteopontin and kallistatin between the groups [101]. One-hundred and forty patients with small AAAs were recruited to FAME-2 (n = 70 per treatment arm) in order to fulfil sample size requirements, 3 of whom were lost to follow-up. Overall adherence to the trial medication regime was reported as 85%, with no statistically significant differences

between the groups. Patients allocated fenofibrate demonstrated a significant reduction in serum triglyceride concentrations after taking the drug for 3 weeks, which persisted for the remainder of the trial, suggesting that an effective therapeutic dose was administered. Despite this, circulating concentrations of osteopontin, kallistatin and other AAA-associated proteins were similar between groups, leading the authors to conclude that the drug provided no direct impact on AAA pathophysiology. An important limitation raised by the authors was the fact that peripheral blood samples were examined, and the possibility that fenofibrate may have exerted beneficial effects within the aortic wall could not be dismissed [100]. To overcome this limitation, the same group of researchers are currently completing a related clinical trial (FAME), in which patients are allocated to a short course of fenofibrate or placebo prior to scheduled open surgical repair of large AAAs [102]. Aortic biopsies collected during surgery will be examined to determine the effect of fenofibrate on AAA pathophysiology, as assessed by the extent of arterial inflammation (such as the number of infiltrating macrophages), and osteopontin concentrations [102].

The FAME study follows a similar design to that of a previously reported small trial assessing the potential benefit of short term statin use in reducing markers of aortic wall proteolysis [53]. This study recruited 40 patients who were randomised in a 1:1 ratio to receive either atorvastatin or placebo in the 4 weeks leading to scheduled open AAA repair. The primary outcome measure for the study was the concentration of matrix metalloproteinase-9, and no significant difference for this marker was observed between groups following treatment [53]. Whilst these data suggest that atorvastatin had no impact on aortic wall markers, it is difficult to generalise findings from this study as the sample size may have been too small to detect subtle differences between groups. Recruitment for this trial appeared problematic as a large number of potential participants were excluded since they were already receiving a statin, however the researchers were able to fulfil *a priori* sample size calculations. In addition, no data regarding adherence to trial medication were presented, and it is therefore difficult to determine whether compliance may have affected the reported outcomes. Of note, a recent meta-analysis has suggested that statins may provide some protection against AAA growth and subsequent rupture although this hypothesis is based on observational data only [103]. Current management guidelines recommend that AAA patients should receive statin therapy to reduce the risk of myocardial infarction, stroke or vascular death, rather than limit AAA growth [104].

12.5.4 Current Trials

Table 12.4 details the design of some contemporary trials assessing potential therapies for AAA. At the time of writing, two of these studies have been terminated. One assessing subcutaneous infusion of canakinumab, a monoclonal antibody against interleukin-1 beta was abandoned owing to a lack of efficacy, whilst another comparing the efficacy of aliskerin and amlodipine could not recruit sufficient

patients (data reported from https://clinicaltrials.gov, accessed 15/10/2018). As with past studies, the ongoing trials are focused on agents aimed to limit inflammation, hypertension or thrombosis. Independent studies investigating the therapeutic effects of doxycycline (at a higher dose than the previous PHAST trial) and ticagrelor have reported that recruitment is completed, and results are eagerly anticipated [105].

More recently an increasing body of data has suggested that the slower AAA growth observed in patients with diabetes may not be solely attributed to the presence of diabetes, but may also be due to the drugs used to treat diabetes [106]. Several independent centres have reported that the prescription of metformin is associated with slower AAA growth, and rates of rupture or surgical repair, whereas this trend is not robust for other commonly prescribed diabetes drugs [107–111]. Interpreting these findings is complicated by the fact that all included patients receiving metformin also had diabetes. Thus the degree of protective effect (if any) exerted solely by metformin is difficult to quantify, however, experimental data suggest that non-diabetic rodents receiving metformin appear to be more resistant to AAA formation and subsequent growth, than those receiving control interventions [111, 112]. Collectively, this body of evidence has provided stimulus for interventional trials assessing the potential effects of metformin on AAA outcomes in patients who do not have diabetes [113]. At the time of writing, one small randomised controlled trial aiming to assess the effects of 12 months of metformin prescription on AAA growth has been announced, and outcomes of this trial are eagerly anticipated (see https://clinicaltrials.gov/ct2/show/NCT03507413).

12.5.5 Interpreting Findings from Clinical Trials

None of the completed clinical trials to date have demonstrated a clinical benefit for any assessed medications, and this likely arises due to multiple factors. Firstly, many potential therapeutics have been selected based on promising results from investigations conducted in rodent models. Thus the possibility that the lack of translation may be due to inherent biological differences between rodents and humans must be considered. Of note, mice appear naturally resistant to cardiovascular disease owing to key differences in lipoprotein metabolism meaning that significant genetic and/or surgical manipulations are often required to predispose them to AAA formation [34]. In addition, AAA development in rodent models is an acute process, but occurs over decades in humans meaning that chronic pathology cannot be easily simulated. Rodent models usually do not include human AAA risk factors such as old age and widespread atherosclerosis which are known to be important in human populations. Haemodynamics observed within the AAA sac of commonly used rodent models have also been shown to differ from those experienced by patients [114, 115]. The design of rodent model experiments may also have contributed to the lack of translation as many previous studies have investigated the ability of potential therapeutics to block AAA formation, as opposed to limiting

progression of established AAAs which is more representative of the clinical situation [35]. Moreover, animal-based studies have not traditionally followed the same rigorous processes required of clinical studies such as presentation of sample size calculations, randomisation of subjects, blinding of assessors to group allocations and detailed statistical reporting, potentially increasing the scope for interpretation bias [116]. This limitation is, however, not restricted to AAA research and can be broadly extended across the biological sciences. In recognition of this limitation, the UK-based National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), has proposed a series of experimental and reporting standards, the ARRIVE guidelines, to improve the transparency of *in vivo* experiments [116]. There is growing acceptance of the ARRIVE guidelines within the scientific literature, revealed by an increasing number of journals requesting evidence of compliance as part of the publishing process, aiming to improve the translational potential of findings from pre-clinical models.

In questioning the current lack of success in identifying an effective AAA therapy, the design and conduct of contemporary clinical trials must also be examined. The difference in AAA growth between treatment groups has been the most commonly employed outcome measure for the trials reported to date. Whilst an objective measure of AAA progression, AAA growth is typically slow (trials reporting an average of 1–3 mm/year), and variable between patients. Hypothesised reductions in AAA growth rate attributable to pharmaceutical intervention are therefore extremely subtle, and are potentially within the limits of error for many abdominal imaging techniques [37]. This can be partly overcome through the establishment of highly reproducible protocols for AAA size measurement, however large sample sizes and long follow-up periods are also required to improve analytical sensitivity. Despite this, many reported and currently ongoing studies have relatively small sample sizes, and follow-up periods are typically less than 2 years (see Tables 12.2 and 12.3). This likely reflects the practical constraints associated with recruiting, and following AAA patients within small catchment areas. Lessons from the past therefore suggest that multi-centre and multi-national trials involving centres with harmonised outcome assessment approaches may be needed to definitively assess therapies into the future [75].

12.6 Conclusion

Although AAA prevalence has decreased over the past decade, AAA remains an important cause of mortality in older adults. Surgical repair is currently the only means to treat AAA. However, surgery is costly and is associated with significant peri-operative morbidity and mortality. Whist endovascular repair has lower perioperative risks, the procedure has limited long-term durability. Most AAAs identified in high-income countries are below the recommended size threshold for elective surgery but subsequently reach this size during surveillance. Effective AAA drugs would improve patient care and may provide a more cost-effective management.

Past clinical trials have not identified any drugs which convincingly limit AAA growth. Challenges in discovering effective AAA drugs include poorly designed pre-clinical studies and clinical trials, difficulties in modelling human AAA in pre-clinical studies and lack of interest from the pharmaceutical industry in drug development in this field. To overcome these hurdles, it is likely that an international collaborative approach will be necessary to ensure that future randomized controlled trials have sufficiently large sample sizes to reliably detect a clinically meaningful outcome.

Recommended Reading

Detailed discussion on AAA pathogenesis and current medical management approaches can be found in references [12, 37].

A detailed overview of the similarities and differences between AAA and atherosclerosis is found in references [45, 46].

Reference [35] provides a recent systematic review detailing the characteristics of available animal models for AAA research.

An overview of insight into AAA clinical trials is provided by reference [16]. *This is supported by recommendations for the standardisation of the design of AAA clinical trials to improve translational potential made in reference* [75].

The current Society for Vascular Surgery guidelines for AAA patient management are provided in reference [11].

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Chapter 13
Pathophysiology and Principles of Management of Hereditary Aneurysmal Aortopathies

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Key Learning Points

- An aortic aneurysm is a progressive dilatation of the aorta which entails a substantial risk for aortic rupture or dissection, i.e. events that come with an ultimate mortality rate of 80%.
- TAAs can be subdivided in syndromic forms, such as Marfan syndrome or Loeys-Dietz syndrome, and non-syndromic forms based on the presence or absence of multi-systemic manifestations, respectively.
- Roughly 30 TAA genes have been identified to date, explaining about 30% of all TAA probands. TAA genes encode proteins involved in ECM homeostasis, the TGF-β pathway or the VSMC contractile apparatus.
- Early clinical and/or molecular diagnosis and serial cardiovascular follow-up with either CT, TTE, TEE or MRI are important actions in TAA management.
- The molecular TAA landscape facilitates gene-tailored therapy. For instance, in case of *SMAD2*, *SMAD3*, *TGFBR1*, *TGBFBR2* mutations, surgical intervention should already be considered for aortic diameters as small as 4.0–4.5 cm.
- Pharmacological TAA treatment usually encompasses β-blocker or losartan administration.

13.1 Introduction

Aortic aneurysms result from structural weakening of the aortic wall [1], and predispose to aortic rupture and dissection because of a progressive increase in wall tension. Only 5% of patients experience warning symptoms prior to aortic rupture

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or dissection, which are estimated to be responsible for 1–2% of all deaths in industrialized countries [1, 2]. Due to the silent and unpredictable disease course of aneurysmal disease, mortality rates are likely to be underestimated [2].

Based on the anatomic location, aortic aneurysms can be divided in two groups: abdominal aortic aneurysms (AAAs) and thoracic aortic aneurysms (TAAs). AAA is the most common form, in which the aneurysm is located below the diaphragm. A positive family history is present in 12% of cases, suggesting a role for genetics in the aetiology of AAA. Monogenic AAA causes remain to be identified, but multiple AAA risk genes have been reported including: *DAB2IP*, *LRP1*, *CDKN2B-AS1*, *CNTN3*, *LPA*, *IL6R*, *SORT1*, *MMP3*, *AGTR1*, *ACE*, *APOA1*, *PEPD*, *CD22 and MTHFR* [3]. Age, gender (males > females), European ancestry, hypertension, smoking and/or diabetes, however, also impinge significantly on AAA risk [4].

TAAs, located above the diaphragm, occur less frequently than AAAs but have been more extensively investigated due to a higher heritability and a younger age at onset. In approximately 20% of TAA patients, a positive family history is identified and in about 30% of these, a mutation is found in one of the roughly 30 currently known TAA genes (Table 13.1). Patients with thoracic aortic aneurysm and dissection (TAAD) often do not present with other risk factors such as hypertension.

TAAD, which is clinically characterized by excruciating pain of sudden onset, can also be classified in two groups based on their anatomic location; type A dissections which affect the proximal aorta and the aortic arch on one hand, and type B dissections which begin beyond the subclavian artery on the other hand. Type A dissections are associated with the most serious complications. Since the aetiology of TAA has been more extensively studied from a genetic perspective, TAA will be the main focus of this book chapter.

13.2 Epidemiology

It is not straightforward to accurately determine the prevalence of TAA(D) as many patients remain asymptomatic, and hence undiagnosed, until aortic dissection or rupture occurs. The current TAAD incidence is estimated to be 2.7/100,000 person-years, but most probably the true figure is higher [5]. In general, males are more frequently affected than women. However, mortality rates are higher for female patients which is thought to be due to atypical clinical presentation leading to diagnostic delay [6]. TAAD is associated with high morbidity and mortality. Dissection/rupture has been shown to occur in about 37% of TAA patients [7], of which about 40% die immediately. Each hour after the initial event another 1% of the patients die if surgical intervention is not performed and an additional 5% die during and 20% shortly after emergency intervention [8]. Preventive surgical intervention reduces the mortality rate down to less than 5%, emphasizing the importance of serial cardiovascular monitoring of TAA patients [9].

Table 13.1 Thoracic aortic aneurysm and dissection related disorders with their respective genotype. OMIM; Online Mendelian Inheritance in Man

Disease	Gene	Inheritance	Cardiovascular features	OMIM	Ref.
Syndromic TAA disorders					
Marfan syndrome	*FBN1*	AD	TAA(D), mitral valve disease	154700	[24]
Loeys-Dietz syndrome type 1	*TGFBR1*	AD	TAA(D), arterial tortuosity, arterial aneurysms	609192	[31]
Loeys-Dietz syndrome type 2	*TGFBR2*	AD	TAA(D), arterial tortuosity, arterial aneurysms	610168	[31]
Loeys-Dietz syndrome type 3	*SMAD3*	AD	TAA(D), arterial tortuosity, arterial aneurysms	613795	[31]
Loey-Dietz syndrome type 4	*TGFB2*	AD	TAA(D), arterial tortuosity, mitral valve disease	614437	[33]
Loeys-Dietz syndrome type 5	*TGFB3*	AD	TAA(D), mitral valve disease	615582	[34]
Loeys-Dietz syndrome type 6	*SMAD2*	AD	TAA(D)	NA	[32]
Shprintzen-Goldberg syndrome	*SKI*	AD	TAA(D)	182212	[37]
Meester-Loeys syndrome	*BGN*	X-linked	TAA(D)	300989	[39]
Vascular Ehlers-Danlos syndrome	*COL3A1*	AD	TAA(D), arterial aneurysms	130050	[42]
Vascular like Ehlers-Danlos syndrome	*COL1A1*	AD	TAA(D), arterial aneurysms	130060	[44]
Classical Ehlers-Danlos syndrome	*COL5A1*	AD	TAA(D), arterial aneurysms	130000	[43]
Periventricular nodular heterotopia type 1	*FLNA*	X-linked	TAA(D), mitral valve disease	300537	[45, 46]
Arterial tortuosity syndrome	*SLC2A10*	AR	Arterial tortuosity and aneurysms	208050	[20]
Cutis laxa	*ELN*	AD	Occasionally TAA, mitral and aortic valve regurgitation	123700	[47]
Cutis laxa type 1	*EFEMP2*	AR	Arterial tortuosity, aortic aneurysms, stenosis	614437	[51]
Non-syndromic TAA disorders					
Familial thoracic aortic aneurysm 6	*ACTA2*	AD	Livedo reticularis, iris flocculi, Moya-Moya disease	611788	[53, 54]
Familial thoracic aortic aneurysm 4	*MYH11*	AD	PDA, TAA(D)	132900	[55]
Familial thoracic aortic aneurysm 7	*MYLK*	AD	TAA(D)	613780	[56]
Familial thoracic aortic aneurysm 8	*PRKG1*	AD	TAA(D), arterial tortuosity, hypertension	615436	[57]
NA	*MAT2A*	AD	TAA(D), BAV	NA	[58]
Familial thoracic aortic aneurysm 11	*FOXE3*	AD	TAA(D)	617349	[59]

(continued)

Table 13.1 (continued)

Disease	Gene	Inheritance	Cardiovascular features	OMIM	Ref.
Aortic valve disease	*NOTCH1*	AD	TAA(D), BAV	109730	[61]
Familial thoracic aortic aneurysm 9	*MFAP5*	AD	Paroxysmal atrial fibrillation, TAA(D)	616166	[61]
Aortic valve disease 2	*SMAD6*	AD	TAA(D), BAV	614823	[62]
Aortic valve disease 3	*ROBO4*	AD	TAA(D), BAV, aortic valve regurgitation/stenosis	607528	[63]
Familial thoracic aortic aneurysm 10	*LOX*	AD	Aortic root aneurysm, BAV, TAA(D)	617168	[64]

13.3 Pathophysiology

Aortic aneurysms are pathological dilatations of the aorta, caused by vascular wall weakness. The aortic wall consists of three different layers: the intima, the media and the adventitia. The innermost layer, i.e. the intima, is composed of a metabolically important endothelial monolayer that is supported by internal elastic laminae. This sub-endothelial elastic matrix harmonizes the motion of the intima and media during cyclic aortic expansion or contraction [10, 11]. The media makes up the largest part of the vessel wall and consists of vascular smooth muscle cells (VSMCs) embedded in extracellular matrix (ECM) proteins such as elastins, collagens and proteoglycans. The ECM provides elasticity and tensile strength, besides sequestering a variety of growth factors. ECM homeostasis is controlled by matrix metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPS) produced by synthetic VSMCs during vascular remodelling. Whereas MMPs degrade the ECM, TIMPs reduce ECM degradation through MMP inhibition [12]. Contractile VSMCs control the vessel's luminal diameter, and hence blood pressure, by mediating vessel contraction or relaxation. The adventitia is the outer layer of the vessel wall and consists of collagen and elastin fibres as well as fibroblasts, immunomodulatory cells, vasa vasorum endothelial cells and pericytes. It contributes to aortic integrity and provides nutritional circulation to the vascular wall [10, 11].

In TAA patients, a shift from contractile VSMCs towards synthetic VSMCs has been observed leading to an imbalance between MMP and TIMP expression. Pathological amounts of MMPs are secreted, resulting in disproportionate ECM degradation [13], ECM disorganisation and growth factor release [14]. Moreover, loss of VSMC contractility reduces the aortic wall's capacity to properly control the luminal diameter. In addition to a shift in VSMC function, excessive VSMC apoptosis (see Glossary) is typically observed. In the VSMC-depleted regions, degradation products of proteoglycans and glycosaminoglycans as well as inflammatory cells can be found [15, 16]. Collectively, these events are hallmarks of a phenomenon called medial degeneration, which weakens the aortic wall and contributes to aortic aneurysm formation and progression according to Laplace's Law: *circumferential wall tension = transmural pressure x vessel radius*. Ruptures or dissections occur when the mechanical stress on the vascular wall overpowers the tissue strength [17].

Endothelial cell dysfunction has also been linked to TAA pathology, but its precise role is less extensively studied. Decreased endothelial proliferation and differentiation, as well as altered endothelial expression of contractile apparatus or ECM proteins, have been observed in TAA patients. As endothelial cells influence VSMC differentiation, they may contribute VSMC dysfunction and, thus, to aortic wall deterioration and TAA formation [18].

13.4 Syndromic Thoracic Aortic Aneurysm Presentations

Patients can present with isolated TAA or syndromic TAA, with the latter being most thoroughly studied over the years. The specific combination of the multisystemic phenotypic traits in syndrome TAA patients, often already present in early childhood, facilitates early establishment of a diagnosis [19]. Most syndromic TAA presentations are inherited in an autosomal dominant manner, with the exception of *BGN* and *FLNA* mutations which are inherited in an X-linked manner [19] and *SLC2A10* and *EFEMP2* mutations which segregate in an autosomal recessive manner [20, 21].

13.4.1 Marfan Syndrome

In 1896, Antoine-Bernard Marfan first described Marfan syndrome (MFS), which is now known as the most common (circa 1:5000) and best studied syndromic TAA condition. Multiple organ systems are affected, i.e. the skeletal, ocular, skin and cardiovascular system. The most frequent musculoskeletal manifestations are skeletal overgrowth of the long bones, arachnodactyly, pectus deformities, joint laxity and facial features. Myopia and ectopia lentis are characteristic ocular features, while striae are a typical skin symptom. The cardiovascular manifestations (i.e. TAA(D) and mitral valve disease), however, pose the greatest threat. In MFS, aortic aneurysms typically occur at the sinus of Valsalva and the proximal aorta [22, 23].

MFS has an autosomal dominant inheritance pattern and is caused by mutations in the *FBN1* gene [24]. *FBN1* encodes a large extracellular glycoprotein, fibrillin-1, that is secreted by endothelial cells and VSMCs. It forms complex microfibrils, providing elasticity and structural support to the aortic wall, but also regulates the bioavailability and activity of growth factors. About 1850 different *FBN1* mutations, which are widely spread over the entire gene, have been described to date (http://www.umd.be/FBN1/), explaining approximately 95% of MFS cases. *FBN1* mutations lead to impaired protein synthesis, secretion and loss of the microfibrillar architecture, compromising tissue homeostasis and strength [25].

FBN1-related structural tissue weakness can not solely explain all MFS clinical manifestations (e.g. skeletal overgrowth, craniofacial dysmorphism and skeletal muscle

hypoplasia). Using a MFS mouse model that fully reproduces the MFS phenotype, an important additional role for dysregulated TGF-β (transforming growth factor-β) signaling in the pathophysiology of MFS was shown [26, 27]. Under normal physiological circumstances, fibrillin-1 is capable of binding TGF-β ligands that are complexed within a large latent complex [22, 23]. Mutant fibrillin-1 excessively releases these TGF-β ligands, thus increasing the amount of bioavailable TGF-β and TGF-β signaling.

13.4.2 Loeys-Dietz Syndrome

Loeys-Dietz syndrome [28] has a significant clinical overlap with other connective tissue disorders such as MFS, Shprintzen-Goldberg syndrome and Meester-Loeys syndrome. Hypertelorism, craniosynostosis, bifid uvula, cleft palate and arterial tortuosity, however, are discriminative LDS features. Moreover, with respect to the aortic phenotype, LDS patients are more severely affected with dissections and ruptures occurring at smaller aortic diameters, at younger ages and throughout the arterial tree [29, 30]. Dissection or rupture has been reported in children as young as 3 months [31], emphasizing the need for early cardiovascular screening in cases where LDS is suspected.

Six autosomal dominant LDS genes have been identified so far, i.e. *TGFBR1*, *TGFBR2*, *SMAD3*, *TGFB2*, *TGFB3* and *SMAD2,* which are now implicated as the causes of LDS types 1–6 [32–35] (Table 13.1). They encode cytokines, receptors or intracellular effectors of the TGF-β pathway. Upon the discovery of causal mutations in *TGFBR1* and *TGFBR2*, the initial LDS classification was based on the presence or absence of outward phenotypic appearance (e.g. cleft palate, craniosynostosis, hypertelorism). Dysmorphic patients who showed prominent craniofacial traits hinted towards a LDS1 subtype, while EDS-vascular traits were directing towards LDS2. Since the discovery of the other currently known genes, classification and diagnosis of the different subtypes has become based upon the underlying gene defect [19].

In all LDS subtypes, the mutation has been shown to cause loss-of-function in genes involved in the TGF-β pathway. This suggest that the primary event in LDS is a lack of, or reduced, TGF-β signaling. However, subsequent biochemical analysis of aortic tissues revealed nuclear accumulation of pSMAD2/3 and upregulation of prototypical TGF-β target genes such as *MMP2*, *MMP9*, *COL3A1* and *CTGF*, revealing paradoxically enhanced TGF-β signaling [36].

13.4.3 Shprintzen-Goldberg Syndrome

Shprintzen-Goldberg syndrome (SGS) is a very rare autosomal dominant connective tissue disorder that was first recognized as a separate clinical entity in 1982. The SGS phenotype involves the craniofacial, neurological, skeletal and cardiovascular systems and has significant overlap with MFS, LDS and vascular Ehlers-Danlos syndrome. The most discriminative features of SGS are craniosynostosis and

developmental delay with intellectual disability. Although the cardiovascular manifestations in SGS are not as severe as in LDS, complications such as progressive aortic root dilation have been reported as a cause of death [37].

SGS is caused by mutations in *SKI*, which encodes a TGF-β repressor [38]. Most SGS patients carry a *de novo* mutation, either in the N-terminal SMAD2/3 binding domain (73%) or the Dachshund-homology domain. These domains are important for binding of SKI with SMADs and other cofactors, necessary for the recruitment of transcriptional co-regulators. Under normal conditions SKI binds the MH2 domain of SMAD2/3, displacing the transcriptional activator p300. This SKI-SMAD complex alters the local chromatin environment so that TGF-β signaling is inhibited. Mutations in SKI render it unable to properly bind the SMAD proteins, resulting in loss of inhibitory feedback of the TGF-β signalling pathway. Although in depth understanding of TGF-β signalling in is currently lacking, the clear involvement of counter regulatory or compensatory TGF-β signalling events contributes greatly to the pathophysiological understanding of TAA development [23, 39].

13.4.4 Meester-Loeys Syndrome

Defects in the X-linked *BGN* gene cause a disorder that strongly overlaps with LDS and MFS, and presents with hypertelorism, bifid uvula, early-onset aortic dilatations (as young as 1 year) and dissections, pectus deformities, joint hypermobility, and striae. Findings which are more characteristic for Meester-Loeys syndrome (MLS) are ventricular dilatation on brain imaging, gingival hypertrophy, mild skeletal dysplasia with platyspondyly (flattened and widened vertebral bodies), phalangeal dysplasia and dysplastic epiphyses of the long bones [40]. Male mutation carriers are more severely affected than females, demonstrated by an early onset of cardiovascular manifestations in males and a variable phenotype in females, ranging from unaffected through aortic root dilatation to death due to aortic dissection.

BGN encodes for the biglycan protein, which belongs to the small leucine-rich proteoglycan class I proteins and has both structural and functional properties in the vascular wall. Biglycan interacts with multiple ECM proteins, including collagen type I/II/III/VI, elastin and microfibrils [40, 41], and is thus involved in ECM assembly. Biglycan also interacts with several growth factors and cytokines, including TGF-β, in order to regulate cell proliferation, migration and differentiation. In the aortic wall of MLS patients, a lack of biglycan has been shown to be associated with increased TGF-β signaling [40].

13.4.5 Vascular Ehlers-Danlos Syndrome

Ehlers-Danlos syndrome (EDS) refers to a group of connective tissue disorders that is most prominently characterized by skin (hyperextensibility, translucency, easy bruising) and ligament/joint (hypermobility) manifestations. A subset of EDS cases

present with aortic aneurysmal disease. Aneurysms are most prevalent in vascular EDS (vEDS, EDS type IV), which has the worst prognosis amongst all EDS subtypes and is one of the most severe connective tissue diseases [30].

vEDS represents approximately 5% of EDS cases, and has an estimated prevalence between 1:50000 and 1:100000. A high mortality rate is seen in young patients due to early rupture of arteries (with or without aneurysms) and hollow organs, usually during the third decade of life [42]. In contrast to MFS, LDS, MLS and SGS, mostly medium-sized abdominal vessels such as renal, iliac, femoral, mesenteric and hepatic arteries are involved, but aortic involvement is also regularly reported. Owing to significant soft tissue fragility, surgical intervention in vEDS patients is associated with severe complications and relatively high mortality rates.

vEDS is inherited in an autosomal dominant manner and is caused by mutations in the *COL3A1* gene [43]. Several dozen mutations of *COL3A1* have been identified to date, all leading to structurally defective collagen III pro-α1-chains. The mutation spectrum encompasses mostly glycine-affecting missense mutations, splice site mutations and (multi)-exon deletions. Mutations causing *COL3A1* haploinsufficiency lead to milder phenotypes [43].

Occasionally, mutations in *COL1A1* and *COL5A1*, i.e. respectively arthrochalasia (pathological loosening of joints) and classical EDS genes, have been found in patients who are clinically diagnosed with aneurysmal phenotypes [44, 45]. These genes encode collagens, which upon formation of homo- or heterotrimeric fibrils produce the ECM of internal organs and skin, providing tissue tensile strength.

13.4.6 Periventricular Nodular Heterotopia Type 1

Periventricular nodular heterotopia (PVNH) is a neuronal migration disorder that often comes with difficult-to-treat seizures. It can occur with or without EDS-like connective tissue anomalies, including TAA. The condition is caused by loss-of-function mutations in the X-linked *FLNA* gene, encoding the cytoskeletal component filamin A that connects the VSMC contractile apparatus to cell membranes and the ECM [46, 47]. Recently aortic dissections have also been reported in *FLNA* patients [47].

13.4.7 Arterial Tortuosity Syndrome

The main characteristics of arterial tortuosity syndrome (ATS) are the elongation as well as increased twisting and turning of major arteries [48], including the thoracic and abdominal aorta. Additionally, ATS can present with stenosis, dilatation or dissection of the aorta and/or pulmonary arteries, craniofacial dysmorphism, a soft and doughy skin, marfanoid skeletal findings (e.g. scoliosis, joint laxity and arachnodactyly), hypertelorism and hypotonia [49]. Compared to LDS patients, ATS patients

have more generalized tortuosity of the major blood vessels. ATS clinical presentation and severity can be highly variable, ranging from early mortality during childhood to limited features at advanced age [48].

ATS is a rare syndrome that is caused by autosomal recessive loss-of function variants in the *SLC2A10* gene [20]. *SLC2A10* encodes the facilitative glucose transporter GLUT10. Abnormal transport of GLUT10 substrates hinders mature glycoprotein and proteoglycan production, leading to abnormal ECM deposition and loss of vascular wall integrity, arterial tortuosity and aneurysm development. Downregulation of decorin, a proteoglycan and TGF-β inhibitor, has been observed in VSMCs and fibroblasts of ATS patients, suggesting TGF-β involvement in the pathogenesis of ATS [20].

13.4.8 Autosomal Recessive Cutis Laxa Type 1

Cutis laxa is a connective tissue disorder characterized by inelastic and loose skin. Although autosomal recessive cutis laxa type 1 (ACRL1) is categorized as a cutis laxa subtype, this condition is mainly characterized by vascular anomalies and, less commonly, lung emphysema and diverticula of the urinary and gastrointestinal tract [50, 51]. The predominant vascular findings are aortic aneurysms as well as arterial tortuosity and stenosis [52]. ARCL1 is caused by autosomal recessive mutations in the *EFEMP2* gene [53]. *EFEMP2* codes for fibulin-4, an ECM protein that is involved in elastic fibre formation.

13.5 Non-syndromic Disorders

Familial TAAD (FTAAD) refers to non-syndromic TAAD with a positive family history. In the absence of a family history, mutations in FTAAD genes can be suspected when aneurysms are detected in young patients, or occur in the absence of other known risk factors such as hypertension or atherosclerosis. FTAAD inherits in an autosomal dominant manner and typically comes with reduced penetrance.

ACTA2 was the first described FTAAD gene. Mutations in this gene account for a remarkable 14–21% of FTAAD cases, but are associated with low penetrance (~50%). In addition to TAAD, stroke, premature coronary artery disease, AAA, bicuspid aortic valve, patent ductus arteriosus, livedo reticularis, and iris floccule are common *ACTA2*-related symptoms [54, 55]. *ACTA2* encodes aortic smooth muscle actin, a major constituent of the contractile apparatus that is involved in artery shape maintenance.

MYH11 mutations are found in less than 2% of FTAAD patients [56], most commonly in pedigrees in which both TAAD and patent ductus arteriosus, i.e. failed closure of the arterial shunt between the aorta and the pulmonary artery, coincide. Also in this case, reduced penetrance is frequently observed. Additionally, a

considerable number of *MYH11* variants of unknown significance have been identified. *MYH11* encodes smooth muscle myosin heavy chain, which is an important constituent of the thick contractile filaments in VSMCs.

Loss-of-function mutations in the short isoform (130 kDa) of *MYLK* explain less than 1% of FTAAD patients. In most mutation carriers, little or even no aortic enlargement occurs prior to dissection. Hypertension, however, has been suggested to be an important dissection-provoking factor in *MYLK* cases. *MYLK* encodes smooth muscle myosin light chain kinase (MLCK) which initiates VSMC contraction upon interaction with calcium-calmodulin complexes [57].

Only one single *PRKG1* mutation, p.Arg177Gln (where a single base mutation has changed arginine to glutamine), has been reported to cause FTAAD to date [58]. Besides aortic aneurysms and dissections at young age (15–51 years), this fully penetrant gain-of-function mutation is associated with tortuosity and hypertension. *PRKG1* encodes for a type I cGMP-dependent protein kinase, which dephosphorylates the regulatory light chains (RLCs) of VSMCs. The p.Arg177Gln mutation leads to constitutive activation of the enzyme and, hence, to a pathological decrease in RLC phosphorylation.

Mutations in *MAT2A* and *FOXE3* have also been linked to FTAAD [28, 59]. To date, only one extended family harbouring a *MAT2A* mutation has been reported [59]. TAAD penetrance is low, with only seven out of 15 mutation carriers >30 years of age being affected. Therefore, it has been suggested that TAAD development in individuals with *MAT2A* loss-of-function variants might require co-occurrence with another genetic or environmental TAAD risk factor. Since some affected mutation carriers also had a bicuspid aortic valve (BAV), and thus BAV might be considered such a provocative factor. Little is known about the precise role of the protein encoded by *MAT2A*, (methionine adenosyltransferase II), in the cardiovascular system.

FTAAD-causing *FOXE3* mutations specifically affect amino acids at the C-terminal end of the forkhead DNA-binding domain. Dominant *FOXE3* mutations either at or outside the N-terminal domain, however, cause ocular disease [60]. Remarkably, so far only male *FOXE3* mutation carriers have been found to be affected by TAA. While the role of the transcription factor *FOXE3* in the cardiovascular system has been poorly studied, some experiments point towards *FOXE3* involvement in VSMC development and differentiation.

MFAP5 haploinsufficiency (see Glossary) is another genetic cause of FTAAD [61]. *MFAP5* encodes an ECM component called microfibrillar-associated protein 5 (also known as microfibril-associated glycoprotein 2 (MAGP2)), which localizes to fibrillin-containing microfibrils and interacts also with growth factors such as TGF-β and bone morphogenetic protein (BMP). As for other FTAAD genes, penetrance is less than 50% [61]. Mild systemic features and lone paroxysmal atrial fibrillation have also been observed in some carriers but it remains to be confirmed that these are truly related to *MFAP5* deficiency.

BAV is characterized by an aortic valve with two leaflets instead of the normal three and is the most common congenital heart defect with a prevalence of 1%. In about 20% of BAV patients, dilatation of the aortic root or ascending aorta occurs

[62]. Previous studies have linked mutations in *NOTCH1* [61], *SMAD6* [62] and *ROBO4* [63] to BAV. However, the majority of BAV and BAV/TAA genetics remains obscure. 15% of the *LOX* mutation carriers also presented BAV in addition to TAA [64]. *LOX* codes for protein lysine 6 oxidase, (lysyl oxidase), an extracellular copper enzyme that initiates crosslinking of collagen and elastin [64].

Finally, mild mutations in several syndromic TAA genes (e.g. *TGFBR2*, *FBN1*, *SMAD3*, *TGFB2*) have been found in TAA patients without outward features. Therefore, the distinction between syndromic and non-syndromic causes of TAA is somewhat artificial.

13.6 Pathogenesis of Thoracic Aortic Aneurysm/Dissection

Summarizing the current knowledge on the genetic basis of TAA(D) (Table 13.1), it can be stated that TAAD is caused by defects in genes coding for proteins belonging to three overlapping and interacting functional groups, depicted in Fig. 13.1. These groups encompass the structural integrity and/or homeostasis of the ECM, the regulatory function of VSMCs in the aortic wall and the TGF-β pathway [19, 41]. Fibres of the ECM are able to connect with VSMCs in the aortic wall in order to form a matrix-cell complex and synchronize the mechanical properties of the aortic wall's components. Abnormal ECM composition due to mutations in genes encoding for structural proteins (e.g. Fibrillin-1), may lead to loss of physical VSMC-ECM interaction as well as increased MMP expression and subsequent collagen and elastin degradation. VSMCs will react on these changes by switching from a normal contractile phenotype into a pathogenic synthetic phenotype, which promotes VSMC proliferation and migration. On the other hand, mutations in the genes encoding for the contractile apparatus of the VSMCs can disrupt the contractile machinery, also favouring the synthetic VSMC phenotype and reducing vascular wall elasticity due to improper contractility and increased expression of ECM components or MMPs. The TGF-β pathway mediates the expression levels of various proteins involved in ECM homeostasis and is implicated in aortic contractile-to-synthetic VSMC phenotypic switching [41].

13.6.1 Structural Integrity of the ECM

Elastic and collagen fibres account for 60% of the aorta's bulk dry weight and are indispensable for its normal physiological compliance. Assembly of these fibres depends on the proper formation of intramolecular and intermolecular crosslinks. To date, numerous genes encoding components of the ECM (e.g. *FBN1*, *MFAP5*, *COL3A1, BGN, EFEMP2, LOX*) have been associated with TAAD development. Mutations in these genes alter normal elastin or collagen fibre formation and stability as well as crosslinking. Elastic fibres consist of microfibrils and elastin polymers

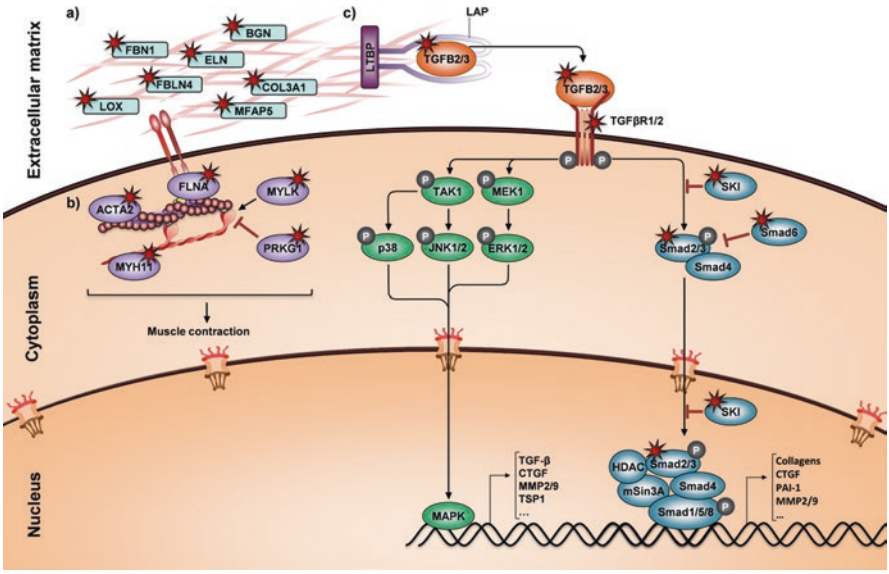

Fig. 13.1 Overview of the genetic basis of thoracic aortic aneurysm and dissection related disorders. Three interacting functional groups can be distinguished in which gene defects can lead to TAA(D)s: structural integrity of the ECM, the VSMC contractile unit and the TGF-β pathway. (**a**) Structural integrity and/or homeostasis of the extracellular matrix can be disrupted by mutations in genes such as fibrilin-1 (*FBN1*), biglycan (*BGN*), elastin (*ELN*), fibulin-4 (*FBLN4*), lysyl oxidase (*LOX*), collagens (*COL3A1, COL5A1, COL1A1*) and microfibrillar-associated protein (*MFAP5*). (**b**) Mutations in genes involved in the regulatory function of VSMCs, i.e. actin alpha-2 smooth muscle (*ACTA2*), filamin A (*FLNA*), myosin heavy chain 11 (*MYH11*), myosin light chain kinase (*MYLK*) and protein kinase cGMP-dependent 1 (*PRKG1*), reduce the capability to withstand mechanical forces during contraction. (**c**) The TGF-β signalling pathway can be divided in the non-canonical (left) and canonical (right) pathway (see Glossary). Multiple genes related to the latter are involved in aneurysm pathogenesis, including TGF-β ligands 2 and 3 (*TGFB2/3*), TGF-β receptors 1 and 2 (*TGFBR1/2*) and mothers against decapentaplegic homolog 2, 3 and 4 (*SMAD2/3/4*), in which loss of function mutations paradoxically lead to increased TGF-β signalling. In addition, SKI proto-oncogene (*SKI*) and SMAD6 inhibit the signalling pathway by preventing nuclear translocation and SMAD2/3-SMAD4 complex forming, respectively. Proteins encoded by genes known to cause TAA(D) are indicated with a red asterix. *LTBP* latent transforming growth factor β-binding protein, *LAP* latency associated peptide domain, *VSMC* vascular smooth muscle cell. (Adapted from Verstraeten et al., 2017 [4])

that give, respectively, strength and elasticity to the vascular wall. Microfibrils are formed by head-to-tail longitudinal polymerization and lateral binding of fibrillin-1 and fibrillin-2 molecules [65]. In MFS patients, mutant fibrillin-1 is erroneously incorporated into microfibrils or abnormally expressed, leading to disruption of the elastin-contractile unit. To form elastin polymers, tropoelastin (ELN) binds to fibulin-4/5 complexes associated with lysyl oxidase (LOX) and LOX-like proteins [65]. Furthermore, structural integrity loss has also been associated with *MFAP5* mutations, disrupting the adherence of smooth muscle cells to elastin fibers. In addition, MAGP2 is able to bind reversibly to multiple members of the TGF-β/BMP

signaling pathway, such as TGF-β1/2 and BMP, and thereby altering signaling intensities.

13.6.2 Regulation of the VSMC Contractile Unit

Within the aortic wall, VSMCs are embedded in the ECM and are circumferentially arranged in multiple layers in between the elastic lamina of the aortic wall. VSMCs exhibit significant plasticity in order to regulate the lumen diameter of the aorta, and thus blood pressure, by regulation of their contraction. In healthy individuals, VMSCs are mostly found to be in their contractile state but pro-inflammatory stimuli and vascular injury can direct the cells into their synthetic, non-contractile phenotype [66, 67].

The mechanism by which VSMCs develop tension and contract is called actomyosin cross-bridge cycling. Increases in intracellular calcium concentrations by Ca^{2+} entry from the extra-cellular fluid triggers calcium calmodulin-dependent contraction of the smooth muscle cells. Ca^{2+} binds calmodulin to form a complex which activates MLCK. The involvement of two enzymes, i.e. Ca^{2+} /calmodulin-dependent MLCK and myosin light chain phosphatase (MLCP) both regulate the extend of contraction. Activated MLCK is responsible for the phosphorylation of the two RLCs which, together with two essential light chains, make up the thick filaments of the contractile unit. RLCs are wrapped around each α-helical neck region of the myosin heavy chains (MHC). In the unphosphorylated state, binding of the myosin motor heads to actin is prevented by intramolecular interactions of the RLCs and the myosin motor heads. Phosphorylation of RLCs displaces the two myosin motor heads and therefore enables cyclic binding to the actin filaments with a consequential cell contraction and force development. A reduction of this force is obtained by a decrease of intracellular Ca^{2+} levels, returning MLCK to an inactive state and a MLCP dependent dephosphorylation of the RLCs [68].

Thin filaments of the VSMC contractile unit consist of polymerized α-smooth muscle actin (α-SMA), a SMC specific isoform encoded by *ACTA2*. Altogether they can make up to 40% of the total cellular protein in SMCs. Mutations in *ACTA2* are found to disrupt amino acids in all four actin subdomains, which are predicted to produce structurally-altered, unstable actin filaments, an increase in monomeric actin pool and a slower sliding of the myosin across mutant actin filaments. Therefore, the mutant actin will decrease the contractile ability of SMCs in response to pulse pressures [54, 55].

Thick filaments are part of the second major component of the VSMC contractile unit, consisting of a SMC-specific isoform of MHC dimers encoded by *MYH11*. Disease-causing *MYH11* mutations are predicted to disrupt accurate polymerization of myosin into thick filaments. The duplication of *MYH11* gene has also been shown to increase autophagy and VSMC degradation, endoplasmic reticulum stress and MHC expression. Additional disease modifiers, either genetic or environmental, can be an explanation why only a subset of the *MYH11* duplication carriers cause

heritable aortic disease. However, the exact mechanisms for this are currently still unclear [68].

Mutations in amino acids 923–1914 that lead to haploinsufficiency of MLCK, diminish the contractile reserve of VSMCs, relative to the RLC phosphorylation signaling. A bigger intracellular Ca^{2+} influx is needed to obtain the same fraction of functionally active MLCK and thus contractile force. Therefore, a modest Ca^{2+} influx leads to reduced aortic VSMC contraction [68, 69].

Relaxation of VSMCs on the other hand, is obtained by a decrease in RLC dephosphorylation which is controlled by type I cGMP-dependent protein kinase (PRKG-1). Both PKG-1α and PKG-1β are encoded by *PRKG1*, however, the PKG-1α splice variant is the major isoform present in aortic VSMCs. Nitric oxide stimulates soluble guanylyl cyclase with subsequent increase in cellular cGMP levels. Upon binding with cGMP the catalytic domain of PKG-1α is released and activated, leading to the activation of the regulatory myosin binding subunit of the phosphatase responsible for the dephosphorylation of the RLCs.

Mutations in *MAT2A* (which encodes methionine adenosyltransferase IIα (MAT2α) that catalyze the synthesis of *S*-adenosylmethionine (SAM)) [25], are predicted to decrease the amount of SAM produced and lead to aortic disease. Multiple hypotheses have been described to explain this clinical phenotype in affected individuals. One hypothesis is that the cause of the phenotype is reduction of DNA, RNA and protein methylation in VSMCs, as SAM serves as a methyl donor in methylation reactions. Hypomethylation in SMCs occurs with phenotypic modulation and proliferation and is associated with altered regulation of SMC differentiation thereby increasing the predisposition to aortic wall weakness. Another hypothesis is that decreased SAM levels can increase oxidative stress in VSMCs by reducing glutathione activity, which increases VSMC sensitivity to angiotensin II (AngII) leading to increased AngII signaling and to aortic aneurysms and dissections. A third hypothesis is a loss of intracellular cysteine pools due to limited demethylated SAM conversion into homocysteine. As a consequence, the cysteine-rich fibrillin-1 deposition into the ECM is greatly reduced with consequential weakening of aortic wall as seen in MFS patients [59].

FOXE3 deficiency caused by C-terminal mutations limits neural crest-derived VSMC proliferation, survival and differentiation during development of the ascending aorta and arch. In physiological circumstances, increased biomechanical forces on VSMCs of the ascending aorta induce *FOXE3* expression, stimulating anti-apoptotic pathways. Pathologically-decreased FoxE3 levels increase VSMC apoptosis, which is seen in medial degeneration [28].

13.6.3 TGF-β Signalling Pathway

An important secreted polypeptide with widespread contributions to vascular development is transforming growth factor-beta (TGF-β), which is essential in cell development, growth, differentiation, migration, apoptosis and production of ECM. Three

different TGF-β ligands exist in humans, TGF-β1, TGF-β2 and TGF-β3 encoded by their respective *TGFB1*, *TGFB2* and *TGFB3* genes and exert a similar biological activity. TGF-β ligands are secreted as part of a tripartite large latent complex, composed of the mature TGF-β dimer, a propeptide dimer of its processed amino terminal propeptide (latency associated peptide) and a single molecule of latent TGF-β binding protein-isoforms 1,3 or 4 (*LTBP* 1,3 or 4) [70]. This complex formation occurs intracellularly and TGF-β remains associated with the pro-peptide when secreted, to prevent premature activation of its high affinity receptor. The ECM is able to capture the large latent complex by binding with disulfide bonds of its cysteine residue to components of the ECM such as fibronectin and microfibrils composed of fibrillin-1 [65].

Upon release from the large latent complex, TGF-β becomes active and is able to bind to type II TGF-β receptor unit (TGF-βR2) which then changes its conformation and phosphorylates type I TGF-β receptor unit (TGF-βR1), also known as activin receptor-like kinase 5 (ALK5). In the canonical (see Glossary) TGF-β signaling pathway receptor-regulated mothers against decapentaplegic homolog 2 and 3 (SMAD2/3) proteins are directly activated by the kinase activity of TGF-β-induced protein through phosphorylation of a serine residue at the carboxy terminus. Once associated with SMAD4, the trimeric complex (SMAD2/3/4) translocates to the nucleus where it will partner with other transcription factors and regulate TGF-β-mediated gene transcription [4, 70]. Canonical signaling is negatively regulated by SKI or SMAD7 by preventing nuclear translocation or initiating degradation of the trimeric complex respectively [71]. Dependent on the proteins recruited, TGF-β ligand binding to its receptor can also initiate noncanonical signalling cascades, including RhoA and the mitogen-activated protein kinases (MAPK) ERK, JNK and p38 [70, 71]. These kinases phosphorylate the regions between the two functionally active SMAD domains, i.e. Mad-homology 1 and 2 (MH1/2). However, the noncanonical TGF-β pathway still remains poorly understood [71].

Insights in the involvement of the TGF-β pathway in aneurysm pathogenesis were mainly obtained by the observation that *TGFBR1/2* mutations result in LDS. Nuclear accumulation of phosphorylated SMAD2 (pSMAD2) and enhanced expression of TGF-β driven gene products such as connective tissue growth factor in aortic wall tissue of LDS patients, indicate enhanced TGF-β signaling [32]. Analogous observations were made for mutations in the more recently identified LDS genes, *TGFB2/3* and *SMAD2/3*, suggesting a presence of other compensatory mechanisms that further dysregulate the pathway [34–36]. Multiple hypotheses have been proposed to explain this paradox, for which further experimental validation is needed.

A first hypothesis involves possible downregulation of auto-inhibitory pathways of the TGF-β pathway. TGF-β binding to its receptor can activate both the canonical and non-canonical pathway. Mutations in genes of the canonical pathway, can cause an initial decrease in TGF-β signaling but will result in a reduction in feedback inhibition in order to restore canonical signalling. Ligand expression and activation is subsequently increased leading to excessive activation of non-canonical signaling cascades [1, 26]. The second possible hypothesis is a shift in TGF-β ligand use.

Similar to the cell-autonomous compensation, reduction of one TGF-β ligand due to mutations can cause pathological upregulation of the other ligands leading to an overall increase in TGF-β signaling [34].

Thirdly, paracrine overdrive between neighboring cell-types with different sensitivity to a perturbation of TGF-β signalling may explain the paradoxical overall increase in TGF-β signaling. Clinical observations demonstrate that very specific sites of the aorta have a predisposition to developing aneurysms, irrespective of haemodynamic stress. These sites correspond anatomically to regions where cells of divergent origins can interact, so-called transition regions. At the aortic root and the base of the pulmonary artery VMSCs are derived from specialized cardiogenic mesoderm, (secondary heart field, see Glossary). The ascending aortic wall is chimaera between secondary heart field VSMCs and VMSCs derived from ectodermal cardiac neural crest (CNC) with gradually more CNC-derived VSMCs in the more distal ascending aorta and aortic arch. An abrupt switch to VSMCs derived from somatic mesoderm occurs at the proximal descending thoracic aorta and to splanchnic mesoderm just below the diaphragm. Cells from different origins react differently upon TGF-β stimulation with cells of one lineage being more prone to perturbed TGF-β signaling compared to cells from another lineage. More sensitive cell types might attempt compensating for the initial loss in signalling by secreting excessive amounts of TGF-β ligand. Neighbouring cells that are intrinsically less vulnerable to heterozygous LDS mutations are then stimulated by the compensatory TGF-β ligand increment, leading to an excessive activation of the TGF-β signaling pathway [1, 72].

Fourth, there is important cross-talk to other TGF-β related pathways. Involvement of related pathways such as activin or angiotensin II signaling cascades can cause the observed enhanced pSMAD2 expression in aortic media [70]. Finally, a less well investigated hypothesis implies increased TGF-β receptor turnover. Another way of regulating signal transduction is the internalization of TGF-β receptors, which determines the amount of active receptors at the surface of the cells. A first pathway of receptor internalization is the clathrin-mediated pathway, regulated by the SMAD anchor for receptor activation protein (SARA), which leads to the recycling and increase of TGF-β receptors available at the cell surface. Alternatively, the caveolin-mediated pathway can internalize TGF-β receptors, mediated by SMAD7 interactions, resulting in proteosomal degradation of the receptor. Mutations in the TGF-β2 receptor is thought to alter interactions with either SARA (promotion) or Smad7 (inhibition) leading to an overstimulation of the clathrin-mediated pathway and an increased TGF-β signaling [73].

13.7 TAA Management

Early diagnosis of a TAA is extremely important as aortic rupture or dissection can rapidly lead to dramatic consequences such as death. Although the incidence of thoracic aneurysmal diseases appears to be increasing, this is more likely due to

better awareness and incidental diagnosis rather than an actual increase in affected individuals [74, 75]. Moreover, improved follow up, surveillance and surgical techniques have contributed significantly to reduced mortality rates.

13.7.1 Clinical Diagnosis

Aortic aneurysms usually remain unnoticed until dissection or rupture occurs. Many patients are diagnosed at routine check-up, or when a syndromic disorder is diagnosed which has a predisposition for the development of aneurysms. Vascular monitoring in these patients, in addition to medical treatment and/or surgical interventions, is very important to ensure timely intervention and to reduce complications. Currently there are several complementary non-invasive diagnostic tools for the assessment of TAA [76]. By complementing echocardiography with computed tomography (CT) or magnetic resonance imaging (MRI) for aortic imaging, diagnosis as well as accurate assessment of the location, extent and growth of the aneurysms can be determined. 6 months after initial diagnosis, repeat aortic imaging is performed to determine baseline rates of aortic growth, indicative for the risk of dissection or rupture. Thereafter, yearly imaging is generally recommended [4].

Each imaging technique has its own strength and limitations; therefore, a combined approach is recommended during long-term follow-up. Baseline transthoracic echocardiography (TTE) is an excellent imaging technique for diagnosis and follow-up of TAA's and should be performed in all patients with suspected or known aortopathy [4]. Proximal aortic segments, aortic valve morphology/function, aortic annulus, root and proximal ascending aorta have an excellent axial resolution with TTE. Visualization of the more distal aorta and, however, is limited. Improved image quality can be obtained by transesophageal echocardiography (TEE), but this is more invasive and as such is less used in routine TAA surveillance [77].

CT is most commonly used for TAA diagnosis and monitoring due to its accurate and precise imaging of the aortic root, visualization of the tortuous arteries and assessment of aortic morphology. However, reproducible and standardized methods are needed for exact location of the imaging, ECG-gating and whether the aortic wall is included in the measurement of aortic diameters. Furthermore, centre-line measurements are needed in order to not to overestimate aortic size [77].

Similar to CT, MRI is also frequently used for TAA assessment because of its excellent temporal and spatial resolution. Measurements of aortic size, aneurysm location, tissue characterization and aortic wall morphology can accurately be obtained. An additional strength of MRI is the evaluation of aortic valve function and morphology, cardiac structure and function and inflammation and oedema in the aortic wall [76]. However, this technique is less often performed due to the long imaging acquisition time, limited availability in most emergency departments, claustrophobic patients and incompatibility with metal devices such as pacemakers and some aneurysm clips [77].

Biomarkers are currently being investigated for the prediction and follow up of aortic dissections. MMP9 and TGF-β levels are, for example, increased in patients with aortic dissections. MMP9 increases within 1 h of the onset of aortic dissections, remaining elevated for the next 2 months, while TGF-β levels can be a predictor aortic rupture and expansion after dissection [77].

13.7.2 Molecular Diagnosis

Molecular confirmation when aortic disease is suspected is increasingly important for optimal patient management. For example, patients harbouring *ACTA2* mutations are at increased risk of premature stroke and coronary artery disease [55]. Similarly, patients with LDS commonly develop aneurysms beyond the aorta [78]. As such, the frequency and location of cardiovascular imaging should be planned accordingly. Genetic defects are also being increasingly taken into account when deciding on surgical intervention. Overall, surgery is recommended when aortic diameters reach 5 cm, when the aorta enlarges at an extremely rapid pace (≥5 mm per year), or when severe aortic valve insufficiency or stenosis occurs [8]. Patients with mutations in *SMAD2*, *SMAD3*, *TGFBR1*, and *TGFBR2*, however, should receive surgery earlier (that is, when the ascending aorta reaches a diameter of 4.0–4.5 cm). Given that *TGFB2* and *TGFB3* mutation carriers generally present with mild aortic phenotypes, one might expect that standard surgical thresholds would be appropriate for these patients**.** However, this remains to be validated experimentally. Surgical guidelines for patients with familial TAA are currently less well defined, largely owing to the very small number of patients with mutations in each of the identified genes. However, patients with mutations in *ACTA2*, *MYH11*, *MYLK,* or *PRKG1* are recommended to receive surgery before their aortas reach a size of about 4.5 cm because they generally undergo dissections at slightly dilated, or even normal, diameters [79]. While surgical outcomes are excellent in MFS and LDS patients, the risk of complications are much higher in vEDS patients, who thus need more careful consideration of the indications for surgery.

13.7.3 Medical Treatment

β-Blockers such as atenolol are widely used to delay the progression of TAA. These drugs lower blood pressure and heart rate, which in turn decreases aortic wall stress. Although β-blockers are considered the gold-standard treatment in the field, different clinical trials have shown variable or even conflicting outcomes since the initial reports [80, 81]. Patients who are intolerant of β-blockers might be treated with other antihypertensive agents such as calcium-channel blockers and angiotensin-converting-enzyme (ACE) inhibitors [82]. ACE inhibitors are also controversial in the field and no large randomized trials support their efficacy.

The latest therapeutic strategy for TAA was launched after the identification of dysregulated TGF-β signalling in syndromic aneurysmal disease. Given that TGF-β acts downstream of angiotensin signalling and increased expression of both angiotensin II and type 1 angiotensin II receptor were observed in MFS aortic tissue, angiotensin blockade was considered a promising therapeutic approach for MFS [83]. After the drug's efficacy was established in mice, numerous clinical studies were conducted to investigate the efficacy of losartan therapy in patients with MFS. A large study by the Paediatric Heart Network did not find significant differences between a high dose of beta-blocker and regular dose of losartan [84]. More recently, the AIMS study in the United Kingdom showed a benefit of irbesartan on top of existing beta-blocker treatment [85].

13.8 Conclusion

The understanding of the pathophysiology of hereditary aortopathies has grown exponentially over the past decades. Identification and functional characterization of disease genes pinpointed dysregulated ECM homeostasis, TGF-β signalling and VSMC contraction as key disease processes. Important knowledge gaps with respect to the molecular mechanisms underlying TAA formation remain though. Owing to the advent of next-generation sequencing we anticipate that more disease genes as well as modifier genes explaining significant intra- and interfamilial variability in TAA severity, will be discovered. This will enable further delineation of the disease pathways involved in TAA formation, identification of novel therapeutic targets as well as more personalized disease management.

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Further Reading

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Chapter 14
Pathophysiology, Classification and Principles of Management of Acute Aortic Syndromes

Mark Hamilton

Key Learning Points

- Be able to describe the underlying anatomical and pathological processes that occur in AAS and the risk factors for development of AAS.
- Be able to clearly describe the various classification systems for AAS, and demonstrate an understanding of the utility of the various systems.
- Describe the diagnostic modalities available for AAS, and their relative strengths and weaknesses
- Demonstrate an understanding of the medical management of AAS

14.1 Introduction

Acute aortic syndrome describes a number of discrete but related pathological processes in the thoracic aorta [2]. These include thoracic aortic dissection [3], penetrating aortic ulcer [4] and intra-mural haematoma (IMH) as the main interlinked variants of thoracic aortic pathology. AAS is a relatively uncommon condition overall, with incidence ranging from 2.6 to 3.5/100,000 per annum [5]. The diagnosis carries a significant morbidity and mortality risk, both in the acute and chronic phases of disease.

This chapter will outline the pathophysiology of AAS, describe the current classification systems for AAS, the role of imaging in diagnosis, and some of the current controversies in management of AAS (see Chap. 13 for further information on the genetics of AAS).

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14.2 Anatomical and Molecular Considerations in the Thoracic Aorta

There are differences at both ultrastructural and molecular levels between the thoracic and abdominal aorta. Embryologically the thoracic aorta is more complex in its development than the abdominal aorta, and vascular smooth muscle cells (VSMCs) in the thoracic aorta are predominantly derived from neuroectoderm, compared to mesoderm in the abdominal aorta [6]. This is important because of the pivotal role that VSMCs play in aortic wall strength and extracellular matrix metabolism, particularly the interaction of VSMCs, elastin and collagen molecules. There is also evidence that VSMCs undergo some degree of de-differentiation and phenotype switching in aortas that develop AAS. Our understanding of the molecular influences of these changes is evolving, with numerous cytokines being implicated in the development of aortic dissection. These include platelet derived growth factor [7], MMP2 [8], the complement cascade—particularly C3-C3a [9] and osteopontin [10].

There are also differential proportions of elastin and collagen in the two segments. In the normal course of events, elastin is a robust fibre and once produced has a half-life of 74 years [5]. Thus further production of elastin is minimal after completion of maturation/growth. Degradation of elastin in the thoracic aorta and increased levels of collagen deposition, under the influence of VSMCs, is one of the hallmark pathologic processes in AAS.

The differing embryologic origin of VSMC has implications for the way in which signaling pathways influence the activity of VSMC and their response to a number of mediators such as Transforming Growth Factor Beta 1 (TGFβ1), an important modulator of the extracellular matrix (ECM) in the thoracic aorta. Neuroectodermal VSMC growth is potentiated by TGFβ1, as is Collagen I production, leading to increasing arterial stiffness. Phenotypically, thoracic VSMCs enter a secretory phenotype under the influence of TGFβ, which also occurs with osteopontin [10] and platelet-derived growth factor (PDGF) [5]. This is in comparison to mesodermal VSMC's where TGFβ1 inhibits growth and has no influence on collagen deposition. Because VSMC's are influential in aortic strength, varying the concentration of VSMC's and subsequent differential alterations in ECM composition influence the sites of dissection or aneurysm development.

There is a variable pattern of elastic lamellar units (the functional elastic unit in the aorta, combining elastin lamellae and VSMC's) throughout the aorta, with higher levels of elastic lamella and VSMC's in the thoracic aorta than in the abdominal segment. Similarly, there is a decrease in the elastin:collagen ratio in the abdominal aorta compared to the thoracic aorta [6]. There is also an influence of haemodynamic cyclical strain on the secretion of mediators such as TGFβ1 and hence arterial wall architecture. This may relate to the influence of high blood pressure, wall tension and shear stress on the secretion of cytokines.

14.3 Haemodynamics of Thoracic Compared to Abdominal Aorta

Dissection flaps occur in the regions of the aorta subject to the greatest fluctuations in pressure over time. Due to the torsional manner in which the heart contracts and the physical effects of cardiac motion on the arch of the aorta, the areas subject to the greatest changes in pressure are the ascending aorta and the proximal descending aorta, particularly in association with increased angulation as is seen in the Type III aortic arch [11] or increased tortuosity. This was demonstrated in a model created by Qiao et al. based on a thoracic aortic aneurysm patient [12]. This model demonstrated differential shear and flow at varying points in the thoracic aorta, particularly the outer curves of the ascending and proximal descending aorta. There has been further work in computational biomechanics and fluid dynamics in aortic dissection with the aim of predicting which individual patients will develop further dissection in the future [13].

The alterations in elastic recoil ability, collagen concentrations and function in the aorta that are present in a number of genetic or inflammatory aortic pathologies, combined with the magnitude of the force involved in blood flow (related to absolute blood pressure, pulse pressure and dP/dT) results in the most likely sites of dissection being where the physical forces on the aorta are greatest and the diminution in aortic strength is maximal. VSMC apoptosis (see Glossary), which is influenced by TGFβ1 is greatest at the convexities of the ascending and descending aorta, particularly in patients with bicuspid aortic valves. This may alter aortic strength and stiffness at these sites, predisposing to dissection or aneurysm at these sites. It is likely that a combination of underlying connective tissue or genetic abnormalities, plus particular anatomical conformations of the aortic arch (e.g. the Type III arch configuration) [14] predispose to increased rates of AAS [11].

Arterial tortuosity is strongly associated with a number of aortopathies and congenital connective tissue disorders and is becoming more recognized as a risk factor for AAS. Arterial tortuosity is felt to be a marker for increased risk of developing aortic complications in both syndromic and non-syndromic aortic diseases [15]. Loeys-Deitz syndrome (LDS), Marfan Syndrome (MFS) (although less commonly reported than LDS), Cutis Laxa related to abnormality of the Fibrillin-4 gene (FBLN4/EFEMP2), arterial tortuosity syndrome, and a number of rarer syndromes all have increased rates of AAS and tortuosity [15]. A study by Shirali et al. [16] demonstrated some increased AAS risk from tortuosity, increased aortic length and volume in non-syndromal aortas. The increase in risk is less than in syndromal aortic tortuousity, where there was a strong correlation between increased aortic tortuosity index and type B dissection (although not with aortic root dilatation) [17]. In Marfan syndrome and LDS, there are efforts to classify tortuosity for risk stratification purposes.

14.4 Risk Factors for the Development of AAS

Well-recognised risk factors for non-traumatic AAS include:

1. Poorly controlled hypertension—present in >70% of cases of AAS. Surges in blood pressure such as are seen in strenuous Valsalva, or cocaine use (~1.5% of AAS) [18], are also correlated. Similarly, amphetamine use may also be implicated. There are also reports of marijuana-associated AAS, possibly from hypertension [19].
2. Genetic syndromes associated with connective tissue abnormality such as MFS, LDS, Turner Syndrome, and Ehlers-Danlos Syndrome (EDS). The incidence of genetic syndromes is approximately 5% of total cases, predominantly occurring in the younger cohort.
3. Pre-existing aortic aneurysms and atherosclerosis of the aorta with approximately 30% of patients with AAS demonstrating atherosclerosis in the aorta.
4. Vasculitides or inflammatory aortopathies such as giant cell arteritis or Takayasu arteritis.
5. Family history—the presence of non-syndromal genetic predisposition such as Familial Thoracic Aortic Aneurysm Dissection (FTAAD)—a set of genetic polymorphisms associated with increased risk of aneurysm and dissection [20] (see Chap. 13).
6. Pregnancy and childbirth in patients with underlying genetic predisposition. A Dutch study reported a cardiovascular-related maternal mortality during pregnancy or post-partum of 3/100,000 and nearly half were related to aortic dissection [21]. Maternal mortality is as high as 30% in AAS in pregnancy, with an associated 50% foetal mortality rate. Management of pregnancy in aortic syndromal patients is complex and an algorithm has been outlined by Wanga et al. [22].
7. Instrumentation or catheterization of the aorta.

14.5 Epidemiology

Based on large cohorts of data available through registries such as IRAD [23], epidemiological data around AAS demonstrates a male predilection (66%), a generally older population with a mean age of all patients of 63 years (women were on average 4 years older than males) and a peak in the range of 40–70 years. The most common underlying comorbidity is hypertension at 72%, with atherosclerosis the second most common association (40%). MFS is present in 5% of patients, predominantly in the younger age cohort (<40 years), along with other connective tissue disorders such as LDS, EDS and Turner Syndrome. Although aortic dilatation (>5.5 cm root) has been felt to be a predictor of dissection, the vast majority of dissections occur in patients with aortic diameters smaller than this [24]. Current AHA/ACS guidelines suggest repair of the ascending aorta at diameters

>5.5 cm. Diabetes on the other hand appears to be negatively associated with AAS, presumably in similar ways to its negative correlation with infrarenal aortic aneurysm [25].

14.6 Pathophysiology of Acute Aortic Syndrome

The modern understanding of AAS is based on significant advances in imaging and genetic and molecular biology that have occurred in the last two decades. While our understanding of the underlying pathology of AAS has altered, the pathognomic lesion remains the same. There is haemorrhagic incursion into the media from either a linear, partly circumferential intimal tear, intramural de-novo haemorrhage in the setting of an intramural haematoma (IMH), or a focal ulcerated lesion leading to haemorrhage through the intima (and occasionally the medial and adventitia) in penetrating aortic ulcer [4]. There is a continuum between these pathological processes, and there may be underlying molecular and genetic factors in common. Certainly, PAU and IMH often occur together.

In the setting of true thoracic aortic dissection [3], a dynamic pulsatile flow of blood into an anatomical cleavage plane leads to an extending false lumen which can be either blind or communicating with the true lumen via fenestrations. This lumen may in turn be patent, partially thrombosed or completely thrombosed. The pressure differential between lumens (and therefore flow volume through each lumen) will vary depending on a combination of these factors.

14.7 Classification Systems for AAS

Acute aortic syndromes can be classified in a number of ways, including chronicity, anatomy and on the basis of the underlying pathology and complications.

14.7.1 Temporal Classification (Acute/Chronic)

In the traditional reporting literature, acute dissections are those present for less than 14 days and chronic are those present for longer. In 2013 it was suggested by the IRAD investigators that a more useful temporal classification was to separate the disease process into four groups—hyperacute (0–24 h), acute (2–7 days), subacute (8–30 days) and chronic (>30 days). This was on the basis of an observed difference in mortality between these phases, presumably due to alterations in the plasticity of the aorta and the dissection septum during the subacute phase [26]. The implication is that this more nuanced approach to assessing chronicity may guide therapy more appropriately in various stages of the disease

process. The analysis demonstrated an ongoing decline in survival after the beginning of the traditional chronic stage, suggesting that vigilance in the sub-acute phase is necessary to improve long term survival [23]. There has also been some evidence suggesting that there are improved early outcomes with endovascular management when the dissection flap is still relatively acute and mobile, with enhanced remodelling [27].

14.7.2 Complicated Versus Uncomplicated Dissection

In the modern era of medical, surgical and endovascular management of AAS, it is necessary to risk stratify patients on the basis of the presence or absence of complications in the early phases of AAS, given the emerging evidence from multiple trials reporting that early management of complicated, or high risk, uncomplicated dissections may be beneficial in the long term.

Complications of AAS include rupture, periaortic haematoma, haemorrhagic pleural effusion, end-organ malperfusion, refractory pain, malignant hypertension despite medical therapy, or shock. Approximately 30% of patients with AAS will present with complications [28]. The presence of these clinical complications is correlated with a mortality of 17% compared to 4% for uncomplicated acute dissection. Mortality in one series was strongly correlated to the presence of a pleural haematoma. As many as 24% of patients with Type B dissection will develop a complication requiring crossover to surgical treatment within the first 14 days of presentation—the so-called sub-acute phase [29]. It is pragmatic to stratify patients based on presence of complications, and also on perceived high-risk clinical presentation, appearances and behavior of the dissection. Some of the predictors of aneurysmal degeneration included age <60 years, Caucasian race, Marfan syndrome, and high levels of fibrin degradation products (FDPs) (>20 mg/mL) on admission.

14.7.3 Penn Classification

In 2012, Augoustides et al. proposed the Penn classification system for type B dissections on the basis of the presence or absence of complications (branch vessel malperfusion and/or rupture). Uncomplicated dissections were further divided into low or high risk on the basis of adequacy of hypertension control, aortic diameter >40 mm, false lumen size and patency, intimal tear location and the presence of what was termed ulcer-like projections (presumable penetrating aortic ulceration). These features were felt to increase risk of development of aortic complications [30].

14.7.4 DeBakey Anatomical Classification

The DeBakey classification system separates classical TAD into three types, with two subtypes of Type 3 (Fig. 14.1 and Table 14.1). Initially described by De Bakey and colleagues in 1965 [31], this classification is based on both the anatomy of the entry tear and the extent of the dissection. It is an anatomical classification and has been simplified on the basis of outcome measures and prognosis into the Stanford Classification.

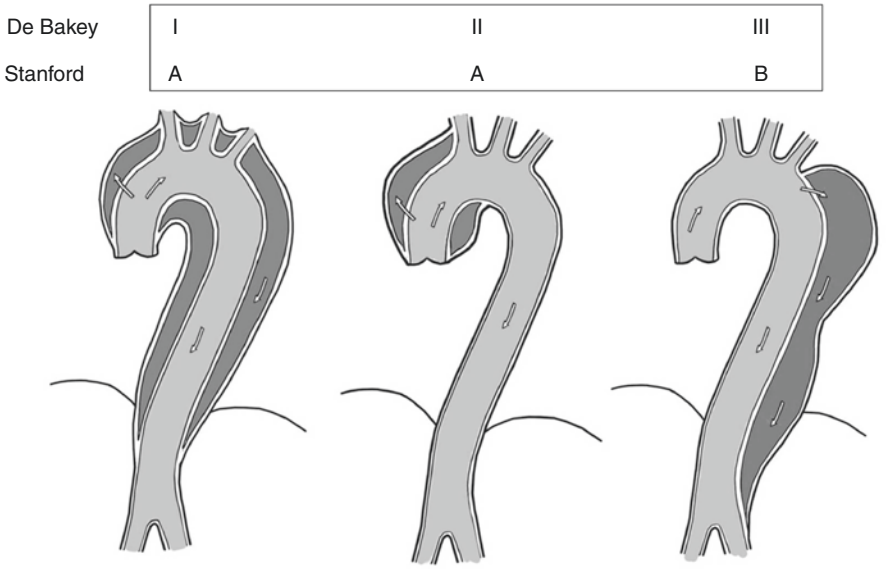

Fig. 14.1 Diagrammatic representation of aortic dissection class 1, divided into De Bakey and Stanford classifications. Based on Figure 4 from Erbel et al. 2014 [5]

Table 14.1 Relationship between Stanford and DeBakey classification of class I dissection

Stanford type	DeBakey equivalent	Site of involvement
A	Type I and II	Ascending aorta +/− Arch
B	Type III	Descending thoracic aorta distal to left subclavian artery
Subtype a		Confined to aorta above diaphragm
Subtype b		Extends through diaphragm into visceral or abdominal aorta

14.7.5 Stanford Classification

The Stanford classification arose from the recognition that prognosis was largely dependent on the involvement of the ascending aorta and was published by Daily and colleagues in 1970 [32]. The De Bakey Classification was thus simplified into two subclasses, Type A and B depending on involvement of the ascending aorta and arch (Table 14.1 and Fig. 14.1). Although the Stanford classification has allowed stratification into immediate surgical treatment or potentially conservative management groups, it fails to take into account the variations of thoracic aortic pathology that comprise AAS. In 1999 the European Task Force on aortic dissection undertook to address this with an extensive literature review, and formulation of a more complex but inclusive classification [33]. Approximately 60% of AAS are classified as Stanford Type A, independent of whether they are true TAD, or IMH/PAU. There are variations between the different pathologic processes (e.g. IMH is predominantly Type B, classical dissection is more commonly Type A).

14.7.6 European Society of Cardiology Task Force on Diagnosis and Treatment of Aortic Diseases

In 1999, Svensson et al. [34] published a new classification of thoracic aortic pathology that included not only classical TAD but also a number of recognised subtype pathologies. This classification had become possible due to advances in imaging technology which allowed visualization of intramural lesions of the aorta that were not previously possible. These lesions make up part of the continuum of aortic dissection, and may progress from one presentation to formal classical TAD. This system should be considered an adjunct to the Stanford classification. The 2001 ESC guidelines for treatment of aortic pathology were updated in 2014 to include other non-thoracic pathology such as infrarenal aortic aneurysm [5]. These guidelines are comprehensive and wide ranging. This classification is outlined below (Table 14.2 and Fig. 14.2).

14.7.7 "DISSECT" Classification

A single mnemonic based system has been introduced by Dake et al. [35] in 2013, intended to guide therapy in the context of emerging evidence for endovascular management of AAS. Although more complex than previous systems, it provides a complete framework for consideration of aspects of dissection. It includes;

1. **D**uration of dissection
2. **I**ntimal tear position
3. **S**ize of aorta

Table 14.2 Summary of aortic dissection classification systems

Stanford classification	
Type A	Dissection of the ascending with or without involvement of the descending aorta
Type B	Dissection of the descending aorta
DeBakey classification	
Type 1	Dissection of the entire aorta
Type 2	Dissection of the ascending aorta
Type 3	Dissection of the descending aorta
New classification	
Class 1	Classical aortic dissection with an intimal flap between true and false lumen
Class 2	Medial disruption with formation of intramural haematoma/haemorrhage
Class 3	Discrete/subtle dissection without haematoma, eccentric bulge at tear site
Class 4	Plaque rupture leading to aortic ulceration, penetrating aortic atherosclerotic ulcer [4] with surrounding haematoma, usually subadventitial
Class 5	Iatrogenic and traumatic dissection
Classes 1–5 represent a subdivision of the Stanford or DeBakey classification	

4. **S**egmental **E**xtent of the dissection
5. **C**linical complications of the dissection
6. **T**hrombosis of the false lumen

By codifying these characteristics, the mnemonic attempts to ensure consideration of all aspects of possible therapy.

14.7.7.1 Anatomical Descriptors of the Thoracic Aorta

The Society for Vascular Surgery reporting standards for TEVAR have outlined a segmental approach to describing aortic anatomy in the context of the site and extent of disease, as well as landing zones for endograft repair in the thoracic aorta (Fig. 14.3) [36]. These are useful in conjunction with the traditional anatomical descriptors of the aorta. Similarly, the STORAGE guidelines [4] have proposed standardised reporting and nomenclature for thoracic aortic interventions.

14.8 Individual Types of Pathology

14.8.1 Classical TAAD (Class 1 Dissection)

The pathognomonic lesion in aortic dissection is a tear in the intima and media, which allows pulsatile surging of blood into the intimo-medial plane of the aorta. Typically, the entry site is transverse but not involving the whole circumference of the aorta. The dissection plane usually extends down the left posterolateral plane of the aorta, in a spiral fashion [37]. These dissections may have communication

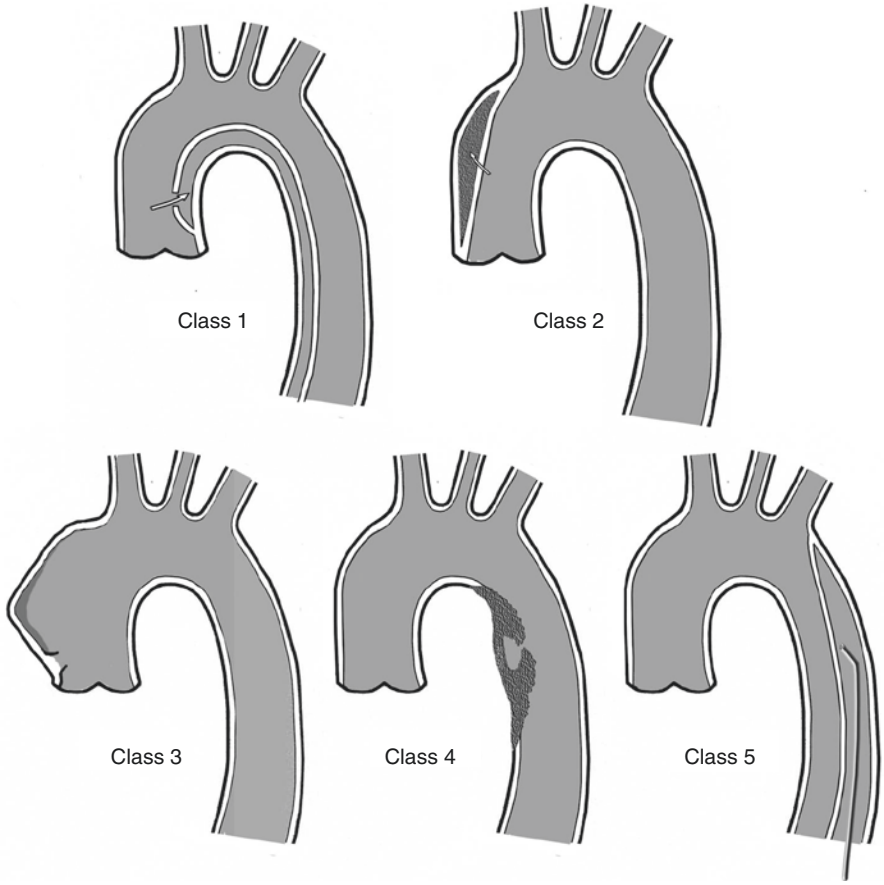

Fig. 14.2 Classes of aortic dissection. Class 1—Classical Aortic Dissection (intimal flap between true and false lumen); Class 2—Intramural haematoma (Medial disruption with formation of IMH); Class 3—Discrete/subtle dissection without haematoma and eccentric bulge at tear site; Class 4—Penetrating aortic ulcer (plaque rupture leading to aortic ulceration or a classical penetrating aortic ulcer with surrounding haematoma (usually sub-adventitial); Class 5—iatrogenic and traumatic dissection. Based on Figure 5 from Erbel at al 2014 [5] and Svensson et al. 1999 [34]

between the false and true lumen, with intimal flap tears being present in >70% of cases at autopsies [33]. The presence of fenestrations in sudden death patients, however, was seen in only 33%. This suggests an increased rate of false lumen pressurization and rupture in patients without fenestrations. Flow in the false lumen is usually antegrade but occurs retrograde in a small proportion of cases, which may lead to involvement of the ascending aorta or arch from an initial Stanford Type B dissection. Differences in the elasticity of the dissection flap and the aortic adventitia, and the increase in pressure in the false lumen (particularly in the blind ending or unfenestrated false lumen) predispose to collapse of the true lumen, with a higher frequency of true lumen compression in non-fenestrated aortic dissection.

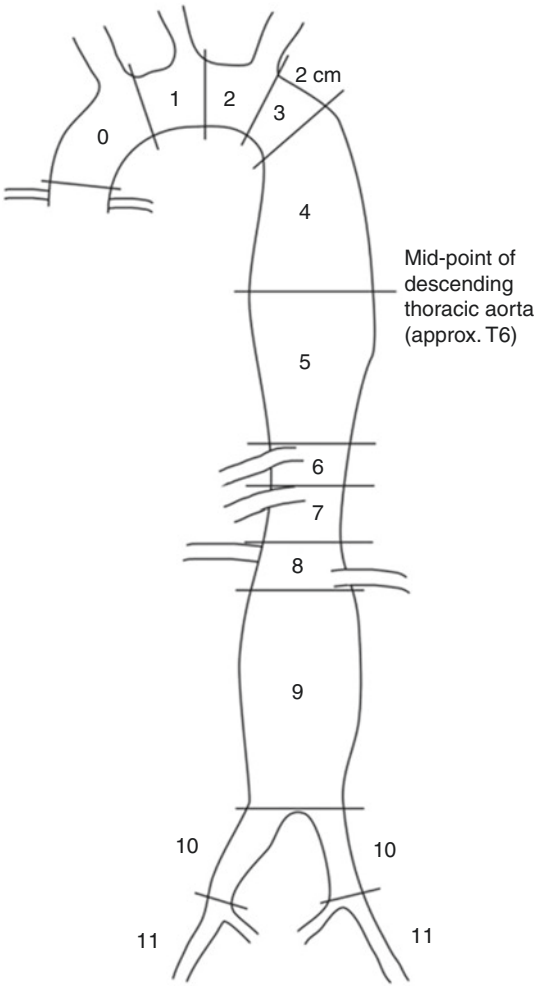

Fig. 14.3 Zones of graft attachment in the aorta and iliac arteries [36]

In 65% of cases of dissection, the intimal tears occur in the ascending aorta, 20% in the descending aorta, 10% in the arch, with 5% starting in the abdominal aorta. There is a male:female ratio of 5:1 with a peak incidence of 50–60 years for proximal dissections, and 60–70 years for distal dissection [37].

14.8.2 Intra-mural Haematoma (IMH) (Class 2 Dissection)

IMH typically occurs in patients with extensive atherosclerotic disease and makes up 5–20% of AAS cases. Several papers have reported that IMH may spontaneously regress (<10%), however a significant proportion progress to true dissection (16–47%) [38]. Occurring in greater prevalence in Asian populations, IMH makes

up 30–40% of AAS in Asian series [39]. It is defined as a circular or crescent-shaped thickening >5 mm of the aortic wall with the absence of dissecting membrane, intimal disruption or false lumen flow [5] (Fig. 14.2).

IMH is usually visualised on CT as a crescent-shaped or concentric thickening of the aortic wall, with attenuation consistent with haematoma. It may involve a longer segment of aorta than classical dissection. It appears as a "dissection without intimal tear", and was previously described as such—however it is now recognised that there may be small atherosclerotic plaque ruptures in the wall of the vessel that are related to the proximal extent of the IMH [40]. It has also been suggested that there may be focal rupture of vasa vasorum in the aortic wall which causes IMH, however with the advent of newer multi-detector CT arrays, previously invisible intimal defects are now being recognised [40]. This leads to the proposition that a ruptured vasa vasorum leads to increased focal transmural pressure and consequent "retrograde" rupture of a pre-existing aortic plaque and intimal disruption [41].

Anatomically, IMH presents as Type A (Ascending aorta or arch, in 30% and 10% of cases respectively), or Type B (distal to the subclavian, 60% of cases). Type A presentations are more common in Asian series [39]. Symptomatic Type A IMH is associated with a high mortality when managed medically (55%), and the argument can probably be made that surgical intervention is appropriate in this cohort. The answer is not so clear in asymptomatic lesions, where mortality with medical management is lower (in-hospital mortality rates of 7% and 5 year survival of 90%). Unfortunately a significant proportion (45%) of cases go on and develop complications over 5 years [42].

Two subtypes of IMH are recognised;

1. Type I showing a smooth aortic intima, with an aortic diameter <3.5 cm and a wall thickness <0.5 cm. The mean longitudinal extent of these lesions is less than 11 cm.
2. Type II lesions occur in the setting of thoracic aortic atherosclerosis and are associated with calcium deposits, rough aortic luminal margins and aortic dilatation to >3.5 cm. The aortic wall thickness is substantially greater than Type I at >1.3 cm with a range of 0.6–4 cm.

IMH seems to be associated with a lower risk of malperfusion syndromes than classical TAD, although complications are common. Between 28 and 47% of IMH progress to overt false luminal dissection. Early aneurysm formation or contained rupture develops in 20–45% of patients [43].

Predictors of progression to TAD include recurrent or persisting pain and presence of PAU. Some IMH may improve spontaneously with medical management only (particularly in Asian series). Younger ages, smaller aortic diameter (<4–4.5 cm) and thinner haematoma (<1 cm) confer better prognosis [33, 44] and may allow non operative treatment with close observation. In one series, a 30-fold increase in progression to rupture was demonstrated if the aortic diameter was greater than 40 mm, and wall thickness >1 cm was associated with a nine-fold risk of progression [45]. IMH has been shown to progress and develop new lesions in a short space of time (24–48 h) so that diligent clinical assessment and repeat imaging is required [4].

The location of the IMH is also prognostic, with IMH in the ascending aorta having a high risk of progression to frank dissection. This usually mandates repair. Exceptions to this seem to be Japanese and Korean series where there is a more benign course with Type A IMH treated with BP control, bed rest and serial imaging [46].

Recent advances in understanding of the contribution of genetic influences on matrix metalloproteinase (MMP) concentrations, elastin and collagen turnover and risk of syndromal AAS, suggest that the genotypic differences between Asian and European groups explains the differences in prognosis, progression and prevalence in IMH.

14.8.3 Penetrating Aortic Ulcer (Class 4 Aortic Dissection)

Penetrating aortic ulcer is defined as ulceration of an aortic atherosclerotic plaque penetrating through the internal elastic lamina into the aortic media. It is also classified as Class 4 Aortic Dissection [5]. PAUs are predominantly distributed in the descending thoracic aorta (62%) and abdominal aorta (31%), with only around 7% in the arch [47, 48]. Ascending aortic PAU are less common but are occasionally seen in association with a Type A IMH. It appears that for a given aortic diameter, the presence of symptomatic PAU confers a worse prognosis than for classical TAD [49].

PAU comprises around 2.3–7.6% of acute aortic syndromes. In a series of 15 patients, 40% suffered aortic rupture, compared to a rate of 7.3% for Type A dissection and <4% for Type B. PAU is consequently considered to be a morbid pathology when present [49]. Approximately 18% of PAU patients present with symptoms consistent with AAS [47] and rupture rates in symptomatic PAU are around 38% [39, 49].

A recent paper by Gabel et al. [50] identified a large number of asymptomatic PAU patients found incidentally on contrast enhanced imaging. The disease progression to symptomatic PAU, or rupture in this previously asymptomatic cohort was only 30%, with 10% undergoing late surgical intervention. They also found that early referral of asymptomatic incidentally discovered PAU for intervention led to better long-term outcomes. This aligns with another study by Nathan [47] which demonstrated a low rupture rate of 4.1% for PAU, and a long term operative intervention rate of 13%. There is radiological progression in approximately 17% of asymptomatic patients, and 43% of symptomatic patients. Thirty-six percent of symptomatic PAU in this series progressed to needing repair. This suggests that incidentally discovered, asymptomatic PAU can safely be monitored and managed medically, with diligent radiological and clinical follow up.

PAU tends to occur in patients with extensive aortic atherosclerotic disease and in an older population than those affected by Type 1 dissection (a mean age of 77 years compared to 54 Type A dissection and 67 for Type B) [49]. Other risk factors include the presence of aortic aneurysm, smoking, chronic obstructive

pulmonary disease, and coronary artery disease [4]. It appears that the pathological lesion (haemorrhage through an atherosclerotic plaque) is limited in its extent around the aorta by the transmural inflammation of extensive surrounding atherosclerosis. Penetration through and dissection towards the adventitia can occur in the setting of medial penetration of the localised plaque haemorrhage.

Both IMH and PAU are recognised to be endpoints of a degenerative aortic pathology, and largely occur in the descending thoracic aorta. In one series [51], 90% of IMH and PAU were confined to the descending aorta. Their presence in the ascending aorta however has the same implications as any other Type A dissection, with commensurately higher morbidity and mortality.

Although such focal pathology as IMH and PAU seem ideally suited to treatment by endovascular means, it is probable that most patients in these groups with pathology in the descending aorta do not require intervention, unless they fulfil criteria that would categorise them in a treatment group for classical TAD (rupture/aneurysm etc.). The presence of aneurysmal dilatation is a strong predictor of the requirement for intervention in the future. A recent interdisciplinary consensus document [52] has laid out an approach to both IMH and PAU, including imaging and treatment options.

14.9 Prognosis of AAS

Data from IRAD demonstrates approximately 25% of medically managed stable type B dissection patients will die within 3 years, with a substantial proportion (30–60%) being aortic related deaths [53]. Overall, 5 year mortality of Type B TAD is 30–40% [54]. This includes death related to complications from aortic surgery in a delayed fashion. The group most likely to suffer complications leading to death were patients over 66 years of age and those with an initial presenting aortic diameter over 40 mm [55]. False lumen diameters of >22 mm have been shown to indicate a poorer long term prognosis, as well as echocardiographic or intra-vascular ultrasound (IVUS) demonstration of entry tears >10 mm in diameter [56]. There has been a recent cohort study that also suggested that left ventricular hypertrophy may be an independent indicator of increased risk of all-cause mortality in uncomplicated type B aortic dissection (TBAD) [57].

Refractory hypertension in patients with TBAD has been shown to denote a worse long-term outcome, with mortality rates of 35.6% in poorly controlled versus 1.5% in well controlled cohort of medically managed patients [58]. This difference did not however seem to be significant in patients treated with TEVAR (3.7 versus 9.1%).

In-hospital mortality of complicated versus uncomplicated TBAD is significantly different, with complicated cases having a mortality of 50% compared to 10% of uncomplicated cases [26]. Age greater than 70 years and hypotension or shock on presentation have been shown to be significant independent predictors of mortality in TAD. As many as 40% of initially uncomplicated TAD managed medically will proceed to develop complications or aneurysm formation over time [59]. Once aortic diameter reaches 60 mm, rupture rates approximate 30% per annum [29].

STABLE-1 [60] reported acceptable 5 years mortality (comparable with that seen in IRAD) and low paraplegia rates in treatment of complicated TAD (either acute or non-acute) with TEVAR, with high rates of thoracic segment false lumen thrombosis and positive remodeling both within and beyond the stented segment. Abdominal aortic false lumen thrombosis was not as common as in the thoracic aorta, however positive remodeling was still seen in the abdominal aorta. Nonetheless thoracic aortic growth was still seen in 35% of chronic TBAD (cTBAD) cohort, and 19% of the non-acute group during post-intervention follow-up. Abdominal aortic growth was seen in 52% and 24% respectively during post intervention follow up. The authors propose that this is related to inflammatory changes occurring in the acute group. Along with many longer term TAD studies, there was a 20% dropout rate, and this leads to inherent flaws in data interpretation, however the data overall suggests that prognosis can be improved by the use of TEVAR in cTBAD in both the acute and non-acute phases out to 5 years.

It is also important to realise there is a significantly increased risk of non-aortic cardiovascular death and morbidity in the AAS cohort, with a 2.4 increased risk of fatal cardiac events, and a 3 times risk of developing a first time non-fatal cardiac event compared to non AAS matched controls [61]. This is presumably due to the baseline higher cardiovascular morbidity and disease burden of these patients.

14.10 Diagnostic Imaging

14.10.1 Computed Tomography

The rapid acquisition times, ECG gating and post-processing of high resolution CT angiography has made it the imaging modality of choice [62]. It allows excellent spatial anatomical visualisation of the aorta and planning for intervention with sensitivity and specificity approaching 100% [62]. There are also some CT features which may suggest an increased risk of later aneurysmal degeneration or complications [63], including features of the false lumen, diameter, entry tear, multiple false

Table 14.3 Comparative diagnostic ability of imaging techniques for aortic dissection

	TOE	CT	MRI	Aortography
Sensitivity	++	++	+++	++
Specificity	+++	++	+++	++
Classification	+++	++	++	+
Intimal flap	+++	–	++	+
Aortic regurgitation	+++	–	++	++
Pericardial effusion	+++	++	++	–
Branch vessel involvement	+	++	++	+++
Coronary artery involvement	++	+	+	+++

TOE Trans-oesophageal echocardiogram

lumens and visceral vessels perfused from the false lumen. The main concerns are the relatively high radiation dose, the use of iodinated contrast, and radiation risks in pregnant women. In non ECG-gated CTA there is the possibility of false positive scans due to pulsation artefact, particularly in the ascending aorta (Table 14.3) [64].

14.10.2 Trans Oesophageal Echocardiography (TOE)/Trans Thoracic Echocardiography (TTE)

There has been extensive interest in the utilisation of echocardiography for initial assessment, and ongoing surveillance of AAS [65]. TTE has the advantage of being rapid and being able to be performed at the bedside. This may have significant utility in patients who are too unstable to transfer to CT [66]. It offers excellent imaging of the ascending aorta and root via the right parasternal and suprasternal views and in well trained hands is able to view IMH and dissection. In the modern era of contrast enhanced harmonic echocardiography, sensitivity and specificity for Type A dissection is 93 and 97% respectively with TTE. It is, however, limited in its views of the descending aorta (sensitivity and specificity of 84 and 94%) [67], and TOE offers superior imaging of the ascending, descending aorta and the arch. However, it is not straightforward to use TOE in the awake patient. The sizing of the aorta is accurate and reproducible (more so than IVUS) [68], and false and true lumens may be defined and flow assessed [69]. It is also useful in the intra-operative setting, allowing modifications in procedure with greater sensitivity than aortography alone [68]. There is evidence that low flow endoleaks are more apparent on TOE than on angiography alone, and that when used along with IVUS it plays an important role in ensuring appropriate true luminal placement of wires and catheters.

There is also an ongoing role for TTE and TOE in surveillance and assessment of aortopathies such as MFS [70] and Turner Syndrome, where it can be utilised to assess size, wall compliance and elasticity, and aid in prediction of long-term risk of complications [71, 72]. Limitations of the use of TOE include some difficulty visualising the carotid and innominate origins on occasion and the inability of ultrasound to penetrate PTFE stent grafts due to the relative impermeability of this material to ultrasound waves. Also, TTE is not able to reliably image the descending thoracic aorta (although it may demonstrate an abdominal aortic dissection flap, particularly in TTE windows achieved from the abdomen).

14.10.3 Magnetic Resonance Imaging (MRI)

There is currently no role for the routine utilisation of MRI in the diagnosis or investigation of AAS in unstable or hyperacute patients [73]. There have been improvements in 4D and contrast enhanced Magnetic Resonance Angiography (MRA) which allow imaging of complex TAD [74] and may be predictive of degeneration

of aortic anatomy. Analogous to TOE, time resolved (4D) MRA can demonstrate flow in each lumen and may be useful for surveillance for this reason.

14.10.4 Intravascular Ultrasound (IVUS)

IVUS can display both true and false lumen anatomy, particularly the relationship of visceral or arch branches to the site of dissection. It has been utilised to assist in the placement of grafts in multi-channel aortic dissections [75], and can demonstrate dynamic or static malperfusion syndromes [69], along with assisting in assessment of dissection flap mobility (and hence likelihood of improved remodeling after treatment) [27]. The adjunctive intraoperative use of IVUS may reduce the procedural dose of radiation and iodinated contrast [76]. It has been shown to be able to guide stent graft size selection, particularly in the setting of AAS, where shocked patients may have significant alterations in aortic diameter with resuscitation, and hence alterations in landing zone diameter [77]. Limitations of IVUS include that eccentric placement of the IVUS catheter within the aorta limits visualisation of the distant wall of the aorta. Also, the relatively high cost of IVUS units and their consumables reduces their cost-effectiveness when compared with TOE.

14.10.5 Biochemical Markers

D-Dimer [78] has been shown to be elevated in AAS, with levels over 500 ng/mL being associated with increased severity and extent of AAS [39] and possibly may have some prognostic implications [79]. D-dimer levels <500 ng/mL can reliably rule out classical TAD within 6 h of symptom onset, however this marker cannot reliably exclude IMH or PAU, or indeed TAD where the false lumen is not fenestrated [64]. There has been some concern about the high rate of false negatives with D-Dimer (9/113) [80] in IMH and it should not be solely relied upon to exclude TAD. C-Reactive Protein (CRP) elevation is seen in TAD [56] and there is some suggestion that the peak CRP level may be predictive of increased risk of complications of TAD [81].

Interleukin-11 (IL-11), MMP-9 and Platelet-derived Growth factor (PDGF) have all been demonstrated to be upregulated in AAS [7, 82, 83], however the only biomarkers that are routinely available for diagnosis or exclusion of AAS are D-dimer and CRP.

14.11 Principles of Treatment of AAS

The basic principles of treatment for AAS remain appropriate first line medical therapy in all instances. This includes adequate analgesia, provision of beta blockers and addition of further supplemental antihypertensives. Recent data suggests that

medical therapy may not in itself be enough to reduce long term mortality, however delayed TEVAR for TAD is still safe and feasible [66]. Unfortunately best medical therapy does not always result in optimal management of blood pressure and heart rate, and refractory hypertension has been shown in the IRAD data to be associated with a worse long-term outcome [58]. Optimal blood pressure targets are still debated, but it is accepted that a target in the range of 130–140 mmHg systolic is reasonable [5]. Lower limits (<130 mmHg) have been shown to reduce 90 day aortic related adverse events in some studies [84], and in the absence of a contraindication, these lower limits are probably optimal.

Multiple agents may be considered appropriate for initial medical therapy. A reasonable approach to the initial medical management of AAS is shown in Table 14.4 [85]. Longer term medical management of AAS patients is also important, given the increased risk of non-aortic events such as myocardial infarction, stroke, heart failure and cardiac death in this cohort [61].

The traditional indications for surgical/endovascular treatment of dissection remain—Type A TAD, either classical, IMH or PAU requires surgery in otherwise fit candidates. Persistently symptomatic or ruptured TBAD, IMH or PAU require surgical/interventional treatment, as do those patients who are unable to be managed optimally with medical treatment. There is suggestion from IRAD that patients with refractory hypertension may be more appropriately dealt with by TEVAR than medical management due to lower all-cause mortality when stented versus medically managed [58].

However, it is apparent that TBAD in its different variations now requires a more tailored approach to consideration of intervention, particularly in light of recent evidence that endovascular treatment of the uncomplicated but high-risk Type B dissection may be beneficial. The INSTEAD [66], INSTEAD-XL [86] and ADSORB [87] trials have all shown to some degree either an improvement in aortic remodeling [66] or a decrease in aortic-related mortality (INSTEAD-XL, ADSORB) in the

Table 14.4 Medical therapies commonly used in aortic dissection (analgesia, heart rate control, blood pressure control) (based on (Strayer, 2017 #85)

Drug	Dose
Fentanyl	1–2 mcg/kg bolus (may need to be repeated), then 0.5–2 mcg/kg/h (titrate loading dose and infusion to pain/ sedation levels
Labetolol	10–50 mg bolus, then 0.5–4 mg/min infusion
Metoprolol	0.1 mg/kg titrated every 5–10 min (1–2 mg/min), up to three doses
Esmolol	500 mcg/kg bolus, then 50–200 mcg/kg/min (can repeat bolus if titrating up infusion)
Clonidine	75–300 mcg 3–6 h up to max 750 mcg/day (useful in very anxious patient)
Hydralazine	5–10 mg IV slow injection. Repeat after 20 min if desired BP not achieved. Infusion rate 200–300 mcg/min (e.g. useful addition to beta-blocker if further reduction in HR undesirable)
Morphine	0.1 mg/kg bolus (may need to be repeated), then 0.1 mg/kg/h
Nitroprusside	0.3 mcg/kg/min, maximum of 10 mcg/kg/min (risk of reflex tachycardia and metabolic complications with cumulative dose)

longer term compared to best medical therapy. It is now therefore reasonable to consider TEVAR for patients with asymptomatic non-ruptured but "high risk" TAD.

Decision making around these high-risk features is not entirely clear. A number of perceived high risk features are laid out in a review of current management of TAD by Alfson et al. [88]. These include features such as large entry tear, aortic diameter >40 mm, patent false lumen with partial thrombosis, false lumen diameter >22 mm (at upper descending aorta), number of intercostal arteries and visceral perfusion from the false lumen. Rapid aortic expansion or increase in size of IMH or PAU are also high-risk features.

Best medical therapy, surveillance and follow-up in congenital and genetic aortic diseases is well codified in an article by Bradley et al. [89]—this is useful for the vascular surgeon who may also be involved in the care of the paediatric patient with syndromal aortic disease.

14.12 Summary

Acute aortic syndrome is associated with high morbidity and mortality. There are a number of discrete but interlinked pathological processes that predispose individuals to AAS, and the prevalence of AAS is much higher in particular populations such as those with congenital aortic or valvular disease, and connective tissue disorders such as Marfan or Loeys-Deitz syndromes.

Early aggressive medical management, and decision-making regarding intervention based on well validated classification systems and guidelines improves outcomes, and there is emerging evidence that suggests that even stable patients with uncomplicated thoracic aortic dissection may benefit from endovascular management of their dissection to improve long term outcomes.

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Chapter 15
Biomarkers in Vascular Disease

Ashraf Cadersa and Ian M. Nordon

Key Learning Points

- A biomarker is a "characteristic that is objectively measured as an indicator of normal biological processes, pathological processes, or pharmacological responses to a therapeutic intervention".
- Biomarkers are indicators of a disease trait (risk factor or risk marker), disease state (preclinical or clinical), or disease rate (progression). They may also serve as surrogate end points used as an outcome measure to assess efficacy of therapy.
- Biomarkers found in body fluids may represent the active disease process or the patient's reaction to the disease. Disease-related biomarkers may be directly due to the disease (e.g. Disease Progression Biomarkers) or be due to biological changes caused by the host as it responds to disease (e.g. Host Response Biomarkers). Disease progression biomarkers are very specific to the disease and tend to be proteins of low abundance. Conversely, host response biomarkers are less specific to the disease itself and are generally high abundance proteins.
- Biomarkers have the potential to enhance all aspects of vascular care of AAA, carotid disease and peripheral vascular disease.
- Identification of blood-based biomarkers capable of identification and individual stratification of risk of progression and rupture would revolutionize the care of aortic aneurysm disease. A blood test for a biomarker of aneurysm expansion or aneurysm sac pressurization post-endovascular repair that could replace serial imaging would reduce the cost and morbidity attributed to graft surveillance.
- Molecular processes such as inflammation, lipid accumulation, apoptosis, thrombosis, proteolysis and angiogenesis have been shown to be highly related with carotid plaque vulnerability. Serum biomarkers reflecting these processes may

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distinguish stable from potentially unstable carotid stenosis and be a powerful discriminator in the selection of patients for carotid surgery in asymptomatic patients.

- There are two potential approaches to biomarker discovery. Firstly, there is a knowledge-based approach exploring known candidates based on our understanding of disease pathophysiology. Alternatively, an inductive approach can be undertaken, using non-hypothesis driven exploration to discover novel differences in genetic, proteomic or metabolomic expression.
- A number of methodologies can be used to discover novel biomarkers for aneurysm disease and atherosclerotic plaque stability. These include genetics, proteomics, metabolomics, bioinformatics and molecular imaging.
- Potential biomarkers for AAA presence and growth include circulating extracellular matrix markers, matrix-degrading enzymes, thrombus-related and inflammatory biomarkers.
- Possible biomarkers for carotid artery plaque behaviour include biomarkers associated with inflammation, lipid accumulation, apoptosis, thrombosis and proteolysis.

15.1 Introduction

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality in the developed world. These diseases encompass the consequences of localized atherosclerosis and aneurysmal arterial degeneration. In both disease states, there is a body of evidence demonstrating a natural life course to their development. Evolution of risk factors contributes to the onset of subclinical disease; subclinical disease progresses to overt and often catastrophic clinical sequelae. Primary and secondary prevention strategies for CVD are public health priorities.

Whilst clinical assessment and cross-sectional imaging remain the cornerstones of patient management, they have limitations. There is increasing interest in the use of novel markers of cardiovascular disease as screening and risk-assessment tools to enhance the ability to identify "vulnerable" patients. Biomarkers are one tool to aid clinical assessment and identify high risk individuals, to ensure prompt and accurate disease diagnosis and to aid prognostic scoring of individuals with disease.

15.2 What Is a Biomarker?

Initially described as a "measurable and quantifiable biological parameter that could serve as an index for health assessment", the definition of a biomarker has since been standardized. *"A characteristic that is objectively measured as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention"* [1].

Biomarkers are indicators of disease trait (risk factor or risk marker), disease state (preclinical or clinical), or disease rate (progression) [2]. They may also serve as surrogate end points used as an outcome measure to assess efficacy of therapy. A biomarker may be a recording taken from an individual (e.g. blood pressure), it may be an imaging test (CT/PET scan), or it may be a biosample (blood, serum, urine). Although each of these measurements constitutes a biomarker, the term biomarker has become synonymous with a novel protein, enzyme or cytokine with discriminatory value in clinical care.

15.3 Types of Biomarker

Biomarkers found in body fluids may represent the active disease process or the patient's reaction to the disease. A disease condition is a combination of biological changes directly due to disease (e.g. Disease Progression Biomarkers) and biological changes caused by the host as it responds to disease (e.g. Host Response Biomarkers). Disease progression biomarkers are very specific to the disease and tend to be proteins of low abundance. Conversely, host response biomarkers are less specific to the disease itself and are generally high abundance proteins (Fig. 15.1). When used in the correct clinical context, both have discriminatory value.

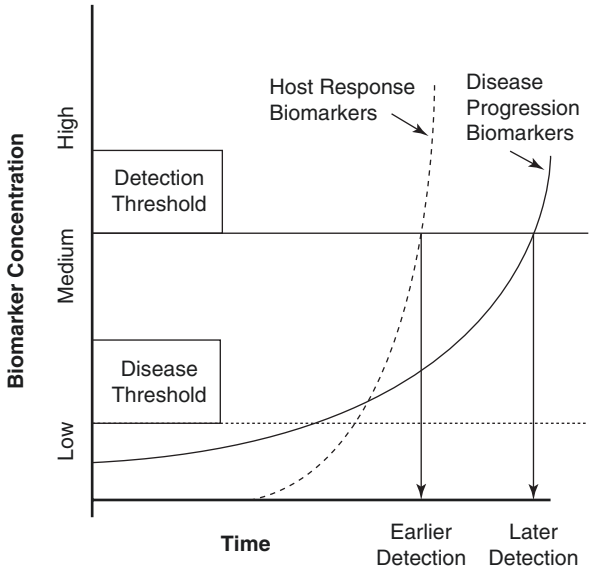

Fig. 15.1 Comparison of host response and disease progression biomarkers

15.3.1 A Classical Clinical Example

Troponin is an established clinical biomarker. The diagnosis of myocardial infarction now stands on a convincing history, electrocardiogram changes and the detection of a protein biomarker for myocardial necrosis. The biomarker is a result of the systemic spillover of structural, myocardial specific, myofilament proteins (troponins). The levels of protein, due to the time course and extent of systemic release, correlate well with myocardial injury. First discovered by Ebashi in 1963, troponin's utility as a biomarker was highlighted in 1989 when a standardized immunoassay for circulating troponin T was developed. It underwent clinical validation against the then best marker of myocardial ischaemia, CK-MB, and was found to improve the efficiency of diagnosis of myocardial cell necrosis [3]. In 2000 the American Heart association incorporated a positive troponin T rise into its definition of myocardial infarction, and it remains the gold standard for the diagnosis of cardiac ischaemia [4].

15.4 Potential Value of Biomarkers in Vascular Disease

Biomarkers have great potential to enhance all aspects of vascular care through AAA, carotid disease and peripheral vascular disease. AAA development is likely to represent a product of genetic predisposition and environment factors. They are characterized by local inflammation, matrix degradation and smooth muscle cell apoptosis [5]. Once established, AAAs grow at a rate of 2.6 mm/year (95% range −1.0–6.1 mm/year) [6]. Generally this growth is insidious and asymptomatic until rupture. During this growth phase, the active processes of AAA formation are ongoing and both local and systemic cytokines and protein levels will be modified in response to, or as a consequence of, this pathology.

The principal challenge in the management of AAAs is that they generally remain asymptomatic until rupture. At rupture, survival is poor, with mortality rates up to 70% [7]. In order to make a significant impact on the outcome of AAA, a number of significant advances are required. Improved detection of AAAs is the first step. Aneurysm screening is now established in the UK and other countries, however there remains doubt over the cost-effectiveness of these ultrasound-based programs. Currently, maximum aortic diameter alone is generally the only means of assessing AAA rupture risk. However the complications of AAA are not simply correlated to aortic diameter alone. Some small AAAs rupture and some large AAAs remain stable for prolonged periods [8, 9]. Patients continue to undergo aneurysm repair on the probability of rupture, with the inevitability that some patients will undergo unnecessary repair. An improved risk model is required. Identification of blood-based biomarkers capable of identification and individual stratification of risk of progression and rupture, would revolutionize the provision of care for AAA.

Endovascular AAA repair (EVAR) has significantly reduced the peri-operative mortality associated with elective AAA surgery. The current standard of care requires regular post-deployment surveillance to ensure that the aneurysm sac is excluded from the circulation and adequately depressurised. This surveillance is dependent on Duplex ultrasound and computed tomographic imaging. A blood test, for a biomarker of aneurysm expansion or aneurysm sac pressurization that could replace serial imaging would reduce the cost and morbidity attributed to graft surveillance.

Stroke is the third leading cause of death worldwide. Approximately 15% of strokes and transient ischaemic attacks (TIAs) are caused by unstable carotid artery plaque. Surgical treatment of a carotid artery stenosis by endarterectomy (CEA) can significantly reduce stroke risk, but is accompanied by morbidity and mortality. Equally, not all carotid plaques will become symptomatic and cause a stroke. Fundamental to the selection of patients for intervention is the identification of plaques conferring an excess risk of neurological events. Currently, selection for carotid intervention is determined by the grade of stenosis and symptomatology. It is broadly accepted to treat high-grade symptomatic carotid stenosis, but in lower grade stenoses and asymptomatic patients, interventions are still a matter of debate. There is growing evidence that the degree of stenosis alone is a poor guide for intervention. Molecular processes such as inflammation, lipid accumulation, apoptosis, thrombosis, proteolysis and angiogenesis have been shown to be highly related with plaque vulnerability. Serum biomarkers reflecting these processes may distinguish unstable from stable carotid stenosis and be a powerful discriminator in the selection of patients for carotid surgery.

15.5 Biomarker Discovery Steps

Biomarkers must be measurable, add new information and aid the clinicians' management of patients. To apply the biomarker to a risk prediction model, it must allow discrimination, calibration and risk stratification (Table 15.1). Discrimination

Table 15.1 Translating biomarker discovery from the laboratory to patients

Phase	Title	Explanation	Estimated numbers required
P1	Discovery	Exploratory studies to identify potential biomarkers	50
P2	Validation	Capacity of biomarker to discriminate between health and disease	100
P3	Pre-clinical	Capacity of biomarker to detect pre-clinical disease	200
P4	Prospective	Prospective screening studies for sensitivity of biomarker	500
P5	Impact	Large scale study to assess impact of biomarker on survival	>1000

Table 15.2 Glossary of "omics" methodologies used to discover novel biomarkers

Technology	Objective	Method	Tissue
Genetics	Gene identification	SNP genotyping Gene array analysis	Nucleated cells, diseased tissue
Proteomics	Protein or post-translational modified protein identification	2D-gel electrophoresis Mass spectrometry	Blood, saliva, tissue, urine
Metabolomics	Identification and characterization of small molecule	Mass spectrometry NMR spectroscopy	Blood, saliva, tissue, urine
Bioinformatics	Link array data to biological pathway	BLAST Hierarchical clustering	Data from combined methods
Molecular imaging	Non-invasive identification of molecular constituents of disease	CT MRI PET SPECT	Patients

SNP single nucleotide polymorphism, *NMR* nuclear magnetic resonance, *BLAST* basic local alignment search tool, *CT* computed tomography, *MRI* magnetic resonance imaging, *PET* positron emission tomography, *SPECT* single-photon emission computed tomography

is the specificity and sensitivity of the marker. Calibration denotes the ability of the marker to assign predicted risks that match actual observed risk, and risk stratification is the power to assign patients into clinically relevant categories.

There are two potential approaches to biomarker discovery. Firstly, there is a knowledge-based approach exploring known candidates based on the understanding of disease pathophysiology. Alternatively, an inductive approach can be undertaken using non-hypothesis driven exploration to discover novel differences in genetic, proteomic or metabolomic expression. The two methodologies are complementary. Dependent on the understanding of molecular biology of disease and cell signaling pathways, there is also cross-over between the "omic" sciences used to trawl for novel candidates (Table 15.2).

15.6 AAA Biomarkers

Candidate biomarkers have been studied based on current understanding of AAA pathogenesis. Examination of aneurysmal aortic wall biopsies has demonstrated pathological processes including medial arterial destruction, accumulation of inflammatory cells, elastin fragmentation, increased concentrations of proteolytic cytokines and in-situ thrombus. Consequently, investigators have explored enzyme,

protein and cytokine alterations on the basis of this understanding. The principle limitation of this approach being that all these features represent the end-stage of AAA development and may not be indicative of factors initiating AAA development or stimulating AAA growth.

The alternative "hypothesis generating" approaches have been applied to AAA biomarker discovery. Samples of body fluids and vascular tissue have been compared between AAA patients and control subjects using genomic and proteomic array techniques. These investigations have proposed novel potential circulating biomarkers of AAA. However, particularly in the proteomic studies, the studies have involved very small numbers of patients and similar number of control subjects. The challenge of finding appropriately matched controls can also reduce the value of some results with inbuilt confounding variables likely to diminish the power of any preliminary discovery. This is particularly the case when using proteomic techniques on aortic wall tissue. Harvesting aortic tissue from appropriately-aged and comorbidity-matched controls in a timely fashion is clearly difficult, and limits this methodology.

15.6.1 Possible Circulating Biomarkers of AAA (Table 15.3)

15.6.1.1 Circulating Extracellular Matrix Markers

Collagen fragmentation is typically found in AAA biopsies. This is associated with synthesis of new type I and III collagen. During collagen synthesis both the carboxyterminal and aminoterminal ends of the precursor molecule are released. These two fragments represent candidate biomarkers for increased extracellular matrix remodeling

Table 15.3 Substrates explored as possible biomarkers for AAA presence and growth

Related process	Biomarker	Proposed significance	Reference
Circulating extracellular matrix markers	Tissue carboxyterminal propeptide of type I procollagen (PICP)	Plasma PICP levels are significantly decreased in AAA vs. controls ($p < 0.01$)	Nakamura M. et al. 2000 [47]
	Aminoterminal propeptide of type III procollagen (PIIINP)	Acceleration of AAA growth is reflected in serum PIIINP ($r = 0.55$)	Satta J. et al. 1997 [48]
	Tenascin-X	AAA is associated with high serum concentrations of tenascin-X	Zweers M.C. et al. 2006 [11]
	Serum elastin peptides (SEP)	SEP levels higher in cases prone to rupture relative to controls (60% specificity) ($r = 0.40$)	Lindholt J.S. et al. 2001 [13]

(continued)

Table 15.3 (continued)

Related process	Biomarker	Proposed significance	Reference
Matrix degrading enzymes	Cystatin-C	Negative correlation with expansion rate (r = −0.24)	Lindholt J.S. et al. 2001 [49]
	MMP-9	Elevated in aneurysmal aortic walls—Correlates with expansion of small AAAs (r = 0.33)	Linholt J.S. et al. 2000 [14]
	Alpha-1 antitrypsin	Alpha-1 antitrypsin correlates with AAA growth (r = 0.55)	Vega de Ceniga et al. 2009 [17]
	P-elastase	P-elastase is positively correlated with the mean annual AAA expansion rate (r = 0.30)	Lindholt J.S. et al. 2003 [18]
Related to thrombus	Fibrinogen	Fibrinogen concentrations are significantly higher in AAA vs. controls (median: 2.89 vs. 2.53 g/L; $p < 0.01$) and correlate with AAA size (r = 0.32)	Al-Barjas H.S. et al. 2006 [19]
	D-Dimer	Annual AAA growth is positively and significantly associated with D-Dimer (r = 0.39)	Golledge J. et al. 2010 [20]
	Homocysteine (HCY)	HCY levels correlate with AAA growth rate (r = 0.28). Hyper HCY is related to rapid AAA growth.	Halazun H.J. et al. 2007 [50]
	Thrombin-antithrombin III complex (TAT)	Elevated serum TAT levels are associated with large AAA diameter (r = 0.57)	Yamazumi K. et al. 1998 [51]
Inflammation	C-reactive protein (CRP)	CRP levels elevated in large AAAs	Norman P.E. et al. 2004 [24]
	Osteopontin (OPT)	Osteopontin level correlates with AAA presence and growth (r = 0.24)	Golledge J. et al. 2007 [29]
	IL-6	IL-6 level is independently associated with AAA and correlated with index diameter (r = 0.28)	Rohde L.E. et al. 1999 [25]
	Osteoprotegerin (OPG)	Osteoprotegerin associated with AAA growth (r = 0.2)	Moran C.S. et al. [28]
	Resistin	Serum resistin concentration is independently associated aortic diameter (r = 0.19)	Golledge J. et al. 2007 [29]
	Ig-G to *C. Pneumoniae*	Aneurysm progression correlated with IgG *C. Pneumoniae* infection (r = 0.45)	Lindholt et al. 2001 [52]

and consequently AAA formation. Small case control studies using radioimmunoassay for these peptide fragments have reported associations with AAA. However, contemporary series have failed to repeat these findings in a larger cohort [10].

Tenascin-X was identified as a candidate biomarker due to its implication in Ehlers-Danlos syndrome, where patients are prone to aortic dissection and aneurysm formation. Elevated serum Tenascin-X has been demonstrated in AAA patients (n = 87) compared to controls. Notably, the highest quartile of serum tenascin-X concentrations were associated with a five-fold increase in AAA risk (OR 5.5; 95% CI, 2.0–13.8) [11].

Serum elastin peptide (SEP) is a degradation product of elastin. The role of SEP as a biomarker has been explored in two separate cohorts, the Viborg aneurysm screened cohort and the patients from the Chichester screened cohort who were unfit for surgery. Using a reproducible ELISA (enzyme linked immunosorbent assay), a clear correlation between SEP and aneurysm growth rate was reported (r = 0.4) [12]. SEP was also found to be elevated in patients with symptomatic AAAs and those who went on to rupture [13]. This study was underpowered to identify a statistically significant biomarker and has not yet been repeated.

15.6.1.2 Matrix-Degrading Enzymes

Histological examination of aneurysm wall demonstrates fragmentation of the extracellular matrix. This has implicated elastases and matrix metalloproteinases (MMPs) in the pathophysiology of AAAs. Specifically, MMP-9 is abundantly expressed in AAAs and is considered to play a pivotal role in their formation. This candidate has been explored as a possible biomarker for AAA presence in case-control studies. The majority of studies confirm an elevated circulating MMP-9 concentration in patients with AAA compared to healthy controls or subjects with occlusive atherosclerotic disease [14, 15] and pooled analysis of this data has verified this finding [16]. However the variability in the findings, sample handling and analysis highlights the principal challenges in primary validation in biomarker discovery.

Alternative elastases have been explored as serum biomarkers. Small studies (n < 50) have raised the possibility of alpha-1 antitrypsin [17] and p-elastase [18] acting as serum biomarkers for aneurysm growth. They have not been repeated in larger cohorts, nor have these findings translated into a tool for prediction of rupture risk or the need for surgery.

15.6.1.3 Proteins Associated with Thrombosis

The role of the intraluminal thrombus commonly found in AAAs is yet to be fully understood. Examination of this thrombus has identified a number of proteases that may be implicated in AAA progression. Therefore, proteins associated with thrombosis have been explored. These proteins may represent either end of the signaling pathway or be a by-product of degradation. The principal markers that have been

evaluated are fibrinogen, D-Dimer, homocysteine and protein complexes implicit in the coagulation cascade.

A positive association between plasma fibrinogen concentration and AAA diameter has been demonstrated (r = 0.323) [19]. The link between smoking and AAA is irrefutable, and raised plasma fibrinogen is induced by smoking. This association may only be a consequence of smoking and elevated fibrinogen has yet to be demonstrated independent of cigarette smoking.

D-Dimer level is a routinely used validated assay in general clinical practice to exclude a diagnosis of DVT. Plasma concentrations of D-Dimer reflect fibrin turnover in the circulation. Its role as a candidate biomarker for AAA has been explored. In a large cohort (n = 1260, 337 with AAA), average annual AAA growth was shown to be positively and significantly associated with D-dimer levels [20]. This study went on to propose possible diagnostic cut-off values for AAA presence for D-Dimer to be used as a screening tool. In their population, a level >400 ng/mL for D-Dimer had an adjusted odds ratio (OR) of 12.1 (95% CI, 7.1–20.5) and >900ng/mL represented an OR of 24.7 (95% CI, 13.7–44.6) for AAA presence. D-Dimer in combination with additional clinical risk stratification may have general value in AAA risk assessment.

Hyperhomocysteinaemia has been identified as a significant cardiovascular risk factor. These findings have evolved from studies into coronary heart disease and stroke. A review of the case-control studies found all series to report elevated homocysteine in subjects with AAA [21]. However this association was weak and failed to reflect a causal role for homocysteine in AAA development. It is likely that elevated homocysteine in AAA patients is reflective of dietary variability or renal clearance rather than the aneurysm.

Biomarkers to identify thrombosis are unlikely to translate into a universal clinical tool. The principal issue is that not all AAAs contain thrombus. Equally, in-situ thrombus is a dynamic substrate and findings from small studies may be temporal and not valid throughout the disease course.

15.6.1.4 MicroRNAs

MicroRNAs (miR) constitute a recently discovered class of non-coding RNAs that play key roles in the regulation of gene expression. At a post-transcriptional level, they act by modifying the expression of a large proportion of protein-encoding genes. miR appear integral to biological processes including cell-cycle control, immune responses and cellular apoptosis [22]. These associations have led to excitement at their possible utility as biomarkers for AAA. Significant altered miR expression has been demonstrated in patients with AAA versus controls [23].

15.6.1.5 Markers of Inflammation

C-reactive protein (CRP) is the most commonly investigated biomarker in cardiovascular disease. It is an acute phase protein implicit in inflammation specifically to

activate the complement cascade in cell death. Its elevation is inextricably linked to other inflammatory cytokines including interleukins (IL-6) and macrophage activation. CRP levels have been shown to be elevated in large aneurysms (40–54 mm), but no association with AAA expansion has been shown [24].

It has been suggested that the AAA itself is one source of IL-6. Circulating plasma levels of this inflammatory cytokine are elevated in AAA compared to controls (all small series, $n < 100$). Also, plasma IL-6 has been correlated to aortic diameter in patients without AAA [25]. IL-6 expression and initial AAA diameter has been used in a mathematical model to predict AAA growth rates. However, the model was based on debatable assumptions such as the AAA wall is taken to be a pure hyperelastic material and the parameters for the mathematical component was based on crude estimates [26]. These findings are contributory to the understanding of AAA pathophysiology, supporting the role of inflammation and of macrophages in AAA progression. However, they lack the specificity to translate into a clinical biomarker.

Other candidates explored include osteopontin (OPN), osteoprotegerin (OPG) and resistin. These have been identified based on the pathophysiology and epidemiology of AAA development. OPN and OPG are both cytokines associated with macrophage activity. Serum OPN levels show an independent but poor correlation with AAA growth ($r = 0.24$) [27]. A similar finding has been reported for OPG; in a cohort of 146 men with small AAAs followed for 3 years, serum OPG showed a significant but weak correlation with AAA growth rate ($r = 0.2$) [28]. The elevated risk of AAA associated with obesity has led to exploration of resistin as a putative biomarker. Serum resistin concentration is independently associated with AAA (OR 1.53; 95% CI, 1.32–1.76) and aortic diameter ($r = 0.19$, $P < 0.0001$) [29].

15.6.2 Biomarkers of AAA Rupture

Biomarkers capable of predicting AAA rupture would offer the greatest clinical value. Observing patients until aortic rupture is rarely performed and unethical. As the rupture of a small aneurysm is a rare event, few ultrasound-based studies have assessed the relationship between increasing biomarker levels and rupture. In the UK small aneurysm trial, an association between cotinine and subsequent AAA rupture was reported [30]. This is a marker of smoking rather than any specific pathophysiological process.

Elevated MMP-9 levels have been reported in the plasma of patients with ruptured AAA compared to an elective non-ruptured population [31]. In this cohort, a four-fold elevation in plasma MMP-9 was associated with non-survival at 30-days compared to those patients surviving surgery. Whether MMP-9 is important in the pathogenesis of rupture or simply a marker of an acute process is unclear.

Advanced imaging techniques such as $^{(18)}$F-FDG PET-CT have explored whether visualizing the inflammatory changes in an AAA can predict growth or rupture [32]. There remains a complex and unclear relationship between inflammation and AAA

progression. The SoFIA (Sodium Fluoride imaging of abdominal aortic aneurysms) study reported that ^{18}Fluorine-NaF PET-CT showed promise in the identification of disease activity in patients with AAA and could have future value as a predictor of future clinical events [33].

15.6.3 Biomarkers Following Endovascular Repair

Endovascular repair (EVAR) has become the preferred strategy for the management of AAAs in many centres. Following stent graft deployment, surveillance is required to ensure aneurysm exclusion and continued depressurization of the aneurysm sac. The role of biomarkers, to replace radiological imaging, has been explored. Decreases in MMP-3 and MMP-9 have been reported after successful EVAR with statistical differences compared to patients with active endoleak [34], whilst elevated plasma MMP-9 concentrations have been shown to correlate with endoleak at 3-months following graft implantation [35]. The principal problem with any biomarker will be its ability to discriminate between benign (type II) endoleaks and more significant (type I or type III) endoleaks.

15.7 Unstable Carotid Plaque Biomarkers (Table 15.4)

The current indication for carotid endarterectomy is Duplex derived grade of stenosis combined with clinical evaluation. There is growing awareness that in isolation this is a poor guide as to whether a patient should receive intervention. Biomarkers capable of discrimination between those carotid plaques which are either currently unstable or may become so in the future would revolutionize risk stratification in carotid surgery. Research into biomarkers for carotid plaque formation remains embryonic. The majority have come from subgroup analysis of large studies into coronary plaque risk analysis. Atherosclerosis is a multi-site disease process throughout the vasculature. Therefore any biomarker for carotid plaque instability would require optimal specificity. This has led to early studies looking at carotid plaque tissue to identify possible candidates that would be specific to carotid atherosclerosis.

Atherosclerotic plaque development results from interaction between modified lipids, extracellular matrix, macrophages and activated vascular smooth muscle cells. (VSMCs) Certain processes in the evolution of atherosclerotic lesions have been associated with plaque vulnerability. These include inflammation, lipid accumulation, apoptosis, thrombosis, angiogenesis and proteolysis [36]. These changes are connected to the morphological characteristics of an unstable plaque. The search for a biomarker has focused on these processes.

Table 15.4 Substrates explored as possible biomarkers for carotid artery stenosis progression and instability

Related process	Biomarker	Proposed significance	Reference
Inflammation	C-reactive protein (hs-CRP)	Hs-CRP associated with progressive atherosclerosis, (upper quintile OR 3.65; 95% CI 1.65–8.08)	Schillinger M. et al. 2005 [37]
	Seum amyloid A (SAA)	SAA associated with progressive atherosclerosis, (upper quintile OR 2.28; 95% CI 1.24–4.20)	Schillinger M. et al. 2005 [37]
	IL-18	IL-18 expression found to be >3× greater in symptomatic plaques than asymptomatic	Mallat Z. et al. 2001 [53]
	IL-6	Serum IL-6 elevated in symptomatic stenosis compared to asymptomatic	Koutouzis M. et al. 2009 [39]
	Neopterin	Plasma levels (nmol/L) higher in complex plaques vs. non-complex plaques (24.2 vs. 19.4; P = 0.01)	Sugioka K. et al. 2010 [54]
	CD-36	Soluble CD36 elevated in patients with echolucent plaques vs. echogenic plaques	Handberg A. et al. 2008 [55]
Lipid accumulation	Lipoprotein-associated phospho-lipase A2 (Lp-PLA2)	Symptomatic carotid plaques are characterised by elevated Lp-PLA2	Mannheim D. et al. 2008 [40]
Apoptosis	Annexin V	Annexin V uptake associated with plaque instability	Keiselaer, B.L et al. 2004 [41]
Thrombosis	Tissue plasminogen activator (t-PA)	Transient increase in t-PA gene expression associated with plaque instability	Sayed S. et al. 2009 [42]
	Fibrinogen	Elevated fibrinogen is associated with carotid disease progression	Sabeti S. et al. 2005 [43]
	Plasminogen activator inhibitor-1 (PAI-1)	Transient increase in PAI-1 gene expression associated with plaque instability	Sayed S. et al. 2009 [42]
Proteolysis	MMP-9	MMP-9 level correlates with plaque instability. MMP-9 > 607 ng/mL best predicted presence of unstable plaque (OR 19.2; 95% CI 3.9–94.2)	Alvarez B. et al. 2004 [45]

15.7.1 Inflammation

Inflammation in the vessel wall is considered to play an essential role in the initiation, progression and the final steps of atherosclerosis, namely plaque destabilization and eventual plaque rupture. CRP may have direct pro-inflammatory effects and contribute to the initiation and progression of atherosclerotic lesions. In carotid

artery stenosis, hs-CRP correlates with the morphological features of rapidly progressive carotid atherosclerosis [37]. CRP has also been shown to predict stroke risk in a healthy elderly population (Framingham Study) [38]. Men in the highest quartile of CRP had double the risk of ischaemic stroke (RR 2.0; P = 0.03), and women had almost 3 times increased risk (RR 2.7; P = 0.0003) compared to the lowest quartile.

Serum amyloid A (SAA) is another acute phase protein. It is elevated in atherosclerotic lesions and has previously been shown to be a biomarker capable of predicting poor outcome in acute coronary syndromes. Serum SAA is associated with progressive carotid atherosclerosis, (upper quintile OR 2.28; 95% CI 1.24–4.20). The pro-inflammatory cytokine IL-6 has pro-atherogenic properties. Histology has demonstrated increased expression of IL-6 in unstable plaque regions. Elevated serum baseline IL-6 levels are associated with a greater stroke risk [39].

15.7.2 Lipid Accumulation

In atherosclerotic plaques, unstable lesions have a greater area occupied by lipid. Systemic lipid lowering in patients with cardiovascular risk using statins has shown a 25% proportional reduction in first event rate for stroke. OxLDL levels have been shown to be related to carotid plaque instability. One link between oxLDL and plaque instability is lipoprotein-associated phospholipase A2 (Lp-PLA2). In carotid artery disease, symptomatic carotid artery plaques express higher levels of LP-PLA2 than asymptomatic plaques [40]. No serum studies have been performed on this possible biomarker.

15.7.3 Apoptosis

The necrotic core at the centre of advanced atherosclerotic plaques contains dead VSMCs and debris. Smooth muscle cells and inflammatory cells die as a consequence of programmed cell death (apoptosis). VSMC apoptosis may weaken the fibrous cap creating an unstable plaque prone to rupture. Apoptotic markers have been explored to identify vulnerable plaques. Annexin V, a marker of apoptosis, has been detected in symptomatic carotid artery plaques. A pilot study utilized exogenous radiolabelling and only examined four patients. The investigation did indicate that molecular imaging with the use of technetium-99m–labeled annexin A5 may be a new method for assessing plaque instability and identifying patients at risk for acute vascular events [41].

15.7.4 Thrombosis

Thrombotic activity in carotid plaques is associated with stroke and transient ischaemic attacks (TIA). Examination of RNA from carotid plaques removed at endarterectomy has shown that expression of thrombomodulatory genes is increased in unstable plaques [42]. These include t-PA and plasminogen activator inhibitor-1. To date no study has examined the possible role of these factors as biomarkers.

Fibrinogen is a key factor in the coagulation cascade, exhibiting proinflammatory properties, and is suggested to play a pivotal role in atherogenesis. Plasma fibrinogen levels have been shown to be related to progressive atherosclerosis. In a cohort of 1268 asymptomatic patients, progressive atherosclerosis was seen in 9.2%. The adjusted hazard ratio for atherosclerosis progression was 2.45 (P = 0.002) for the upper quartile fibrinogen level compared to the lower quartile. Fibrinogen level at follow up was also shown to be associated with progressive disease (P = 0.004) [43]. Elevated fibrinogen, reflecting the level of inflammatory activity, is associated with progression of carotid atherosclerosis.

15.7.5 Proteolysis

Plaque destabilization is associated with proteolysis. Proteolytic enzymes including matrix metalloproteinases appear important in the pathophysiology of atherosclerotic plaque cap rupture and consequent neurological events. It is likely that an imbalance in MMPs may lead to matrix degradation and plaque destabilization. In unstable carotid plaques there is a local increase in active MMP-9 concentration [44]. Elevation of MMP-9 has been shown in the serum of patients with symptomatic carotid artery disease in a small cohort of 40 patients undergoing carotid endarterectomy [45].

15.7.6 Immunity

Neutrophil gelatinase-associated lipocalin [NGAL] is a biomarker that is expressed in atherosclerotic lesions. NGAL is a protein involved in innate immunity. It is expressed in neutrophils and has historically been implicated in acute kidney injury, the pathogenesis of cardiovascular pathology and more recently, plaque vulnerability in carotid atherosclerosis. Elevated serum NGAL and MMP-9/NGAL complexes are associated with plaque vulnerability in patients with carotid artery stenosis [46].

15.8 Challenges in Biomarker Discovery

A cautionary tale of biomarker exploration is described in the field of ovarian malignancy. Proteomic exploration was adopted early and with great enthusiasm in this field of cancer. Despite early reports citing proteins with 100% sensitivity, 95% specificity and a positive predictive value of 94% in a small cohort; these findings have failed to translate into a clinically applicable tool. The initial proteomic fervour was tempered and despite greater than 10 years exploration, clinicians remain reliant on an older protein biomarker, CA-125.

Many candidate biomarkers, based on current understanding of vascular pathophysiology have been explored. None have translated into clinical practice. It is therefore the task of the discovery sciences i.e. proteomics and metabolomics, to further this endeavor. Biomarkers continue to represent one of the most anticipated healthcare concepts. Yet before this potential can be fully realised, numerous challenges need to be resolved. It is unlikely that single biomarkers will be considered adequate for most applications. Multiple protein panels are the new paradigm. Because of variations in sample complexity, the approach to biomarker discovery will continue to be highly dependent on the intended application, each with its own discovery challenges. Body fluids are especially difficult to handle consistently. Serum is vulnerable to temperature and fasting state whilst variations in its protein content are difficult to identify as it is >90% albumin. Plasma is modified by the clotting cascade and haemoglobin breakdown, and urinary protein excretion is principally a product of renal filtration. High throughput consistent sample handling is essential if these biomarker panels are to be elucidated.

15.9 Future Work

The future of biomarker discovery lies in comparative proteomics combined with innovative bioinformatics and mathematical modeling. This chapter has demonstrated a large number of small independent scientific groups generating exciting and unique findings. The principal limitation consistent across the literature is a failure to develop these discoveries through validation in larger mixed populations. Different substrates (blood, plasma, serum) are being explored in different conditions (Snap frozen, embedded, fresh), using varied assays, dependent upon the expertise of the scientific group. Large co-operatives tasked with biomarker discovery with defined consistent protocols across mixed populations offer the most appropriate environment for biomarker discovery.

In recent years there has been enthusiasm about the potential of “big data” and machine learning-based solutions in the hunt for biomarkers and personalized medicine. Industries such as Google DeepMind™ are investing in integrating multiple types of data at a population level to understand further human health and disease. Application of artificial neural networks which allow computers to learn from

observational data is probably the best hope in the hunt for biomarker patterns that can truly influence disease management.

15.10 Conclusion

Biomarkers are likely to have increased utility in the future of vascular surgery. To date no biomarker for AAA or carotid stenosis has been translated into clinical practice. However, with advances in mass spectrometry and proteomic techniques combined with worldwide interest in this discovery science, a significant discovery is likely to not be far away. In the future, the decision to operate on a dilated aorta or carotid stenosis may be guided by the presence of a specific protein in the patient's serum, and no longer simply the morphology of the lesion.

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Chapter 16
Pathophysiology and Principles of Management of Vasculitis and Fibromuscular Dysplasia

Maureen Rischmueller, Sarah Downie-Doyle, and Robert Fitridge

Key Learning Points

- Early recognition and treatment of vasculitis leads to reduced morbidity and mortality.
- Severity and prognosis of vasculitic conditions are dictated by the pathology, size, and distribution of affected blood vessels.
- Giant cell arteritis is characterised by a biphasic inflammatory process, with the glucocorticoid-responsive Th17 (IL-6/IL-17)-mediated pathway driving systemic inflammation in early disease, and poorly glucocorticoid-responsive Th1 (IL-12/IFNγ)-mediated mechanisms promoting chronicity of inflammation in blood vessel walls.
- Aortitis and retroperitoneal fibrosis are characteristic features of IgG4-related disease.

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- Cancer immunotherapy targeting immune checkpoints is a newly recognised cause of vasculitis.
- Fibromuscular disease is a non-atherosclerotic arterial disease which predominantly affects women aged from 45 to 55 at time of diagnosis. FMD may be asymptomatic or cause thrombosis, aneurysm development and/or dissection.
- Fibromuscular disease most frequently affects the renal and cerebrovascular arteries. FMD lesions should be classified according to appearance on angiography as focal or multifocal FMD.

16.1 Introduction

Vasculitis covers a broad array of clinicopathological diseases, from transient localised cutaneous reactions, to clinical manifestations of systemic diseases, to fulminant life-threatening diseases dominated by widespread inflammation, occlusion and rupture of blood vessels. There is great diversity in pathophysiology, clinical presentation, diagnosis and management. Cutaneous lesions such as petechiae, livedo reticularis, purpura, nodules and ulcers raise the suspicion of vasculitis, which also commonly affects organs and tissues including lungs, kidneys, peripheral nerves, muscles, and the gastrointestinal tract. Patients are often systemically unwell with malaise, fever, weight loss, arthralgia, normochromic normocytic anaemia and have raised inflammatory markers. History is important and should include the onset and evolution of symptoms, systems review, preceding infections, exposure to drugs and toxins, and relevant family history. Full blood count, electrolytes, renal and hepatic function, C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and, where relevant, viral titres, autoantibodies, and cryoglobulins should be measured. Urinalysis is important to identify renal involvement, typified by red cells, casts, or proteinuria, as prompt treatment may avert irreversible renal failure. Biopsy remains the gold standard for histopathological confirmation of vasculitis, and in addition to formalin fixation, a fresh sample should be provided for immunofluorescent labelling of immune complexes and complement if small vessel vasculitis is suspected. Imaging is paramount when a biopsy is unobtainable and, in large vessel vasculitis, is useful to determine the extent of disease.

Comorbidities are common, including treatment-related toxicities such as corticosteroid-induced obesity, mood disorders, skin fragility, bruising, osteoporotic fractures, peptic ulcer disease, diabetes, dyslipidaemia, hypertension, accelerated atherosclerosis, cataracts, glaucoma, and immunosuppression predisposing individuals to common and opportunistic infections. Vaccination against influenza, pneumococcus, and pertussis should be up to date, and prophylaxis against

pneumocystis jiroveci pneumonia should be prescribed for patients taking high doses of glucocorticoids. It should be noted that vaccination with live vaccines such as Zostavax (attenuated varicella-zoster virus vaccine) are contraindicated in the setting of immunosuppression because of the risk of disseminated viral disease. Baseline assessment of bone mineral density, and treatment to prevent or manage steroid-induced osteoporosis is standard of care. Small and large vessel vasculitis is associated with an increased risk of contemporaneous malignancy, particularly in elderly patients, and clinical examination supplemented by relevant investigations should be undertaken.

The aetiology of most vasculitides is unknown, with management directed towards the prevention and treatment of organ damage, maintenance of a low inflammatory state, titration of therapy according to clinical, serologic and imaging parameters, management of comorbidities, and prevention and management of treatment-related side effects. This chapter will outline the main types of vasculitis encountered in clinical practice, describe known pathogenetic mechanisms, and present management strategies. In addition to vasculitis, Raynaud's phenomenon, thromboangiitis obliterans and fibromuscular dysplasia will be discussed.

16.2 Primary Versus Secondary Vasculitis

Vasculitis is caused by immune-mediated inflammation of blood vessels. In most cases the underlying cause of this reaction is unknown, and the term "primary" is often applied, whereas in a subset of cases the vasculitis is triggered by a known drug or virus or is a manifestation of an underlying systemic disease and is thus termed "secondary". Categorization into primary *versus* secondary vasculitis becomes problematic, however, as more aetiologies are discovered. Vasculitis has more recently been divided according to the size and type of blood vessel involved, which has merit given that similar organs and tissues are affected. The discovery of anti-neutrophil cytoplasmic antibodies (ANCAs) associated with a subset of patients with small vessel vasculitis, causative viruses and gene mutations in a subset of patients previously diagnosed with polyarteritis nodosa, and the evolution of modern imaging techniques such as magnetic resonance imaging/angiography (MRI/MRA) and positron emission tomography (PET), has led to further subclassification of vasculitis. Table 16.1 shows the names for vasculitides adopted by the 2012 International Chapel Hill Consensus Conference of the nomenclature of vasculitides [1]. Technology-led advances in our understanding of mechanisms of disease initiation and progression, as well as insights emerging from empiric trials of targeted biologic therapies, will continue to inform this area.

Table 16.1 Names for vasculitides adopted by the 2012 International Chapel Hill Consensus Conference on the Nomenclature of Vasculitides [1]

Large vessel vasculitis (LVV)
Takayasu arteritis (TAK)
Giant cell arteritis (GCA)
Medium vessel vasculitis (MVV)
Polyarteritis nodosa (PAN)
Kawasaki disease (KD)
Small vessel vasculitis (SVV)
Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV)
Microscopic polyangiitis (MPA)
Granulomatosis with polyangiitis (Wegener's) (GPA)
Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA)
Immune complex SVV
Anti-glomerular basement membrane (anti-GBM) disease
Cryoglobulinemic vasculitis (CV)
IgA vasculitis (Henoch-Schönlein) (IgAV)
Hypocomplementemic urticarial vasculitis (HUV) (anti-C1q vasculitis)
Variable vessel vasculitis (VVV)
Behcet's disease (BD)
Cogan's syndrome (CS)
Single-organ vasculitis (SOV)
Cutaneous leukocytoclastic angiitis
Cutaneous arteritis
Primary central nervous system vasculitis
Isolated aortitis
Others
Vasculitis associated with systemic disease
Lupus vasculitis
Rheumatoid vasculitis
Sarcoid vasculitis
Others
Vasculitis associated with probable aetiology
Hepatitis C virus-associated cryoglobulinemic vasculitis
Hepatitis B virus-associated vasculitis
Syphilis-associated aortitis
Drug-associated immune complex vasculitis
Drug-associated ANCA-associated vasculitis
Cancer-associated vasculitis
Others

16.3 Large Vessel Vasculitis

16.3.1 Giant Cell Arteritis (Temporal Arteritis)

Giant cell arteritis (GCA) is a granulomatous vasculitis principally involving medium- and large-calibre branches of the aorta, which left untreated, can lead to permanent blindness. It is the commonest form of vasculitis, occurring in patients over the age of 50, and mainly affects people of northern European and Scandinavian descent. There is a female preponderance and a worldwide incidence between 1 and 30 per 100,000. The strongest genetic association is with human leucocyte antigen class II gene loci DRB1∗04, DQA1∗03 and DQB1∗03 [2].

GCA is a T cell driven disease characterised by the formation of vessel wall granulomas, intimal hyperplasia and end-organ ischaemia. T helper cell 1 (Th1) and Th17 pathways and innate immunity appear to be central in its pathogenesis. Activation and maturation of immature vascular dendritic cells (vasDCs) within the normally immunoprivileged arterial wall leads to recruitment and activation of local innate immune cells such as monocytes and fibroblasts, as well as naive CD4 T cells. Adventitial *vasa vasora* critically control vessel wall access and drive differentiation of tissue-invasive T cells, which establish tissue residency within autonomous inflammatory lesions [3]. Antigens have been suspected to drive the local activation of vasculitogenic CD4 T cells, but recent data suggest a more generalized defect in their threshold setting. In health, immune checkpoints provide a physiological brake on T cell activation to curb inflammation-associated tissue destruction. This mechanism has been shown to be disrupted in GCA, as vasDCs fail to express the immunoinhibitory programmed cell death ligand-1 (PD-L1), leaving lesional T cells unchecked. Consequently, programmed cell death protein-1 (PD-1)-positive CD4 T cells can enter the vessel wall, where they produce a broad spectrum of inflammatory cytokines including interferon-gamma (IFN-γ), interleukin-17 (IL-17) and IL-21, and have a direct role in driving intimal hyperplasia and intramural neoangiogenesis. The deficiency of the PD-1 immune checkpoint in GCA, promoting unopposed T cell immunity, contrasts with checkpoint hyperactivity in cancer patients in whom excessive PD-L1 expression paralyses the function of antitumor T cells [4].

Diverse macrophage subsets, secreting matrix metalloproteinases which degrade the internal elastic lamina, and smooth muscle cells within the media, promote the migration and proliferation of myofibroblasts into and within the intima, ultimately inducing wall capillarisation and intimal hyperplasia, leading to luminal compromise [3]. Macrophages release IL-6 and IL-1β, potent cytokines required for differentiation of Th17 effector cells; levels of circulating IL-6 correlate with the severity of the acute inflammatory response in GCA, and fluctuate in line with disease activity. Th1 cells differentiate in the presence of IL-12, thought to be produced by activated vasDCs, and these effector T cells are responsible for the secretion of IFN-γ, a potent activator of macrophages and heavily implicated in promoting

mural infiltration, as well as giant cell and granuloma formation. In contrast to IL-6 and IL-17, which appear to wax and wane with disease activity and are highly responsive to glucocorticoids, elevated levels of IL-12 and IFN-γ persist within the serum of patients and within temporal artery samples despite months of glucocorticoid treatment, and higher levels of these cytokines correlate with ischaemic complications.

16.3.1.1 Biphasic Inflammatory Response in GCA

It has been proposed that there is a biphasic inflammatory process, where the Th17 (IL-6/IL-17)-mediated pathway, which is glucocorticoid-responsive, drives systemic inflammation in early GCA, while Th1 (IL-12/IFN-γ)-mediated mechanisms promote chronicity and are poorly steroid responsive. This might explain the disconnect between early control of systemic inflammation and suboptimal numbers of patients achieving long-term remission, with late development of aortic aneurysms [2]. A recent study of patients who had serial temporal artery biopsies (TAB) before and 3–12 months after initiation of therapy revealed that vascular inflammation persisted in most patients, despite normalisation of CRP and ESR [3].

GCA may occur *de novo*, or in patients with known polymyalgia rheumatica (PMR), a related inflammatory condition characterised by pain and stiffness of the shoulder and hip girdles. The most common presenting features of GCA are of occlusive cranial arteritis, such as temporal headache, jaw claudication, facial pain, amaurosis fugax, diplopia, cerebrovascular accident (particularly of the vertebral circulation) and unheralded unilateral or bilateral blindness, which occurs in up to 20% of patients. Limb claudication due to large vessel vasculitis in the absence of cranial arteritis may be the presenting feature of GCA, and some patients present with generalised lethargy, malaise, unexplained weight loss, or pyrexia of unknown origin. Physical signs may be minimal, but include scalp tenderness, nodularity/decreased pulsation of the temporal artery, and in patients with visual symptoms, fundoscopic changes of anterior ischaemic optic neuropathy, retinal arterial occlusions or choroidal infarction. Presenting features with the highest positive predictive value for GCA include jaw claudication and/or scalp tenderness [2]. Depending on disease duration, normocytic anaemia, thrombocytosis and/or leukocytosis may be evident, and significant elevation of the ESR and/or CRP is characteristic, although occasionally absent. The American College of Rheumatology classification criteria for GCA are shown in Table 16.2 [5].

GCA is considered a medical emergency because of the high risk of irreversible blindness or stroke if treatment is delayed. In patients sustaining monocular blindness, there is a 50% risk of visual loss in the contralateral eye within 2 weeks.

Histopathology of a temporal artery biopsy (TAB) is the gold standard for diagnosis, but treatment should not be delayed, as pre-TAB glucocorticoid exposure does not affect the yield for up to 6 weeks and beyond [6]. The sensitivity of TAB for the diagnosis of GCA varies between 39 and 91% because of skip lesions, and

Table 16.2 1990 criteria for the classification of giant cell (temporal) arteritis[a] [5]

1. Age at disease onset ≥50 years	Development of symptoms or findings beginning at age 50 or older
2. New headache	New onset of or new type of localized pain in the head
3. Temporal artery abnormality	Temporal artery tenderness to palpation or decreased pulsation, unrelated to arteriosclerosis of cervical arteries
4. Elevated erythrocyte sedimentation rate	Erythrocyte sedimentation rate ≥50 mm/h by the Westergren method
5. Abnormal artery biopsy	Biopsy specimen with artery showing vasculitis characterized by a predominance of mononuclear cell infiltration or granulomatous inflammation, usually with multinucleated giant cells

[a]For purposes of classification, a patient shall be said to have giant cell (temporal) arteritis if at least 3 of these 5 criteria are present. The presence of any 3 or more criteria yields a sensitivity of 93.5% and a specificity of 91.2%

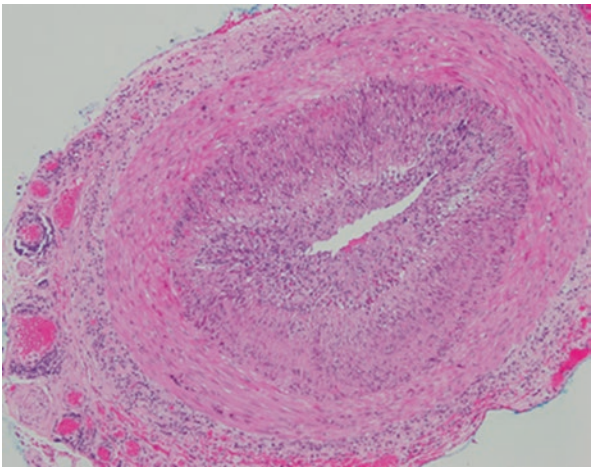

Fig. 16.1 Giant cell arteritis. Histological section taken from temporal artery biopsy shows marked intimal thickening with luminal stenosis associated with dense infiltrate of lymphocytes, histiocytes, and eosinophils, extending to the internal elastic lamina of the media. Only very occasional giant cells are seen

the yield can be improved by performing initial bilateral TAB or achieving a TAB post-fixation length of at least 3 cm [6].

Histologically, the inflammatory infiltrate in GCA is predominantly composed of histiocytes/macrophages and CD4+ lymphocytes, with variable involvement of the three arterial layers, often showing only segmental inflammation in parts of the arterial wall (Fig. 16.1). The lymphocyte population usually includes lesser numbers of CD8+ cytotoxic and CD20+ B cells. Focal fusion of histiocytes and formation of multinucleate giant cells are seen, but these are not required for the diagnosis. The inflammation typically results in breaks, segmental loss and reduplication of the elastic lamina, best seen on elastin stain. This may lead to critical luminal narrowing and aneurysm formation. Following the active phase inflammatory cell infiltrates may be absent but intimal-medial scarring and injury to the elastic lamina often remains [2].

The European League Against Rheumatism (EULAR) taskforce for imaging in large vessel vasculitis guideline recommends temporal artery ultrasound as the

first-line imaging modality for predominantly cranial GCA in centres where experience is at hand, as the test is non-invasive, immediate and cost effective, and in some centres, is replacing the need for TAB [7]. The temporal artery ultrasound finding of a non-compressible halo sign, caused by oedema of the vessel wall, has a reported sensitivity between 77 and 79% and specificity between 96 and 100% for the diagnosis of GCA. This investigation is dependent on training and expertise of the sonographer, however, and the ability to detect a halo sign declines rapidly after the initiation of glucocorticoids, with sensitivity falling to below 50% at 4 days. For confirmation of large vessel GCA not involving cranial vessels, other imaging techniques are required, which are also sensitive to glucocorticoid therapy. High-resolution MRI has the advantages of the absence of radiation, the contemporaneous detection of structural lesions (such as vessel wall thickening and luminal stenosis/occlusion), and contrast enhancement of the arterial wall presumed (but not proven) to reflect active inflammation [7]. Compared with TAB, ^{18}F-fluorodeoxyglucose PET combined with low dose fine cut computed tomography (18F-FDG-PET/CT) performed within 72 h of commencement of glucocorticoids had a sensitivity of 92%, specificity of 85%, negative predictive value of 98%, and identified patients with aortitis, who require closer follow up for late complications such as aneurysm development or aortic regurgitation. Cranial arteritis affects temporal, vertebral, maxillary and occipital arteries, with vasculitis mimics including malignancies found in 20% of patients [8].

Treatment of GCA consists of high dose glucocorticoids, usually 40–60 mg prednisolone daily or 1 mg/kg body weight, augmented by immediate intravenous pulses of high dose methylprednisolone in the presence of ocular involvement. Blindness is almost always irreversible, but is a rare occurrence after 2 weeks of high dose glucocorticoids. Glucocorticoids are slowly tapered following normalisation of symptoms, CRP and ESR, usually commencing at around 4–6 weeks, with ongoing monitoring of symptoms and inflammatory markers. Treatment with glucocorticoids typically continues between 18 and 24 months, with many requiring long-term therapy. When studied in the context of clinical trials, only 15–20% of patients achieve long-term remission, highlighting the need for alternative therapies.

Traditional disease modifying antirheumatic drugs (DMARDs) are not effective in GCA; treatment with methotrexate (MTX) leads to a significant, though modestly reduced risk of relapse and lower cumulative glucocorticoid doses compared with placebo, however does not reduce the rate of drug-related adverse events. Leflunomide (LEF), azathioprine, cyclophosphamide (CYC), mycophenolate mofetil (MMF), and cyclosporin A (CyA) have not shown efficacy. Low dose aspirin is recommended to prevent thrombotic complications, but clinical evidence is lacking [9]. One year of treatment with tocilizumab (TCZ), a humanised monoclonal antibody targeting the IL-6 receptor (IL-6R) was shown in clinical trials to be significantly more likely to lead to sustained remission of GCA than placebo, at just over half the cumulative glucocorticoid dose [10]. Aortic MRA abnormalities resolved in only one third of patients on TCZ, however, and a significant relapse rate was observed after its cessation [11]. TCZ is

nevertheless the first drug to be licensed by the Food and Drug Agency (FDA), the European Medicines Agency and Therapeutic Goods Administration for GCA, and has paved the way for further clinical trials. Tumour necrosis factor inhibitors (TNFi) are not effective for GCA, whereas a monoclonal antibody to the p40 subunit common to both IL-12 and IL-23 (key cytokines regulating Th1 and Th17 differentiation, respectively) showed promise for refractory GCA in an open-label prospective study. Other targets under evaluation include T cell co-stimulation, IL-1β, B cells, Janus kinases (JAK), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-17, and endothelin receptors. A lack of reliable biomarkers which reflect vascular inflammation is an impediment to individual patient management and drug development. Redirecting the focus of diagnostic and therapeutic strategies from the extravascular/systemic disease phase of the disease reflected by raised CRP and ESR, to the vascular inflammation phase, may ultimately improve disease outcomes [3].

16.3.2 Takayasu Arteritis

Takayasu arteritis (TAK, previously known as "pulseless disease") is a granulomatous arteritis affecting the aorta and its main branches as well as the pulmonary vasculature, causing aneurysm formation and tissue infarction. Histopathology is similar to GCA with the presence of transmural lymphocytic infiltration and multinucleated giant cells, but involvement of cranial vessels is not a feature, and patients are typically women under 50 years of age. TAK occurs across all races and geographic locations, with a higher incidence in Asians and South Americans [12].

The strongest genetic susceptibility locus for TAK is *HLA-B∗52*. Non-HLA susceptibility loci include Fc gamma receptor loci *FCGR2A/FCGR3A*, *IL12B*, and *IL6* [13]. Early vascular lesions consist of T cells, natural killer cells, and macrophages. Granuloma formation and giant cells can subsequently be found in the media of elastic arteries. Late-stage ('burnt out') damage demonstrates extensive fibrosis and intimal hyperplasia, which may lead to aneurysm formation or arterial stenosis (Fig. 16.2). In contrast to GCA, a pathogenic role for B cells has been suggested in TAK by the identification of nonspecific polyclonal hypergammaglobulinemia, circulating anti-endothelial antibodies, increased numbers of circulating plasmablasts, B cell infiltration of aortic adventitia, and elevated serum levels of B cell-activating factor in patients with active disease [13].

Patients usually present with symptoms of limb ischaemia, hypertension and/or renal impairment. Signs on examination include differential brachial blood pressures, inability to palpate peripheral pulses, and subclavian, axillary, abdominal and femoral bruits. Diagnosis may be confirmed by imaging of the aortic trunk and vessels by MRI, PET or CT [7]. CRP and ESR are not always elevated. The American College of Rheumatology classification criteria for TAK are shown in Table 16.3 [14].

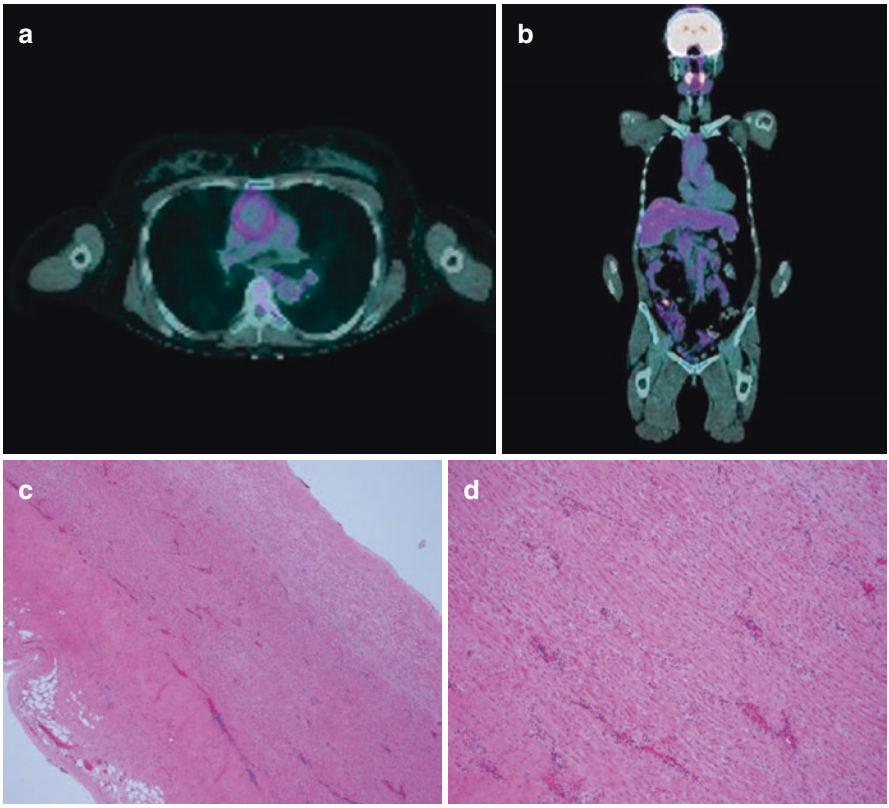

Fig. 16.2 Takayasu arteritis. 18F-FDG-PET/CT showing increased radiotracer avidity associated with the ascending aorta (**a**, **b**). Aortic wall showing patchy lymphocytic inflammatory infiltrate within the media. Elsewhere there is evidence of dense fibrosis of the aortic wall with extensive disruption of the elastic fibre architecture (**c**, **d**)

Table 16.3 1990 criteria for the classification of Takayasu arteritis[a] [14]

1. Age at disease onset ˂40 years	Development of symptoms or findings related to Takayasu arteritis ≤40 years
2. Claudication of extremities	Development and worsening of fatigue and discomfort in muscles of one or more extremity while in use, especially the upper extremities
3. Decreased brachial artery pulse	Decreased pulsation of 1 or both brachial arteries
4. BP difference >10 mmHg	Difference of >10 mmHg in systolic blood pressure between arms
5. Bruit over subclavian arteries or aorta	Bruit audible on auscultation over 1 or both subclavian arteries or abdominal aorta
6. Arteriogram abnormality	Arteriographic narrowing or occlusion of the entire aorta, its primary branches, or large arteries in the proximal upper or lower extremities, not due to arteriosclerosis, fibromuscular dysplasia, or similar causes; changes usually focal or segmental

[a]For purposes of classification, a patient shall be said to have Takayasu arteritis if at least 3 of these 6 criteria are present. The presence of any 3 or more criteria yields a sensitivity of 90.5% and a specificity of 97.8%. BP = blood pressure (systolic; difference between arms).

Glucocorticoids are the mainstay of treatment for TAK. CYC is usually used initially in conjunction with high dose glucocorticoids for severe or organ-threatening disease. Conventional DMARDs have shown limited potential for steroid-sparing in uncontrolled series [12], yet treatment with leflunomide led to a favourable clinical response in 12 of 15 TAK patients with refractory disease [15]. A recent literature review reported the efficacy of TNFi (mainly infliximab, IFX), with remission induction in 70–90% of TAK patients unable to achieve or maintain remission with glucocorticoids and traditional immunosuppressants alone; over 40% of these patients were able to discontinue glucocorticoids, while relapses were described in nearly 40% [15]. As in GCA, IL-6 is highly expressed within inflamed arteries in TAK, and serum IL-6 levels correlate with disease activity. Several case series and case reports suggested that TCZ may be effective for relapsing or refractory TAK, but with mixed radiologic outcomes [12]. Other rare causes of aortitis, such as relapsing polychondritis and Cogan's syndrome, have also been reported to benefit from TNFi and TCZ [12]. Rituximab (RTX), a B-cell depleting monoclonal antibody targeting CD20, may be a potentially effective and safe therapeutic option in patients with TAK refractory to the above immunosuppressive and biologic drugs, but data does not support its use as first line biologic therapy [15].

TAK is a chronic disease and inflammatory activity tends to wax and wane, with subclinical radiographic progression seen in more than two thirds of patients [12, 13], thus patients require long term monitoring and usually immunosuppression. Stenting of arterial stenoses, particularly of renal arteries is often required, preferably when vessel inflammation has been controlled. Collateral circulation may compensate and improve painful limb claudication over time. In both TAK and GCA, the late complications of aortic root dilatation with aneurysm formation and/or aortic valve regurgitation, or abdominal aortic aneurysm, may require surgical intervention. In patients with large vessel vasculitis (GCA or TAK), MRA, CT angiography (CTA) and/or ultrasound may be used for long-term monitoring of structural damage, particularly to detect stenosis, occlusion, dilatation and/or aneurysms. The frequency of screening as well as the imaging method applied should be decided on an individual basis [7].

16.4 Medium Vessel Vasculitis

16.4.1 Polyarteritis Nodosa

Polyarteritis nodosa (PAN) is a rare necrotising vasculitis affecting medium-sized or small muscular arteries. In contrast to microscopic polyangiitis (see below), PAN is not associated with glomerulonephritis or vasculitis of the small vessels (arterioles, capillaries and venules). PAN occurs most commonly in middle-age with a slight male predominance, with decreasing incidence concomitant with reduced prevalence of hepatitis B virus (HBV) infection.

The aetiopathogenesis of classic PAN is not known, and pathway(s) leading to necrotizing inflammation of the blood vessels probably involve both the innate and adaptive immune systems. Newly described loss of function mutations in genes associated with autoinflammatory diseases, such as *CECR1* which codes for adenosine deaminase-2 (ADA2) can lead to a similar phenotype, implicating perturbation of leukocyte growth factors and endothelial instability [16]. HBV infection accounts for 30% of cases previously diagnosed with PAN; vasculitis in this setting has been shown to be caused by viral antigen-containing immune complex deposition on vascular walls with complement fixation and vascular damage. Cutaneous PAN is a designation given to patients presenting with necrotizing vasculitis confined to the skin, with occasional musculoskeletal features but no other systemic involvement [16].

The characteristic presentation of PAN is with ischaemia and infarction of numerous organs including the skin causing palpable purpura, livedo reticularis or subcutaneous nodules, nervous system causing stroke or mononeuritis, kidneys causing infarction, hypertension and renal failure, heart causing myocardial infarction, pericarditis, and congestive cardiac failure, gastrointestinal system causing abdominal pain, infarction and haemorrhage, and testicles causing painful orchitis. PAN rarely affects the lungs. Systemic symptoms such as fever, weight loss, arthralgia and myalgia are common.

Diagnosis is established by a combination of clinical features, biopsy (skin, testes, renal) and/or characteristic findings on digital subtraction angiogram, which has a better resolution than CTA. Histopathology reveals segmental transmural inflammation of muscular arteries with evidence of necrosis and disruption of the internal and external elastic lamina. The latter can lead to aneurysmal dilation. Characteristic mesenteric or renal angiographic findings are of multiple aneurysms and irregular constrictions of larger vessels with occlusions of smaller vessels.

Isolated cutaneous PAN often responds to low dose glucocorticoids. Higher doses of glucocorticoids are the mainstay of treatment for mild classic PAN in combination with MTX, AZA, MMF or LEF as steroid-sparing agents. CYC is used in conjunction with glucocorticoids in moderate to severe cases. There is emerging evidence for infliximab for refractory cases and patients with mutations causing ADA2 deficiency, and reports of benefit with rituximab. Treated systemic PAN has a 5 year survival rate between 75 and 80%; untreated it is usually fatal. Treatment for HBV-associated PAN is with anti-viral therapy coupled with short-term glucocorticoids, which can lead to total remission of vasculitis with no evidence of recurrence.

16.4.2 Kawasaki Disease

Kawasaki disease (KD) is a paediatric vasculitis with coronary artery aneurysms (CAA) as its main complication. It is most common in Japan and Asia, with reported incidence rates of 265/100,000, most patients being between 6 months and 5 years of age. The male:female ratio is approximately 1.5:1 [17].

The aetiology of KD is unknown, with current consensus being that an infectious trigger initiates an abnormal immune response involving innate and adaptive pathways in genetically predisposed children. Several genome-wide association studies and case control studies have identified single nucleotide polymorphisms in genes which code for proteins important in immune activation including *FcgRIIa*, *CD40*, and *BLK*. Other identified pathways of potential importance include vascular endothelial growth factor (VEGF), angiopoietin, transforming growth factor-beta (TGF-β, important in T cell activation and cardiovascular remodelling), and inositol-triphosphate 3-kinase (ITPKC, part of a transmembrane signalling pathway). ITPKC influences NLRP3 inflammasome activation through intracellular calcium levels leading to an increased IL-1β and IL-18 production [17]. Multiple potential infectious triggers have been implicated in KD pathogenesis. In the coronary arteries, immune infiltration of the arterial wall with neutrophils, CD8+ cytotoxic T cells, Ig-A producing plasma cells, and macrophages have been found, accompanied by pro-inflammatory cytokines which vary in proportion and contribution over time [17].

The diagnosis of KD is based on the presence of clinical features of persistent fever in combination with a rash, cervical lymphadenopathy, non-purulent conjunctival injection, changes of the lips and oral cavity (including strawberry tongue, cracked lips, redness of the mucosae), and changes in extremities (swelling and redness of the palms, desquamation in the subacute phase). There is no diagnostic test for KD, and the diagnosis may be delayed or overlooked [17]. Coronary artery lesions are diagnosed by echocardiography in the acute and subacute phases. Close monitoring of CAA is important as ischemic symptoms or myocardial infarction due to thrombosis or stenosis can occur, most likely in the largest, so-called giant CAA. Apart from the presence of CAA, it is unclear whether KD causes an increased cardiovascular risk due to the vasculitis itself [17].

Treatment consists of high dose intravenous immunoglobulin (IVIg) and is directed at preventing the development of CAA. Unfortunately, 10–20% of patients fail to respond to IVIg and these children need additional treatment with a second dose of IVIg or glucocorticoids. Other immunosuppressants under investigation are inhibitors of IL-1β, TNF and calcineurin. Initial addition of high-dose aspirin is advised by the American Heart Association [18].

16.5 Small Vessel Vasculitis

Small vessel vasculitides (SVV) are a group of relatively uncommon disorders characterized by inflammation of small intraparenchymal arteries, arterioles, capillaries, and venules which leads to vascular obstruction, tissue ischemia and infarction. A complex overlapping set of clinical features involving different organs and systems may occur. According to the 2012 Chapel Hill Consensus Conference, SVV are divided into ANCA-associated vasculitides (AAV) and immune complex vasculitides (ICV) [1]. AAV are necrotizing vasculitides frequently associated with proteinase 3 (PR3)-ANCA or myeloperoxidase

(MPO)-ANCA; ICV are characterized by wall deposits of immunoglobulin and complement components, and include IgA vasculitis (IgAV), cryoglobulinaemic vasculitis (CV), anti-glomerular basement membrane disease (anti-GBM), and hypocomplementaemic urticarial vasculitis (HUV) [1].

16.5.1 Anti-neutrophil Cytoplasmic Antibody (ANCA)-Associated Vasculitis (AAV)

ANCA are detected by indirect immunofluorescence (IIF) of fixed neutrophils, producing two main patterns: cytoplasmic (C-ANCA), usually caused by antibodies targeting proteinase 3 (PR3), and perinuclear (P-ANCA), the typical pattern of antibodies targeting myeloperoxidase (MPO). A positive C-ANCA or P-ANCA on screening IIF has low specificity and may be seen in other inflammatory conditions and healthy individuals. Confirmation by enzyme-linked immunosorbent assay is required to confirm PR3 or MPO antibody specificity. There are three clinical subgroups of AAV: granulomatosis with polyangiitis (GPA, formerly Wegener's granulomatosis), eosinophilic granulomatosis with polyangiitis (EGPA, formerly Churg-Strauss angiitis) and microscopic polyangiitis (MPA). These are rare and potentially life-threatening multisystem autoimmune diseases.

Environmental factors play a role in pathogenesis, supported by variation in geographical distribution, and relationship with exposure to silica, hydrocarbons and various drugs. Infections such as *Staphylococcus aureus* have often been noted to precede the onset or flares of AAV, with decreased relapse rates reported after use of cotrimoxazole. Genetic associations include *HLA-DRB1∗04*, *DPB1∗0401*, the proteinase 3 gene (*PRTN3)*, and alpha-1-antitrypsin *gene* (*SERPINA1*) with GPA, *HLA-DRB4* with EGPA, and *HLA-DRB1∗0901* with MPA. Toll-like receptor 9 (*TLR 9)* polymorphism is associated with PR3-ANCA but not MPO-ANCA vasculitis. Epigenetic factors include decreased histone demethylation at PR3 and MPO loci [19]. A key role for neutrophils is indicated by amelioration of disease in mouse models in the setting of neutrophil depletion, and abnormal neutrophil extracellular traps containing PR3 and MPO have been demonstrated upon activating neutrophils with ANCA derived from human sera. Macrophages also have a role in propagation of AAV [20]; the predominant effector cells implicated in EGPA are eosinophils [21].

16.5.1.1 Granulomatosis with Polyangiitis (GPA)

The range of clinical presentations is broad and includes granulomatous inflammation of upper and lower airways and vasculitis of small and medium vessels, of which glomerulonephritis is common. Nasal symptoms include nasal obstruction, bloody nasal discharge, epistaxis, chronic sinusitis, and saddle nose deformity.

Subglottic stenosis, mastoiditis, otitis media, and orbital pseudotumor are seen less commonly. Systemic features such as fever, arthralgia, and malaise may be absent in the early localised phase. Pulmonary involvement occurs in up to 85% of patients ranging from asymptomatic nodules to massive haemoptysis with respiratory failure. Radiologic features include pulmonary nodular infiltrates, cavities, and bilateral ground glass opacities from pulmonary haemorrhage. Renal involvement in the form of rapidly progressive glomerulonephritis is the presenting manifestation in 20% of patients, increasing to 80% over the disease course. Ocular involvement occurs in 50% in the form of episcleritis, scleritis, and orbital disease. Cutaneous manifestations are usually minor [22].

16.5.1.2 Eosinophilic Granulomatosis with Polyangiitis (EGPA)

This syndrome is characterized by eosinophil-rich granulomatous inflammation of the airways along with small- and medium-vessel vasculitis. Three different disease phases are usually described, namely, prodromal, eosinophilic, and vasculitic. The main clinical feature in the prodromal stage is asthma (which may predate vasculitis by years) and allergic rhinitis with or without polyposis. Upper airway involvement is much milder than in GPA. The second phase is of peripheral and tissue eosinophilia, which may be masked by glucocorticoid use for asthma. Vasculitic manifestations occur in nerves, heart, lungs, GI tract, and kidneys. Skin involvement is a dominant feature in the form of nodules; urticaria or ulceration. Neurologic involvement occurs in 60–70% of patients in the form of multiple mononeuropathies or symmetric polyneuropathy; ischemic optic neuropathy, cranial neuropathies, and stroke are less common. Cardiac involvement is seen in up to 20% of patients in the form of myocarditis, pericarditis, endocarditis, valvulitis, and coronary vasculitis, and contributes to half the deaths. Renal involvement in the form of small vessel vasculitis is less common than other AAVs [22].

16.5.1.3 Microscopic Polyangiitis (MPA)

MPA is a necrotising small-vessel AAV. Initially considered a part of PAN, it was recognized as a distinct entity in 1994 [23]. The main manifestations are pauci-immune glomerulonephritis and pulmonary capillaritis. Granulomatous inflammation is absent. Most patients present with rapidly progressive glomerulonephritis and may be oliguric or anuric at the time of presentation, necessitating dialysis. Lung haemorrhage is the most catastrophic manifestation seen in about 10% of patients and is mostly associated with renal involvement. Most patients have constitutional symptoms, with fever, weight loss, arthralgia, and myalgia at the time of presentation. Neurologic involvement is common in the form of mononeuritis multiplex, axonal sensorimotor neuropathy, or cranial nerve involvement. Skin manifestations include purpura, painful ulcers (frequently on the legs) and digital gangrene.

Ocular involvement includes scleritis, episcleritis, blepharitis, conjunctivitis, keratitis, uveitis, and retinal vasculitis [22].

Diagnosis of AAV is based on clinical features supported by positive ANCA and biopsy of the involved organ wherever feasible. EGPA is associated with MPO-ANCA in approximately 50% of cases, whereas GPA and MPA are usually associated with PR3-ANCA. The presence of fibrinoid necrosis or crescentic glomerulonephritis is highly suggestive of AAV.

Glucocorticoids and immunosuppressive drugs used in combination are the mainstay of treatment, and have drastically changed the outcomes of these diseases, which previously had a mortality rate of 90% at 2 years. Remission induction in organ- or life-threatening disease consists of CYC or RTX in combination with glucocorticoids. MMF was shown to be non-inferior to CYC for remission induction, but resulted in higher relapse rates [24]. The PEXIVAS randomized controlled trial, of 704 subjects demonstrated that plasma exchange does not reduce the risk of end-stage renal disease or death in patients with severe AAV treated with glucocorticoids combined with CYC or RTX induction. Compared with a standard dose, a reduced steroid regimen did not substantially increase the risk of death or end-stage renal disease and resulted in fewer serious infections [25]. Maintenance therapy following induction is individualised, options including MTX, AZA, LEF, MMF, or RTX [21].

Mepolizumab is a humanized monoclonal antibody which binds free IL-5, an essential cytokine for eosinophil development and proliferation, which is increased in active EGPA. In a randomized controlled trial, mepolizumab proved effective in prolonging remission in EGPA and allowed for reduced glucocorticoid use, leading to FDA approval in 2017 [26]. IL-5 dependent cell proliferation and survival is dependent on JAK signal transduction, as well as tyrosine kinases including Bruton tyrosine kinase (BTK). Oral inhibitors of these kinases are available, and in addition, the complement component C5a is a potential therapeutic target in AAV.

16.5.2 Immune Complex Small Vessel Vasculitis

16.5.2.1 Immunoglobulin-A Vasculitis (IgAV)

Previously known as Henoch-Schönlein purpura, IgAV predominantly affects capillaries, venules, or arterioles, with IgA1-dominant immune complex deposition. It is the most common childhood vasculitis, affecting 10–20 children per 100,000 annually. More than 90% of patients are under 10 years of age, with a mean age of 6 years; adults tend to have more severe disease. Clinical presentation of IgAV comprises a characteristic tetrad of cutaneous palpable purpura, joint pain, colicky abdominal pain, and renal involvement. Proposed triggers include upper respiratory tract infections, medications, vaccinations, and (in adults) malignancies [27].

The skin rash consists of symmetric erythematous petechiae or papules on the buttocks and lower extremities, which evolve to a typical palpable purpura [28]. Gastrointestinal symptoms occur in about 50% of cases. Abdominal pain is

common and may mimic an acute abdomen. The abdominal pain is thought to be due to immune complex deposition in the gut vessel walls leading to haemorrhage and oedema within the bowel wall and mesentery. Gastrointestinal complications such as perforation, intussusception and infarction may occur [28]. Joint involvement includes arthralgias or arthritis often affecting knees and ankles, which often precede the development of palpable purpura. IgAV kidney involvement is frequent, including haematuria, proteinuria, nephrotic or nephritic syndrome, with onset usually within a few months of the rash. Severe renal involvement occurs in 7% of cases, most commonly adults, and may lead to end-stage renal disease [28]. Hypertension may develop at the onset or during recovery from IgAV; neurological manifestations are rare [27].

HLA class I and class II *HLA-DRB1* alleles appear to influence predisposition to this disease, and non-HLA gene candidates include those coding for cytokines, chemokines, adhesion molecules, T-cells, aberrant glycosylation of IgA1, nitric oxide production, neoangiogenesis, the renin-angiotensin system, and lipid, pyrin and homocysteine metabolism [28].

Upper respiratory tract infections precede the majority of cases, with many pathogens implicated. Vascular deposition of IgA1-containing immune complexes plays a pathogenic role, with complement activation, endothelial damage, perivascular leukocytic infiltrates, chemokines and cytokines important factors in this process, thus suggesting an IgA-mediated dysregulated immune response to an antigen [27].

Diagnosis is based on clinical criteria and non-mandatory tissue biopsy. Histological features involving the skin are those of a leukocytoclastic vasculitis primarily affecting the small superficial vessels. Vessel walls are infiltrated by

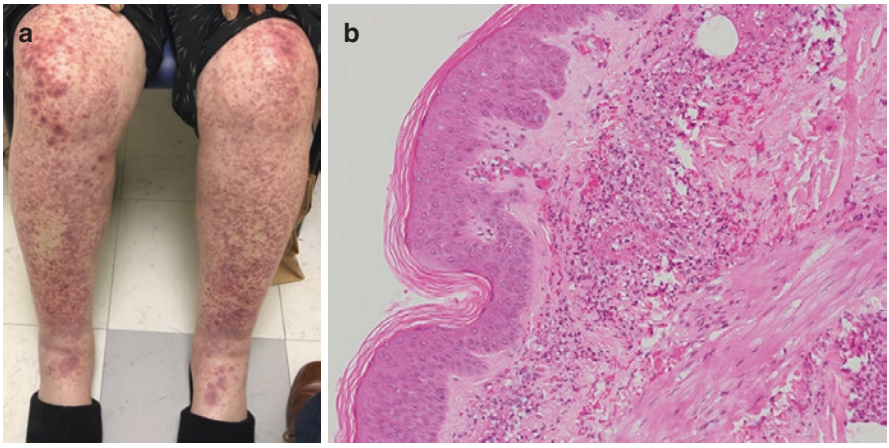

Fig. 16.3 IgA vasculitis. (**a**) Palpable purpura (**b**) skin biopsy showing leukocytoclastic vasculitis (**c**) IgA deposition in vessel walls (**d**) glomerulonephritis with crescent (Periodic Schiff-Methenamine (PASM) stain)

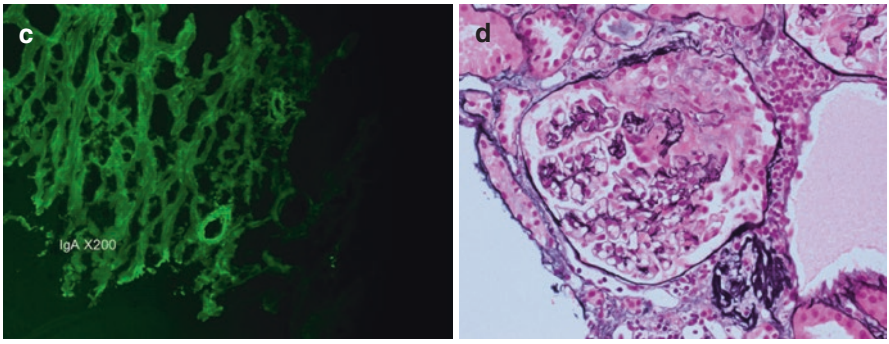

Fig. 16.3 (continued)

neutrophil granulocytes, which partly degenerate and form nuclear dust (leukocytoclasia), located amongst extravasated erythrocytes (purpura) in the surrounding dermis. The vessel walls are thickened and may be necrotic due to exudation of neutrophils and variable amounts of fibrin. On direct immunofluorescence, IgA and, eventually, complement C3 can be seen deposited in the vessel walls. The process is dynamic and not all of these features might be seen in a single biopsy [27] (Fig. 16.3).

Treatment is dictated by the severity of organ involvement. Treatment in patients without renal involvement is supportive, including analgesia, rehydration, surgery for intussusception, and wound care. Compression may be used for leg oedema. Nephritis is treated with glucocorticoids and/or other immunosuppressive drugs, including MMF, CYC, CyA, RTX or dapsone. IgAV has been associated with solid tumours, mostly of the gastrointestinal tract, lungs or urinary tract in men over 60 years of age, and screening for cancer in this subgroup should be considered [27].

16.5.2.2 Cryoglobulinemic Vasculitis (CV)

CV is a small-vessel vasculitis involving mainly the skin, joints, peripheral nervous system and kidneys, characterized by cryoprecipitable immune complexes which may occur in the settings of chronic infection (mainly hepatitis C virus, HCV), or without infection, particularly in the autoimmune rheumatic disease primary Sjögren's syndrome, and lymphoproliferative disorders [21] (Fig. 16.4). These vasculitides result from the deposition of circulating immune complexes, activation of the classic complement pathway and recruitment of neutrophils.

Symptoms from HCV-associated CV are mainly cutaneous, rheumatological and renal. Serological and virological investigations support a pathophysiological role for HCV infection in cryoglobulinaemia: positive anti-HCV testing (80–90% of the

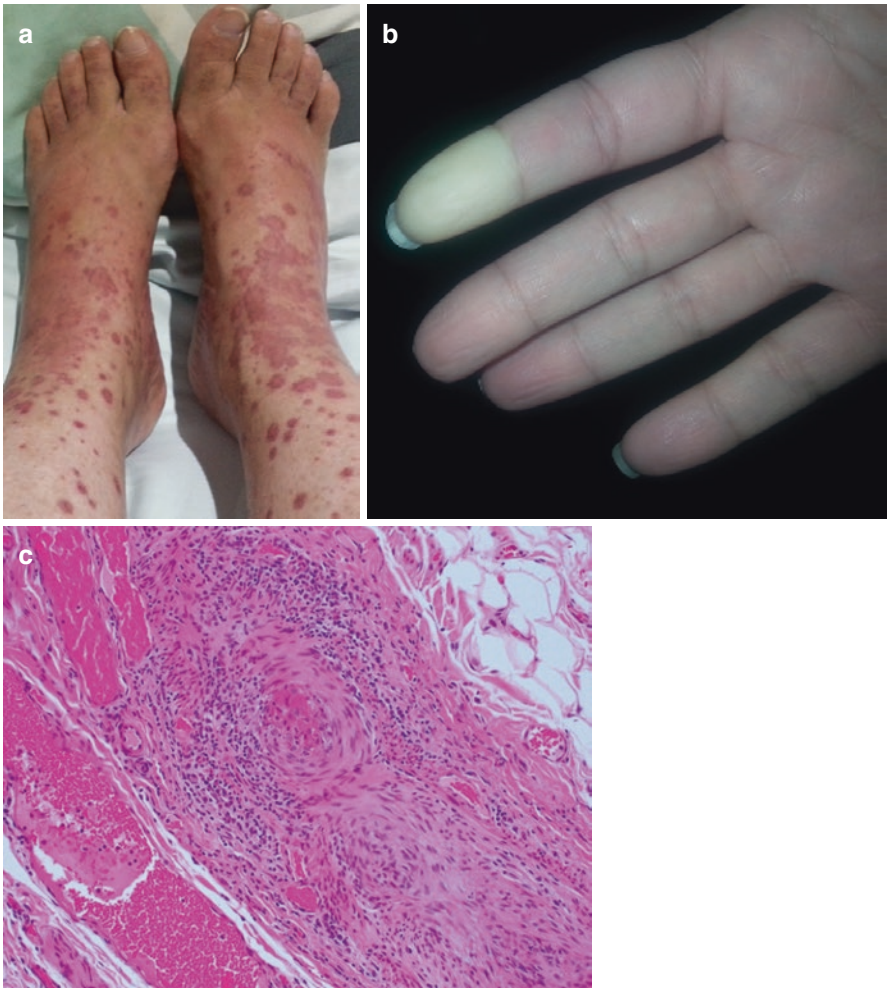

Fig. 16.4 Primary Sjögren's syndrome-related cryoglobulinemic vasculitis. (**a**) Cutaneous palpable purpura (**b**) Raynaud's phenomenon (**c**) vasculitis of the gall bladder wall

patients), circulating HCV-RNA, HCV in lesions and HCV-RNA in cryoprecipitate. The 1b HCV genotype is most frequently associated with cryoglobulinaemia, while genotypes 2–3 are associated with cryoglobulinaemia in coinfected HIV–HCV patients. Reported prevalence of cryoglobulins was 45.7% in HCV patients and prevalence of symptoms associated with cryoglobulins was 27%. Cryoglobulinaemia can also be secondary to HBV infection [29]. Eradication of the hepatitis virus leads to resolution of cryoglobulinaemia; short term glucocorticoids and immunosuppressants may be required for symptoms.

16.5.2.3 Hypocomplementaemic Urticarial Vasculitis (HUV, Anti-C1q Vasculitis)

HUV is an uncommon immune complex-mediated entity associated with anti-C1q antibodies and characterized by leukocytoclastic vasculitis, severe angioedema, pulmonary involvement, arthritis/arthralgia, glomerulonephritis, and uveitis. Treatment is symptomatic for mild disease; severe disease is treated with CYC, AZA or MMF in combination with glucocorticoids. CyA has been found useful in patients with progressive airway obstruction. RTX and plasma exchange followed by IVIg has been used in refractory cases [21].

16.5.2.4 Anti-Glomerular Basement Membrane (Anti-GBM) Disease

A vasculitis affecting glomerular capillaries, pulmonary capillaries, or both, following GBM deposition of anti-GBM autoantibodies. Lung involvement causes pulmonary haemorrhage, and renal involvement causes glomerulonephritis with necrosis and crescents [1].

16.6 Variable Vessel Vasculitis

16.6.1 Behcet's Disease

Behcet's disease (BD) is a systemic inflammatory disorder characterized by multiorgan involvement including oral and genital ulcers, uveitis, skin lesions, central nervous system and vascular manifestations [30, 31]. Superficial and deep venous thrombosis are the most frequent vascular manifestations, affecting 15–40% of patients. Thrombosis characteristically occurs in unusual sites, including the inferior and superior vena cava, suprahepatic veins with Budd-Chiari syndrome, portal vein, cerebral sinuses and right ventricle. Arterial involvement, affecting 3–5% of patients, typically presents with aneurysms affecting peripheral, visceral and pulmonary arteries, the first sign of which may be massive haemorrhage. Vascular events in BD are promoted by inflammation of the vessel wall, with neutrophils playing a key role in the pathogenesis of thrombotic events, and coagulation components such as fibrinogen, thrombin, factor Xa and factor VIIa amplifying the inflammatory cascade [30].

Pathophysiology of BD involves activation of both innate and adaptive immune pathways triggered by several putative pathogens, with consequent interaction between T lymphocytes (Th1 and Th17 phenotypes) and activated neutrophils. The HLA class I allele *HLA-B∗51* is the major genetic risk factor in many populations, especially along the ancient Silk Route which ranged from East Asia to the Middle East and the Mediterranean area. The *HLA-A∗26* allele is associated with

BD independently of *HLA-B∗51*, and polymorphisms in genes encoding IL-10, IL-23 receptor, IL-12 receptor, and STAT4 have been associated with BD in Turkish, Japanese and Korean populations. Disease-associated non synonymous variants in the familial Mediterranean fever gene *MEFV*, and *TLR4*, support the involvement of innate immune responses and bacterial sensing mechanisms in BD pathogenesis [31].

BD typically runs a relapsing and remitting course, and the goal of treatment is to promptly suppress inflammatory exacerbations and recurrences to prevent irreversible organ damage. Ocular, vascular, neurological and gastrointestinal involvement are associated with a poor prognosis, however disease manifestations may diminish over time [32]. Control of vascular thrombosis in BD is achieved with immunosuppressant drugs rather than anticoagulants, in particular AZA or CyA in conjunction with low-dose glucocorticoids for venous thrombosis, while treatment with CYC or TNFi have been successfully used for arterial involvement [30]. TNFi are also effective for control of mucocutaneous, ocular, neurological and refractory venous thrombosis. IL-1βi may be useful for refractory disease, especially uveitis, and IL6Ri for CNS disease [31]. The 2018 updated EULAR recommendations for BD, based on low-level evidence [32], recommend that in addition to TNFi for refractory venous thrombosis, anticoagulants may be added, provided the risk of bleeding in general is low and pulmonary artery aneurysms have been ruled out. For the management of pulmonary artery aneurysms, high dose glucocorticoids and CYC are recommended, and IFX for refractory disease. Because of a high mortality rate in surgically treated patients, intervention should be reserved for life-threatening situations, with arterial embolization the preferred option. For both aortic and peripheral artery aneurysms, medical management should be optimized when possible before surgical intervention, and medical management may be sufficient for small, asymptomatic aneurysms. For both pulmonary and peripheral artery aneurysms, the choice of surgical intervention between graft insertion, ligation and bypass surgery is dictated by the size and location of the aneurysm and the surgeon's experience. Synthetic grafts are preferable since venous grafts have a higher risk of thrombosis in patients with BD. The first episode of cerebral venous thrombosis should be treated with high-dose glucocorticoids followed by tapering. Anticoagulants may be added for a short duration, after screening for vascular disease at other sites [32].

16.6.2 Cogan's Syndrome

Cogan's syndrome is characterized by ocular inflammatory lesions, including interstitial keratitis, uveitis, and episcleritis, and inner ear disease, including sensorineural hearing loss and vestibular dysfunction. Vasculitic manifestations may include arteritis (affecting small, medium, or large arteries), aortitis, aortic aneurysms, and aortic and mitral valvulitis [1].

16.7 Vasculitis Associated with Systemic Disease

16.7.1 Autoimmune Rheumatic Diseases

Small vessel vasculitis may occur in the setting of known autoimmune rheumatic diseases such as rheumatoid arthritis, systemic lupus erythematosus and primary Sjögren's syndrome. Biopsy is usually not required, and if performed will usually show a non-specific leucocytoclastic vasculitis. Vasculitis usually occurs in patients with active systemic disease, to which treatment is directed. In some cases more aggressive treatment is required for the vasculitis itself. Cryoglobulinaemic vasculitis (discussed above) occurs in 4% of patients with primary Sjögren's syndrome, and is a predictor of the development of lymphoma and death [33].

16.7.2 IgG4-Related Disease (IgG4-RD)

IgG4-RD is a rare systemic sclerosing disorder, initially described in Japan and now increasingly recognised throughout the world. Early recognition and treatment is essential to minimise irreversible organ damage and unnecessary surgical intervention. IgG4-RD occurs most commonly in middle aged to elderly men, with a two to four-fold male preponderance [34]. It is characterized by mass-like sclerosing lesions which can involve almost any anatomic site, and many fibroinflammatory and sclerosing disorders previously considered as distinct entities are now included within the spectrum of IgG4-RD, including sclerosing cholangitis, idiopathic retroperitoneal fibrosis and mesenteritis, some forms of aortitis and periaortitis, and sclerosing sialadenitis of salivary and lacrimal glands including Kuttner tumor and Mikulicz syndrome [34]. Four predominant clinical phenotypes of IgG4-RD have recently been described [35]: (1) pancreato-hepatobiliary disease (31% of patients); (2) retroperitoneal fibrosis and/or aortitis (24%); (3) head and neck-limited disease (24%); (4) classic Mikulicz syndrome with systemic involvement (22%). Aortitis occurred in 10.3% overall, and retroperitoneal fibrosis in 15.8%. In addition to sclerosing abdominal and thoracic aortitis and periaortitis, IgG4-related vasculitis occurs with a predilection for the first and second aortic branches including carotid and coronary arteries.

Recent work on the pathophysiology of IgG4-RD identified an essential role for oligoclonally expanded CD4+ cytotoxic T lymphocyte populations (CD4+ CTLs), which secrete pro-fibrotic cytokines including IL-1β, TGFβ and IFN-γ, and are capable of perforin- and granzyme B-mediated cytolysis. They accumulate in tissue lesions, representing the dominant T cell population in affected tissues [36]. Patients with IgG4-RD also have elevations of oligoclonally expanded populations of somatically hypermutated plasmablasts, many of which are IgG4+, and because of the profound clinical responses and decline in CD4+ CTLs observed following B-cell depleting therapy, it is considered likely that activated B cells drive the activation of CD4+ CTLs in affected tissues [37]. Upon relapse, distinct plasmablast clones emerge, suggesting *de novo* recruitment of new plasmablast clones rather than neoplastic oligoclonal proliferations.

T follicular helper (Tfh) and T regulatory (Treg) cells also likely play a critical role in IgG4-RD, particularly with respect to class switching of B cells and induction of aberrant lymphoid follicle formation in tissues. Tfh cytokines IL-21 and IL-4 play a role in germinal center formation, B-cell differentiation, plasmablast induction, and production of IgG4. IL-10 and TGFβ production by Treg cells also likely contributes to IgG4 class switching and fibrosis, respectively.

The role of plasmablasts and the IgG4 molecule in IgG4-RD remains uncertain. They may act to perpetuate the immune response through reactivity to specific antigens and antigen presentation to CD4+ CTLs. However, given that IgG4 has been shown to be a relatively inactive immunoglobulin subclass, without the ability to fix complement or crosslink antibodies, it has been hypothesized that IgG4 production and the frequent IgG4+ plasma cells may be a secondary or reactive phenomenon to cytokine production, rather than being intrinsically pathogenic. It has also been suggested that IgG4 has anti-inflammatory properties and may be produced in an attempt to mitigate the inflammatory response. It remains uncertain as to which autoantigens might be responsible for the robust immune response in IgG4-RD [34].

Elevation of serum IgG4 level is the most well-known laboratory feature used to support a diagnosis of IgG4-RD, however up to half of patients with biopsy proven and clinically active IgG4-RD have normal serum IgG4 concentrations [34], and elevated serum IgG4 levels are neither sensitive nor specific for IgG4-RD. Peripheral eosinophilia, polyclonal hypergammaglobulinemia, elevated serum IgE levels, elevated CRP, and hypocomplementemia are relatively common. Histopathology is required for a definitive diagnosis of IgG4-RD and should include two or more characteristic features: (1) a dense lymphoplasmacytic infiltrate, (2) fibrosis that is typically storiform in pattern (i.e. a matted, irregularly whorled pattern), (3) obliterative phlebitis, (4) an increased number of IgG4+ plasma cells per high power field, and (5) an IgG4+/IgG+ plasma cell ratio of >40%. Obliterative phlebitis is the least often identified histologic feature of IgG4-RD, although the most specific [34].

Given the limitations of available biomarkers, a combination of imaging modalities, including CT, MR and ultrasound is the cornerstone for evaluating disease burden and activity. 18F-FDG PET/CT has been validated for IgG4-RD assessment and exclusion of concomitant malignancies, and provides anatomical and functional information on the extent of organ involvement and disease activity [34].

Due to uncertainty about the molecular mechanisms sustaining IgG4-RD and lack of controlled trial data, treatment is largely based on clinical experience and expert opinion. Prompt intervention is strongly advised in cases affecting vital organs (even sub-clinically) to prevent irreversible organ damage. Given the relapsing-remitting nature of the condition, maintenance therapy should be considered. Immunosuppression is generally advised for cases with prominent lymphoplasmacytic infiltrates on histological examination because they are more likely to respond to pharmacological therapy. Conversely, surgical debulking should be considered for long-standing, end-stage fibrotic lesions since they typically respond poorly to immunosuppression. Temporary stenting is frequently required for bile duct or ureteric strictures.

Glucocorticoid therapy leads to swift clinical responses in the majority of patients with IgG4-RD regardless of the clinical presentation and organ involvement, thus representing the first line therapy for inducing remission. IgG4-RD relapses in up to 46% of cases during or after glucocorticoid tapering, and conventional steroid-sparing agents such as AZA, MMF, MTX, and CYC have been used as maintenance therapy. In a prospective open-label trial, 97% of patients responded to RTX at 6 months even in the absence of concomitant glucocorticoid treatment, and RTX is indicated as a second-line treatment in IgG4-RD patients with recurrent or refractory disease [34].

16.8 Vasculitis Associated with Probable Aetiology

Apart from syphilitic and tuberculous aortitis, a causal relationship between infection and vasculitis has been proven in only few situations, such as HBV with PAN and less commonly SVV and CV, and HCV with CV, discussed above. Relationships between PAN and other infections, particularly streptococcal species, Klebsiella, Pseudomonas, and Yersinia have been suggested, however causation has not been strongly established [29].

16.8.1 Drug-Induced Vasculitis

Members of virtually every pharmacological class have been implicated in the development of drug-induced vasculitis, and the mechanisms involved are mainly related to immune complex deposits with antigen excess. Most frequently implicated are sulphonamides, penicillin, allopurinol, and thiazides. Penicillin causes vasculitis by conjugating to serum proteins and mediating immune complex vasculitis. Drug induced AAV has also been reported, particularly with propylthiouracil, hydralazine, allopurinol, penicillamine, and levamisole in cocaine [38], including a fatal case of methimazole-associated MPO-AAV [39]. Several cross-sectional studies reported a prevalence of propylthiouracil-induced AAV between 20 and 64%. It is essential to withdraw the inciting drug, which usually results in resolution, however immunosuppressant therapy may be required [40]. A high level of suspicion is required, and a clue that AAV is drug-induced is the co-expression of more than one autoantibody, typically MPO-ANCA, antiphospholipid antibodies, antinuclear antibodies (ANA), and sometimes PR3-ANCA.

16.8.2 Vasculitis Associated with Cancer Immunotherapy

Immunotherapy has revolutionized the treatment of a number of types of metastatic cancer. The family of therapeutic agents known as checkpoint inhibitors (CPIs), which target “exhausted” tumour specific T lymphocytes to incite them to an active

effector phenotype and thence destroy malignant cells, is associated with a new group of immune-related adverse events (irAEs) in almost any organ system. Among these irAEs, rheumatic complications are common and seem to have features that are distinct from irAEs in other organ systems, including a highly variable time of clinical onset and the capacity to persist, possibly indefinitely, after cessation of CPI therapy [41]. Nearly every major category of rheumatic disease, including vasculitis, is mirrored by a category of irAEs resulting from CPIs. GCA with or without polymyalgia rheumatica has been reported after treatment with both anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and anti-PD-1 CPIs. Symptoms mirror the traditional forms of disease, including hip and shoulder girdle stiffness, temporal headache, jaw claudication and one incidence of amaurosis fugax. In TAB samples, arteritis, disruption of the elastic lamina, and intimal proliferation have been detected.

Evidence exists that checkpoint dysfunction might contribute directly to GCA pathogenesis and disease activity (discussed above), providing insights into how CPIs might cause autoimmunity. For patients with pre-existing rheumatic diseases who are treated with conventional or biologic DMARDs, as well as for patients with incident rheumatic irAEs who require DMARDs or other immunosuppressive drugs, it is not known whether concomitant immunosuppressive therapy will negate the anti-tumour response of CPI therapy. Retrospective studies do not provide evidence of an adverse effect on tumour kinetics resulting from either glucocorticoids or TNFi. For patients with the most severe irAEs, particularly those that are chronic, the effect of long-term high-dose immunosuppression or other biologic therapies is not known [41].

16.9 Vasculitis Mimics

16.9.1 Raynaud's Phenomenon

Raynaud's phenomenon (RP) is a common disorder manifest by triphasic colour change of the fingers and toes from white to blue to red, occurring in 5% of the population [42]. RP may be bilateral, may be incited by cold or emotion, and usually presents with normal pulses. It is more prevalent in women than in men, and there may be a genetic predisposition. RP can be divided into primary (PRP) and secondary (SRP), the latter associated with rheumatologic as well as noninflammatory conditions. PRP compared with SRP tends to occur at a younger age, is painless, and is rarely associated with tissue breakdown. The most common disorders associated with SRP are systemic sclerosis (scleroderma), systemic lupus erythematosus, primary Sjögren's syndrome, and anti-synthetase syndrome- systemic autoimmune diseases associated with significant morbidity and increased mortality. Immediate referral for further evaluation and diagnosis is therefore recommended for patients suspected of having SRP, which may be the first manifestation of one of these conditions. Transition from PRP to SRP may rarely occur, but is unlikely when the original symptoms are minimal, antinuclear antibodies are absent, and nailfold capillaroscopy is normal [42].

Vasomotor tone in the digital circulation results from interaction between endothelium, smooth muscle, autonomic and sensory nerves, thus RP can result from alterations in various pathways. There are physiological differences between PRP and SRP: nutritional capillary blood flow is normally protected from cold-induced sympathetic vasoconstriction; this protection is mildly impaired in patients with PRP, and severely interrupted in systemic sclerosis. This difference probably reflects the presence of endothelial dysfunction in patients with systemic sclerosis; dysfunctional endothelial cells have reduced reactivity to vasodilators, nitric oxide, and prostacyclin and can express increased thrombotic and inflammatory activity, including the increased release of the vasoconstrictor endothelin-1.The maintenance of nutritional capillary blood flow is normally ensured by the conduction of vasodilatation to upstream vessels that results from flow-mediated activation of the endothelium. Impairment of this protective mechanism, combined with structural limitations of the vascular supply in patients with systemic sclerosis, probably contributes to compromised nutritional blood flow in patients with this disease, leading to tissue injury and ulceration [42].

The diagnosis of RP is usually made by history or by witnessing an episode or photograph (Fig. 16.4). Avoidance of cold remains the most effective therapy for RP; systemic and local warming are highly effective at increasing blood flow in the skin. A variety of factors can potentially aggravate the disorder and should be avoided, including smoking and the use of sympathomimetic drugs.

Drug therapy is initiated when nonpharmacologic approaches are ineffective in reducing the severity of vasospastic attacks which are impacting quality of life. Although there is paucity of clinical trials, current evidence supports the use of calcium channel blockers, phosphodiesterase type 5 (PDE-5) inhibitors and topical nitrates, alone or in combination. There is also some evidence to support the use of selective serotonin reuptake inhibitors such as fluoxetine, and angiotensin II–receptor blockers. Prostacyclin inhibits vasoconstriction, thrombosis, inflammation, and pathologic vascular remodelling, and stimulates the release of endothelium-derived nitric oxide. A systematic review supported the use of intravenous prostacyclin analogues in patients with severe SRP, with reduced severity of vasospastic attacks, increased healing and prevention of digital ulcers. Endothelin-1 receptor antagonists failed to reduce the frequency of vasospastic attacks, but decreased the development of new digital ulcers in scleroderma [42].

16.9.2 Thromboangitis Obliterans (TAO, Buerger's Disease)

TAO is a segmental inflammatory condition affecting small and medium-sized arteries, veins and nerves. It typically affects people under the age of 50 years who use tobacco, usually in the form of cigarettes. Atherosclerotic risk factors other than smoking are usually absent. Incidence varies by geographic location, with the highest prevalence of greater than 10 per 100,000 observed in the Middle East, Asia, Mediterranean, and Eastern Europe. Clinical manifestations of TAO include pain in

a digit or extremity, digital ischemia, RP, distal digital ulceration, and extremity claudication. As TAO progresses, it involves more proximal portions of the extremity, the most dreaded consequence being extremity gangrene and amputation. Cocaine, amphetamine, and cannabis use can present with features mimicking TAO.

The pathogenesis of TAO is largely unknown. In the early stages of disease, there is increased expression of vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and E-selectin on endothelial cell membranes. This may lead to activation of the innate immune response and later to a highly cellular intraluminal thrombus. Other purported mechanisms include delayed type hypersensitivity or toxic angiitis induced by smoking, aberrant Notch signal activation, endothelial dysfunction, anti-endothelial antibodies, impaired endothelium-dependent vasodilation, abnormalities in endothelin, prothrombotic factors, and proinflammatory cytokines [44]. The diagnosis of TAO is clinical, with imaging to exclude other causes of digital ischemia such as large vessel occlusion and proximal sources of emboli. Commonly more than two extremities are involved, and subclinical disease should be sought. Markers of inflammation and autoantibodies are usually absent, and imaging demonstrates normal inflow arteries. While CTA and MRA may be helpful in excluding atherosclerosis, diagnostic arteriography is often needed to demonstrate the distal involvement of arteries. A typical presentation is that of segmental arterial occlusion and corkscrew collaterals (Martorell's sign).

Distinguishing features from atherosclerosis include disease distribution and involvement of both upper and lower extremities, superficial venous thrombosis, and greater severity of pain. Biopsy, usually reserved for atypical cases, demonstrates a highly cellular thrombus with relative sparing of the vessel walls [43].

The evolution of TAO is often categorized into three stages. In the acute phase, inflammation affecting the small-calibre and medium-calibre (1- to 5-mm diameter) arteries and veins is observed. The primary features of TAO during the acute phase include an occlusive, highly cellular arterial thrombus, polymorphonuclear cell infiltrate with leukocytoclasis, giant cells, and microabscess formation; marked inflammation of the entire vessel wall and neurovascular bundle. Multinucleated giant cells can be seen, but fibrinoid necrosis and granuloma are not observed. Although the external elastic lamina may show some disruption the internal elastic lamina remains intact. During the intermediate or subacute phase, there is progressive organization of the occlusive thrombus, with partial recanalization and disappearance of the microabscesses. A prominent inflammatory infiltrate is still present within the thrombus but is less in the vessel wall. Immunoglobulin and complement are deposited along the inner aspect of the internal elastic lamina. The chronic phase or end-stage lesion is characterized by thrombus organization followed by recanalization, prominent vascularization of the media, and perivascular fibrosis. Regardless of the pathologic stage, the internal elastic lamina and the architecture of the vascular walls are well preserved in TAO, in contrast to atherosclerosis and systemic vasculitis, and inflammatory cell infiltration is found predominantly in the intimal layer and the thrombus [45].

The cornerstone of treatment of TAO is complete abstinence from tobacco. Although high level evidence is lacking, intravenous prostacyclin analogues have

been shown to improve ulcer healing and pain, and the endothelin-1 receptor antagonist bosentan reduced new ischaemic lesions [46]. Distal surgical revascularisation may prevent the need for amputations and improve quality of life, however revascularisation is often technically not feasible because of diffuse, segmental arterial involvement and the distal nature of the disease. Distal arterial spasm during dissection and poor-quality veins owing to phlebitis are other disease-specific handicaps [43]. Recent evidence suggests that endovascular treatment is a valid strategy leading to an acceptable limb salvage rate for TAO patients, and surgical bypass to distal target vessels could play a role in cases of previous failed endovascular treatment or extensive soft tissue loss of the foot [47].

16.10 Fibromuscular Dysplasia (FMD)

FMD is a noninflammatory, non-atherosclerotic arterial disease of unknown aetiology in which there is distorted architecture and abnormal proliferation of the arterial wall of medium- or small-sized arteries. Over 80% of affected individuals are women and the mean age at the time diagnosis is 52 years. The true prevalence of FMD is unknown; however an incidence of 3–4% was found in a series of potential renal donors who underwent CT Angiography. FMD has traditionally been divided histopathologically into several types according to which arterial layer is affected and to the arteriographic pattern of disease. Medial fibroplasia was the most common type, comprising 80–90% of cases in the renal arteries. However, the recent First International Consensus on the diagnosis and management of fibromuscular dysplasia [48] stated that in the contemporary endovascular era, where tissue was rarely obtained for histopathological examination, FMD should be classified into two types on the basis of angiographic appearance: (a) focal FMD (approximately 30% of cases) or (b) multifocal FMD (which is characterised by areas of stenosis and dilatation- the “string of beads” appearance). FMD is characterized by intra-arterial fibrotic “webs” that give rise to a “beaded” appearance on imaging studies where the beads are larger than the lumen of the artery.

FMD may affect any major arterial bed and clinical manifestations depend on its distribution. Hypertension, a result of renal artery involvement, remains the most important clinical consequence of FMD. In some patients, the condition remains asymptomatic and incidentally discovered when imaging is performed for other reasons, while in others it may present with arterial dissection, tortuosity, aneurysm formation and/or end organ ischemia. In one series, renal arteries were affected in 75% and the extracranial carotid arteries in 70% of patients with FMD. Intracranial aneurysms have been reported in over 10% of cases in the United States FMD registry. Aneurysms and/or dissection were present in about 40% of patients in the US and European registries [49, 50]. FMD may affect the mesenteric, iliac, femoral or popliteal arteries and result in visceral aneurysm formation or dissection, intermittent claudication or (rarely) critical limb ischemia.

Spontaneous coronary artery dissection (SCAD) is the cause of 10–25% of cases of acute myocardial infarction in women under 50 years of age and 50% of AMIs occurring in the post-partum period. There is a significant association of FMD with SCAD, and thus the International Consensus recommended "imaging of all vessels from brain to pelvis, at least once" in patients who have had SCAD [48].

In contemporary FMD registries, only 2–7% of patients report an affected relative. There are currently no genetic tests that are specific for FMD. Current and past smoking is associated with FMD. There may be a role for TGF-beta pathways in the pathogenesis of the disease.

The diagnosis of FMD usually relies on a combination of clinical and imaging findings. CTA is the investigation of choice for assessment for renal and carotid/vertebral FMD. MRA can be used if CT is contraindicated. Ultrasound in highly expert hands can be used as a diagnostic test for investigation of renal FMD. However, duplex scanning is of limited use in the diagnosis of cerebrovascular FMD, due to the inability to image high cervical internal carotid lesions, vertebral and intracerebral lesions. Angiography, with pressure gradient measurements across lesion(s), is recommended when intervention for renal artery lesions is indicated.

16.11 Summary

Much progress has been made in recent years in understanding pathogenetic mechanisms underlying the broad range of diseases we call vasculitis. The classification is ever evolving as new discoveries are made. The advent of biologic therapy and establishment of clinical trial consortia to enable randomised, double blind, controlled clinical trials in these rare diseases has led to major treatment advances resulting in reduced morbidity and mortality. Basic science research has enabled treatable causes such as viruses and genetic defects to be discovered to enable cures in many cases of diseases which were once uniformly fatal. New challenges in this area are the ability to identify, treat and monitor patients with IgG4-related disease more effectively, identify strategies to prevent or safely treat irAEs caused by cancer immunotherapy without disrupting the anti-tumour effects, increase support for research registries and sample repositories to promote basic research as well as collaborative clinical research for these rare diseases, and improve access to groundbreaking but expensive biologic therapies. Recognition that rather than a monophasic illness, GCA is a chronic disorder with only a minority of patients achieving long-term remission, has thrown down the gauntlet for deeper understanding of its pathogenesis to discover new therapeutic targets. Finally, given that immune mechanisms are involved in vasculitis mimics with or without an identifiable trigger, such as TAO, further research may identify treatment targets for disease amelioration. Application of big data bioinformatic methodology incorporating genomic, epigenetic, transcriptomic and metabolomic data, holds promise to advance our understanding of the pathogenesis and management of vasculitis.

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Chapter 17
Sepsis and Septic Shock

Benjamin Reddi

Key Learning Points

- Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection
- Sepsis is common and associated with high mortality
- Sepsis involves activation of both inflammatory and anti-inflammatory pathways promoting broad-ranging dysregulation of cardiovascular, coagulation, neuronal, bioenergetic, endocrine and other systems
- Early source control plus rational, timely antibiotic selection are crucial to maximise survival
- Management of circulatory shock is complex, therapy should be titrated and responsive to individual patient parameters

17.1 Introduction and Definitions

Sepsis is the primary cause of death from infection. It has been traditionally conceptualised as an excessive host inflammatory response provoked by infection and until recently *sepsis* was defined as the development of two or more systemic inflammatory response syndrome (SIRS) criteria (Box 17.1) as a consequence of infection, and *severe sepsis* as sepsis complicated by organ dysfunction [1]. Sepsis and severe sepsis were considered increasingly perilous stages of a pathobiologic natural history culminating in *septic shock* 'sepsis-induced hypotension persisting despite adequate fluid resuscitation' and death.

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Box 17.1
SIRS (systemic inflammatory response syndrome) defined as two or more of:

- Temperature >38 °C or <36 °C
- Heart rate >90/min
- Respiratory rate >20/min or $PaCO_2$ <32 mmHg
- Leucocyte count >12,000/mm^3 or <4000/mm^3 or >10% immature bands

Table 17.1 Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score

	Respiratory	Cardiovascular	Liver	Coagulation	CNS	Renal
Score	PaO_2/FiO_2 mmHg	Mean arterial pressure/ catecholamines	Bilirubin µmol/L	Platelets $\times 10^3/\mu L$	Glasgow coma score	Creatinine µmol/L
0	≥400	MAP ≥70	<20	≥150	15	<110
1	<400	MAP <70	20–32	<150	13–14	110–170
2	<300	Dopamine <5[a] or any dobutamine dose	33–101	<100	10–12	171–299
3	<200[b]	Dopamine 5.1–15[a] or adrenaline ≤0.1[a] or noradrenaline ≤0.1[a]	102–204	<50	6-9	300–440 or urine output <500 mL/day
4	<100[b]	Dopamine >15[a] or adrenaline >0.1[a] or noradrenaline >0.1[a]	>204	<20	<6	>440 or urine output <200 mL/day

[a]Catecholamine doses are given as µg/kg/min for at least 1 h
[b]With respiratory support

Recently this paradigm has been challenged. Not only is sepsis now recognised to involve activation of both inflammatory and anti-inflammatory pathways but, in addition, broad-ranging dysregulation of cardiovascular, coagulation, neuronal, bioenergetic, endocrine and other systems. These manifestations are not captured by a simple inflammation-based definition and, not surprisingly, the SIRS-based definition shows poor divergent and convergent validity in identifying patients at risk of poor outcome [2, 3].

Improved understanding of the pathobiology of sepsis is recognised in the development of the Third International Consensus Definitions for Sepsis and Septic Shock (SEPSIS-3) which recommend that sepsis be defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [4]. Organ dysfunction is defined as an increase in the Sequential [Sepsis-related] Organ Failure Assessment (SOFA) score of 2 points or more (Table 17.1) [5] and 'infection' as the invasion of sterile tissue by organisms resulting in infectious pathology. Thus defined, sepsis is associated with an in-hospital mortality >10%. Septic shock is defined as a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality. Patients

with septic shock can be identified with a clinical construct of sepsis with persisting hypotension requiring vasopressors to maintain MAP ≥65 mmHg and having a serum lactate level >2 mmol/L despite adequate volume resuscitation. With these criteria, hospital mortality is in excess of 40% [4]. Although the SEPSIS-3 definition better discriminates those patients with presumed infection at high risk of poor outcome, there remain challenges. No simple and unambiguous clinical criteria or biological, imaging, or laboratory features uniquely identify a septic patient and it is not clear how a clinician identifies a 'dysregulated host response' at the bedside. Furthermore, commonly no causative organism is identified and the diagnosis of infection, and thus sepsis, remains presumed. Nevertheless, the new definition utilises objective, easily obtained variables, reflects the complex pathobiology of sepsis and identifies a population of patients with infection at high risk of death. It is widely accepted as the basis for sepsis research and quality assurance.

17.2 Epidemiology

A recent meta-analysis found a population incidence rate of around 288/100,000-person years for hospital treated sepsis. Poor representation of low and middle-income countries in the published data notwithstanding, the authors extrapolate global estimates of 31.5 million cases of sepsis per annum with 5.3 million attributable deaths [6]. Data from the USA indicate that the incidence of septic shock has been increasing over the last decade and as many as 50% of patients hospitalised with septic shock die [7] with survivors frequently suffering marked long-term cognitive decline and functional impairment [8]. Patients frequently require intensive care unit (ICU) management making this condition a significant financial burden.

Risk factors for developing sepsis include extremes of age (<2 or >55 years), concurrent chronic and serious illness (such as cancer, diabetes), impaired immunity (including breach of natural barriers: burns, indwelling lines, surgical wounds etc.) and protein calorie malnutrition.

17.3 Aetiology

A causative organism may only be identified in as few as 50% of patients with sepsis [9]. Likely organisms vary according to the primary site of infection, mode and location of acquisition, immune and vaccination status of the host and local microbial ecology. Hence, a reasoned history often suggests likely culprits and antimicrobials can be tailored accordingly.

Accurate contemporary, global information regarding primary sites of infection and causative agents for sepsis are lacking. A recent study enrolling over 3000 patients with septic shock from Europe, Australasia and Saudi Arabia identified the

most common primary site of infection to be the respiratory tract (34%) followed by abdomen (25%), blood (17%), urinary tract (8%) and skin and soft tissue (7%) [10]. Although less common, CNS and endocardial infections are notable for their high morbidity and mortality. An international point prevalence study of infections in ICU patients found similar proportions although in contrast to the septic shock group respiratory tract infections accounted for 64% of infections [11]. In the latter study, microbiological culture results were positive in 70% of infected patients; 62% of the positive isolates were gram-negative organisms, 47% were gram-positive, and 19% were fungi. Patients who had longer ICU stays prior to the study day had higher rates of infection, especially infections due to resistant *Staphylococcus*, *Acinetobacter*, *Pseudomonas* and *Candida* species.

Antimicrobial resistance patterns vary enormously around the world and even between neighbouring institutions. Organisms resistant to practically all commonly used antimicrobials have now been described and empiric antibiotic regimes need to reflect this unfortunate trend.

17.4 Pathogenesis

17.4.1 Innate Immunity in Sepsis

The first response to an invading pathogen is mediated by the innate immune system. Pattern recognition receptors (such as Toll-like receptors (TLRs) and C-type lectin receptors) on the surface of macrophages, neutrophils, dendritic cells and natural killer cells recognise structurally conserved molecules that are broadly shared by pathogens such as lipoteichoic acid, peptidoglycan, lipopolysaccharide, flagellin, viral RNA and mannan (a fungal cell wall carbohydrate). Intracellular pattern recognition receptors (such as NOD-like receptors) which recognise conserved bacterial and viral molecular patterns have also been identified. As well as these pathogen associated molecular patterns (PAMPs), receptors also bind endogenous ligands, termed *damage* associated molecular patterns (DAMPs), that enter the microenvironment when host cells undergo non-apoptotic death. These molecules, which include fragments of nuclear and mitochondrial DNA, cytosolic heat shock proteins and chromatin associated high mobility group box 1 (HMGB1) instigate the inflammation associated with trauma, burns and pancreatitis. Pattern recognition receptor signalling activates transcription factors such as NFκB which induce inflammatory cytokine expression and promote immune cell maturation and proliferation [12]. Humoral elements of the immune system such as complement and the contact system of the coagulation cascade also bind to conserved elements of the microbial surface and contribute to innate immunity and the inflammatory milieu [13].

Cytokines and complement fragments attract and activate local and circulating immune cells which amplify the inflammatory cascade and exert direct microbicidal activity by liberating substances such as lysozyme and reactive oxygen species

(ROS). As well as direct antimicrobial activity, many cells of the innate immune system (for example macrophages and dendritic cells) act as antigen presenting cells, displaying antigen bound to major histocompatibility complex (MHC) to T-cells of the adaptive immune system. T-helper cells co-ordinate both the ongoing innate immune response and a more antigen specific T- and B-lymphocyte response.

17.4.2 Immunosuppression in Sepsis

Most patents who die of sepsis in the ICU have unresolved foci of infection at post-mortem. Furthermore, patients admitted to hospital with sepsis frequently develop secondary nosocomial infections often with minimally virulent or opportunistic pathogens such as *Stenotrophomonas*, *Acinetobacter*, *Candida* or reactivated *Cytomegalovirus*. It has been suggested that following an initial pro-inflammatory phase, sepsis manifests a subsequent anti-inflammatory phase [14]. In support of this hypothesis it has been shown that inflammatory cells extracted from patients with sepsis produce *less* tumour necrosis factor α (TNFα), interleukin-1 (IL-1) and IL-6 in response to lipopolysaccharide than controls. Furthermore, levels of the anti-inflammatory cytokine IL-10 are elevated in patients with sepsis and the IL-10/TNFα ratio correlates with mortality. Delayed type hypersensitivity reactions are impaired in sepsis and patients dying of sepsis exhibit profound apoptosis-induced loss of CD4 and CD8 T-lymphocytes, B-lymphocytes and dendritic cells with a shift towards immunoregulatory T-lymphocyte phenotype. Notably, therapies which suppress inflammatory cytokine activity such as TLR-4 and TNFα antagonists have failed to improve outcomes. These findings suggest that sepsis and septic shock does not always reflect a maladaptive hyper-inflammatory response to infection but, at least in some cases, may identify a group of patients generating an inappropriate immune suppressive, anergic phenotype. Indeed preliminary data indicate that T-lymphocyte stimulating and anti-apoptotic agents (such as IL-7 and programmed death ligand-1 antagonism, respectively) might be more effective [14].

17.4.3 Microvascular Alterations in Sepsis

TLRs are also expressed on endothelial cells where they promote leucocyte trafficking, tissue factor pathway activation and endothelial permeability. Endothelial permeability is also increased by leucocyte and platelet derived arachidonic acid derivatives, platelet activating factor (PAF), complement products and glycocalyx shedding. Tissue oedema not only leads to hypovolaemia and increased blood viscosity but impairs oxygen and metabolite diffusion, changes tissue architecture, blocks capillary blood flow and lymphatic drainage, and is associated with renal, cerebral and cardiac dysfunction [15]. Glycocalyx shedding as a consequence of bacterial toxin and leucocyte mediated damage further exacerbates tissue oedema

and has been particularly noted in the kidney, liver and lung. Glycocalyx shedding also interferes with endothelial shear stress flow monitoring and vasomotor regulation.

Microvascular perfusion is further compromised by a shift to a local prothrombotic profile: IL-6, ROS and bacterial toxins reduce ADAMTS-13 (a disintegrin and metalloproteinase with thrombospondin motifs) activity resulting in highly thrombotic von Willebrand Factor multimers. TNFα promotes tissue factor expression on macrophages and endothelial cells. Tissue factor pathway inhibitor, thrombomodulin and protein C activity are reduced, whilst plasminogen activator inhibitor is increased. Meanwhile endothelial cell damage and apoptosis leaves pro-thrombotic extracellular matrix exposed and inflammatory cells secrete PAF. The net consequence is a tendency for thrombus formation and microvascular occlusion [16] although reduced platelet function has also been documented [17]. Cross-talk between the coagulation and immune systems promoting amplification and dissemination of local coagulopathy can lead to the systemic consumptive coagulopathy characteristic of disseminated intravascular coagulation.

Microvascular occlusion can also arise from the release of neutrophil extracellular traps (NETs), webs of chromatin barbed with antimicrobial proteins which trap and kill microorganisms. Whilst beneficial in containing and clearing infection, NETs have been observed to occlude capillaries and exacerbate tissue damage [18]. In addition, regional vasomotor regulation (including metabolic and myogenic regulation) is impaired by glycocalyx damage, endothelial damage, downregulation of endothelial nitric oxide synthase (eNOS) and secretion of local vasoconstrictors such as endothelin and thromboxane A_2. NETs, clots, reduced erythrocyte deformability, leucocyte aggregates, extrinsic compression from tissue oedema and vasomotor dysregulation lead to a decrease in the number of functional capillaries and heterogeneity in microvascular perfusion with blood shunted past islands of ischaemic, hypoxic tissue through areas of relatively luxuriant flow. Patchy ischemia of this nature can be directly visualised in myocardium, renal cortex, gut and other vascular beds [19].

17.4.4 Mitochondrial Dysfunction in Sepsis

Mitochondria are responsible for oxidative phosphorylation and ATP synthesis, regulation of ROS and control of apoptosis. Activity of complex I, II and IV of the mitochondrial electron transport chain is significantly reduced in critically ill patients with a corresponding decrease in ATP levels, possibly under the influence of ROS, NO, TNFα and IL-1. Whether this is an adaptive response to limit oxygen consumption, ROS-mediated damage, or to promote aerobic glycolysis, or a consequence of cell injury which contributes to multiorgan dysfunction in sepsis, remains uncertain [20].

Microvascular shunting and mitochondrial dysfunction underlie the observation that an oxygen extraction deficit and multi-organ dysfunction (MOD) can persist

despite resolution of macrovascular haemodynamic variables. Evidence of tissue hypoxia frequently co-exists with globally supranormal oxygen delivery and elevated mixed venous oxygen saturation.

17.4.5 Pathological Vasodilation in Septic Shock

Septic shock is characterised by systemic hypotension and tissue hypoperfusion. Uncontrolled arterial vasodilation dissipates the head of pressure required to appropriately distribute blood flow, whilst reduced venomotor tone (coupled with fluid loss through leaky endothelium) reduces stressed venous volume, ventricular preload and cardiac output.

Pathological vasodilation is a manifestation of both increased baseline vessel calibre and reduced sensitivity to endogenous and administered vasoconstrictors. Inflammatory cytokines promote the production of NO from the cationic amino acid L-arginine by activating inducible NOS (iNOS) in neutrophils and vascular smooth muscle cells. Compared to eNOS, iNOS produces far greater amounts of NO (nanomolar rather than picomolar) and lacks feedback control. iNOS inhibitors improve vascular tone and blood pressure in patients with septic shock but not their outcome [21], perhaps reflecting the complexity of trying to interrupt an endogenous pathway which exerts both useful and toxic effects. Endothelium derived prostacyclin, the anaphylotoxins C3a and C5a (products of the complement cascade), PAF, adrenomedullin and other endogenous vasodilators all contribute to pathological vasodilation. Septic shock is associated with initial spikes in plasma corticosteroid and vasopressin levels followed by relative deficiencies in both [22, 23], whilst ROS oxidise and deactivate circulating noradrenaline. Moreover, even in vitro, sensitivity to α_1-receptor agonists is reduced in arterioles from animals with septic shock potentially as a consequence of receptor downregulation, increased myosin phosphatase activity and increased ATP sensitive K^+ channel conductance [24, 25].

17.4.6 Sepsis Induced Cardiac Dysfunction

In early sepsis, cardiac output may be impaired despite normal contractility because of reduced preload secondary to venodilation, capillary fluid leak and inadequate diastolic filling time. This may be partly overcome with fluid loading and judicious vasopressor use. Sepsis is also associated with elevation in pulmonary vascular resistance, a possible consequence of endothelial injury; elevated right ventricular afterload contributes to impaired RV contractility and RV dilation (which may, in turn, compromise LV filling). In up to 50% of cases of sepsis, however, intrinsic myocardial systolic and diastolic function are depressed as part of the MOD syndrome complicating sepsis. Postulated mechanisms of myocardial dysfunction in sepsis and septic shock are listed in Table 17.2 [26, 29]. Significantly,

Table 17.2 Mechanisms of myocardial dysfunction in sepsis [26, 27]

Pathophysiological mechanism	Causative factors
Downregulated β_1-adrenergic receptors and downstream pathway activity	NO, TNFα, IL-1, other 'myocardial depressant factors (MDF)
Dysregulated Ca^{2+} transients, Ca^{2+} leak from sarcoplasmic reticulum	NO, ROS, MDF DAMPs, PAMPs binding to TLR Leucocyte binding to cardiomyocyte adhesion molecules [28]
Direct cardiomyocyte injury	Bacterial toxins, ROS, leucocyte degranulation, NETs, microvascular dysfunction, MDF
Suppressed mitochondrial activity, reduced oxygen utilisation and loss of redox homeostasis	DAMPs, PAMPs binding to TLR ROS, NO, cytokines, MDF
Mechanically compromised contraction and relaxation	Myocardial oedema

cardiomyocyte death is a rare event in sepsis and does not explain the degree of functional depression observed. A fundamental question is the extent to which observed alterations are protective versus maladaptive. For instance, NO reduces myocardial oxygen consumption, acts as a free radical scavenger, promotes coronary blood flow and improves ventricular compliance [29]. Interestingly survivors tend to have lower ejection fraction and larger LV end diastolic volume (EDV) than non-survivors suggesting that operating with higher ventricular preload is somehow protective in sepsis despite being energetically less favourable in health [27].

17.4.7 Other Sepsis Induced Organ Dysfunction

Sepsis is a multisystem disorder which can cause dysfunction in any organ system. Examples include encephalopathy, acute respiratory distress syndrome (ARDS), acute kidney injury (AKI), acute liver injury, loss of gut integrity, polyneuropathy and myopathy.

Up to 65% of patients with septic shock will develop AKI. Once thought to be primarily driven by microcirculatory insufficiency, it is now recognised that AKI often develops in the context of normal or high renal blood flow. Although microvascular dysfunction and local inflammation can drive tubular injury the surprisingly bland histopathology and often rapid recovery suggest that some of the tubular dysfunction observed is functional and perhaps adaptive; cytokines, PAMPs and DAMPs induce cell cycle arrest and downregulate metabolism [28]. Encephalopathy frequently accompanies sepsis and is mediated by cerebral endothelial changes with increased blood:brain barrier permeability, ischaemic lesions, microabscesses and microglial activation [30]. Common themes in the multi-organ dysfunction (MOD)

syndrome are a causative role for inflammatory cytokines and PAMPs/DAMPs, microvascular and endothelial dysregulation, suppressed mitochondrial activity and often functional impairment rather than necrosis, the latter suggesting that some elements of MOD reflect adaptive changes to reduce cellular stress. Genetic evaluation is a promising future research direction, as certain polymorphisms in genes coding for cytokines are associated with more severe MOD.

17.5 Clinical Manifestations

Taking a rational history of symptoms, risk factors and exposures, accompanied by systematic clinical examination, often identifies the presence and source of infection. Patients progressing to sepsis typically present with dysregulated temperature, tachycardia, tachypnoea and leucocytosis/leucopaenia with organ dysfunction manifest as altered mental status (restlessness, delirium, obtundation), respiratory failure, oliguria, ileus, jaundice or bleeding diathesis. Although a classical transition from a hyperdynamic circulation with bounding pulses and warm peripheries to a low cardiac output state, with cold, mottled skin, lactic acidosis and narrow pulse pressure is recognised, patients can present, and die, at any point along this spectrum. Hypotension must be considered in the context of pre-morbid blood pressure and the influence of chronic illness and concurrent medication use recognised. For instance, a patient with coronary artery disease will be less tolerant of diastolic hypotension, and beta-blocker therapy may mask tachycardia.

17.6 Investigation

Investigation should focus on the anatomic source of infection, the causative organism and complications of the primary infection. Laboratory signs of sepsis per se are often non-specific and arise from the underlying cause of sepsis or consequent hypoperfusion and/or organ dysfunction. Common findings are listed in Box 17.2. Both C-reactive protein and procalcitonin (PCT) have similar sensitivity (0.77 vs. 0.78), specificity (0.79) and area under the receiver operating curve (0.85 vs. 0.86) for identifying sepsis [31]. Serum PCT is elevated in response to bacterial infection and falls during recovery. Furthermore, levels are prognostic. A recent randomised controlled trial and subsequent meta-analysis of over 4000 patients concluded that overall mortality from sepsis was reduced when antimicrobial initiation, de-escalation or cessation was guided by algorithms incorporating PCT measurement [32, 33].

Sterilisation of blood cultures can occur within minutes of administering antibiotics and it is recommended that, provided substantial delay to antimicrobial administration is not incurred, blood, cerebrospinal fluid, sputum, urine or wound cultures

Box 17.2
Laboratory findings consistent with the presence of sepsis

- Leukocytosis or leukopenia
- Normal white cell count with >10% immature forms
- Thrombocytopaenia
- Elevated prothrombin and activated partial thromboplastin time, low fibrinogen, elevated fibrin degradation products
- Hyperglycaemia
- Elevated urea and creatinine
- Hyperbilirubinaemia, hypoalbuminaemia, deranged liver enzymes
- Elevated troponin
- Adrenal insufficiency (hyponatraemia, hyperkalaemia, normal anion gap acidosis)
- Euthyroid sick syndrome
- Elevated C-reactive protein
- Elevated pro-calcitonin (see text)
- Elevated lactate (tissue hypoperfusion, catecholamine driven aerobic glycolysis, reduced pyruvate dehydrogenase activity, mitochondrial dysfunction)

be taken before antibiotics are given when sepsis is suspected. Identifying the organism permits de-escalation of antibiotic therapy reducing antibiotic resistance, side effects, costs and potentially mortality [34]. It is recommended that two or more sets (aerobic and aerobic) of blood cultures be taken. Blood cultures may be drawn together, and yield has not been shown to be improved if timed to temperature spikes [35]. Diagnostic methods such as polymerase chain reaction assays and microarrays are being translated into clinical practice and will hopefully better inform antibiotic prescribing [36].

Other investigations that may be valuable include plain X-ray, CT or MR imaging, echocardiographic examination of heart valves, bronchoalveolar lavage or aspiration of body fluids (e.g. joints).

17.7 Treatment

Sepsis and septic shock are medical emergencies. Resuscitation and treatment should take place immediately and concurrently. The concept of ‘Early Goal Directed Therapy’ (EGDT) was applied to management of septic shock following a seminal study published over a decade ago suggesting that protocolised fluid administration, vasopressor therapy, inotrope use and blood transfusion to achieve target mean arterial pressure, central venous pressure and central venous oxygen

saturation was associated with improved survival [37]. Although the benefits of 'protocolised care' in this single centre study were not replicated in subsequent larger, multi-centre trials this may reflect in part an improvement in the 'usual care' afforded to patients with sepsis and septic shock [38]. The EGDT concept served to establish sepsis as a clinical entity demanding timely, multifaceted treatment and triggered an effective research program which has culminated in the development of the evidence based Surviving Sepsis Campaign (SSC) Guidelines [39] and a progressive reduction in sepsis mortality. Unless otherwise specified the management approach described in this chapter reflects the recommendations of these evidence based guidelines.

17.7.1 Initial Resuscitation

As with any acutely unwell patient the airway should be immediately assessed and secured if necessary. Adequate arterial oxygenation should be achieved using supplementary oxygen or assisted ventilation as appropriate. Severe sepsis is the most common cause of ARDS. A lung protective ventilation strategy [40] should be used with consideration of prone positioning, neuromuscular blockade and judicious titration of positive end-expiratory pressure.

Circulatory management is a critical and challenging aspect of sepsis and septic shock management. In patients with septic shock an initial fluid bolus of 30 mLs/kg administered within the first 3 h appears safe and is recommended, albeit on limited evidence. More rapid infusion may be warranted in some patients. Crystalloids and colloids appear similarly efficacious [41] although hydroxyethyl starch (HES) is no longer recommended since HES 130/0.42 administration has been associated with poorer outcomes [42]. Balanced crystalloid solutions are a rational option [43, 44]. Where patients have already received substantial volumes of crystalloid there may be a case for continuing fluid resuscitation with albumin solution [39]. Following initial fluid resuscitation, blood pressure, heart rate, tissue perfusion (including brain, kidney, gut, skin), plasma lactate and mixed venous oxygen saturation (SvO_2) are re-evaluated to identify whether circulatory insufficiency persists and to guide the decision between further fluid therapy, vasopressor use or inotropy.

The potential benefit of volume expansion, related to an increase in cardiac output and oxygen delivery, must be balanced by the risk of aggravating lung and tissue oedema. Fluid therapy should be carefully titrated, prescribing limited volumes with regular re-evaluation are preferable to large volumes with infrequent monitoring. Using static measures of fluid status such as central venous pressure to guide fluid administration in sepsis has been shown to be physiologically flawed and clinically unreliable [45–47]. Instead, dynamic assessment predicts more accurately which patients are likely to respond to a fluid bolus with an increase in stroke volume. This can be achieved by passive leg raise, assessment of cardiopulmonary interactions or directly measuring the change in cardiac output following fluid

administration [48, 49]. Latterly, recognising the association between positive fluid balance and mortality in sepsis [50], a concept of 'de-resuscitation' has gained currency, emphasising the need to limit fluid administration in patients who are no longer shocked or fluid responsive, and to instigate diuresis once vasopressors are weaned.

In shocked patients with an inadequate or detrimental response to fluid therapy, vasopressor support may be warranted. Below a critical mean arterial pressure (MAP) threshold autoregulation is exhausted and vital organ perfusion is limited by MAP. Multiple studies have evaluated the benefits of different MAP targets in patients with septic shock treated with vasopressors, ranging from 65 to 85 mmHg. Higher pressures have been associated with greater cardiac output, varying effects of tissue perfusion indices and higher rates of arrhythmia [51–53]. The only study powered to identify mortality difference found no benefit in targeting 65 rather than 85 mmHg, but a reduction in the need for renal replacement therapy amongst patients with chronic hypertension in the higher target pressure group [54]. Although an initial blood pressure target of 65 mmHg is reasonable for most patients, higher targets might be considered in patients with a history of chronic hypertension in whom the effective autoregulation range is shifted towards higher pressures. However, blood pressure is not an ends in itself. Once a pressure target is attained, the patient should be re-evaluated for adequacy of tissue perfusion using the clinical indices described above (end-organ function, lactate etc.) and the target reconsidered accordingly. Lactate clearance in particular appears to be associated with outcome and resuscitation paradigms centred on promoting resolution of lactic acidosis have shown promise [55]. Once euvolaemia is established, noradrenaline is recommended as a first line vasopressor augmenting both vasomotor tone and cardiac output (through an increase in cardiac preload and contractility) [56]. Vasopressin is noradrenaline-sparing but has an unclear effect on patient-centred outcomes. Vasopressin is an option for cases of refractory vasodilatory shock but its use can be complicated by splanchnic, myocardial and digital ischaemia [57, 58]. Exogenous angiotensin II has also shown promise as a therapeutic option in vasodilatory shock [59]. Because vasopressor sensitivity is variable throughout the circulation, and some regions are already relatively ischaemic, administering high doses of exogenous vasopressors risks critically impairing vital organ perfusion especially if hypovolaemia and reduced cardiac output are not addressed.

Although septic shock may be associated with augmented or 'hyperdynamic' circulation because of reduced systemic vascular resistance and tachycardia, some patients have either pre-existing or sepsis-related ventricular dysfunction. Echocardiography is an important early adjunct to clinical examination. Findings should be interpreted in the context of the prevailing loading conditions, which greatly influence stoke volume and ejection fraction and may be profoundly deranged in sepsis. Systolic dysfunction may respond better to inotropic support and strategies to augment cardiac output [60] rather than further fluid or vasopressor administration. Dobutamine is a rapidly effective, titratable inotrope which improves indices of tissue perfusion, although it's use can be complicated by tachyarrhythmia and excessive vasodilation. Dosage should be titrated by monitoring indices of

tissue perfusion in response to manipulation of cardiac output. As noted above, normal/high SvO_2 can be misleading in the presence of impaired oxygen extraction and may not reliably identify impaired cardiac output. Conversely, low SvO_2 (<65%) suggests low cardiac output. Levosimendan promotes contractility, relaxation and reduces pulmonary artery pressure whilst circumventing the β-receptor and exhibiting a favourable oxygen consumption profile. However, it is costly, difficult to titrate and associated with arguably worse outcomes than standard care [61].

Preliminary human studies indicate that selective β_1-adrenergic receptor blockade (titrated to keep heart rate < 95 bpm) may improve cardiac function, lactate clearance, microvascular blood flow and even survival [62]. Putative mechanisms include augmented diastolic filling and improved myocardial efficiency, inhibition of inflammatory and pro-apoptotic pathways, and mitigating myocardial catecholamine toxicity and cytosolic Ca^{2+} overload.

The use of vasoactive and inotropic agents usually requires continuous invasive arterial pressure monitoring and frequently central venous access. Pulse contour analysis devices or echocardiographic monitoring may be helpful. Because of the need for frequent re-evaluation and advanced physiologic monitoring, patients with severe sepsis and septic shock are generally best managed in an intensive care environment.

17.7.2 Antimicrobial Therapy

The benefits of early initiation of antimicrobial therapy in patients with septic shock are widely recognised. Each hour delay in delivery of antibiotic is associated with a mortality cost [63] and administration within 1 h is recommended as a minimum target. Furthermore, indices of organ dysfunction and length of stay are adversely affected by delayed antibiotic therapy [39]. The data relating timing of antibiotics and outcome in patients with less severe sepsis is less clear. Sepsis is often overdiagnosed [64] and in light of data suggesting that overtreatment may increase mortality [65] it is arguable that when a patient is not shocked and has a lower probability of infection, spending time gathering more data to confirm the diagnosis and obtain appropriate microbiology samples is justifiable [66].

Initial antibiotic cover should have broad activity against the likely causative pathogens. Inappropriate empiric therapy is associated with up to five-fold increase in mortality [67]. The choice will be determined by factors such as the patient's clinical presentation, immune status, recent healthcare facility admission and antibiotic use, and local microbial ecology. Patients who are considered vulnerable to infection with multidrug-resistant gram-negative pathogens such as *Pseudomonas*, *Acinetobacter* or *Klebsiella* should receive a supplementary gram-negative agent in addition to the empiric regime to increase the probability that they receive at least one active agent. Similarly, vancomycin, teicoplanin, or another anti-MRSA agent can be used when risk factors for MRSA exist. Broadening cover in this way, to ensure potential organisms are covered by one of the administered agents, is distinct from the concept of

'combination therapy' where antibiotics of different classes are combined to cover the suspected organism with more than one agent. The latter approach may be of benefit in patients with septic shock but is not recommended for most other serious infections. Infections associated with high levels of toxin mediated pathophysiology, such as streptococcal toxic shock syndrome or necrotising soft tissue infections may benefit from treatment with both a bactericidal and protein synthesis inhibiting antibiotic (e.g. penicillin and clindamycin). In patients at risk of invasive *Candida* infection consider empiric antifungal cover, generally echinocandins in critically ill patients.

Once the causative pathogen and it's antimicrobial sensitivities are defined antibiotic therapy should be refined to the narrowest spectrum effective agent. If cultures are negative monitored de-escalation may be appropriate based on clinical response. Data regarding de-escalation is scant but there are clear benefits to avoiding unnecessarily prolonged antibiotic exposure for both society and the patient. Unnecessarily prolonged antibiotic therapy promotes *C difficile* colitis, superinfection with multi-drug resistant organisms and is associated with increased mortality [34]. Treatment with appropriate antibiotics for 7–10 days appears adequate for most serious infections complicated by sepsis or septic shock. Rapid clinical resolution and early effective source control may permit shorter courses (for instance surgically treated intra-abdominal sepsis and uncomplicated pyelonephritis). There may be benefit in longer courses when source control cannot be achieved, clinical resolution is slow, immune deficiency exists or in cases of *S aureus* bacteraemia or fungal infection. Daily assessment for de-escalation of antibiotic therapy is effective and may improve survival. Input from an infectious disease specialist is worthwhile. As noted above, PCT measurement can be used to shorten duration of antibiotic therapy in patients with resolving sepsis or support the decision to discontinue antibiotics when ongoing evidence for a presumptive diagnosis of sepsis is weak.

17.7.3 Source Control

When septic shock arises from infection in a site amenable to source control the time to intervention is a critical determinant of survival [68, 69]. Specifically abscess drainage, debridement of infected tissue, removal of an infected device or definitive control of an ongoing source of microbial contamination should be undertaken as soon as possible; in some cases haemodynamic stability cannot be achieved without source control and prolonged efforts at medical stabilisation may be counterproductive.

17.7.4 Corticosteroids

Septic shock appears to be associated with relative adrenal insufficiency [22]. The putative benefits of corticosteroid supplementation are two-fold: (1) A generally

anti-inflammatory effect—corticosteroids suppress chemokine and cell adhesion molecule expression, antigen presentation and lysosome degranulation, phospholipase A_2 mediated production of arachidonic acid derivatives and pro-inflammatory cytokine production (e.g. TNFα, IL-1, IL-2, IL-8). Lymphocytic T-cells are shifted from pro-inflammatory Th1 to anti-inflammatory Th2 phenotype [70]. (2) Augmenting vasomotor tone—corticosteroids promote α_1-adrenergic receptor sensitivity by increasing inositol trisphosphate (IP_3) release and potentiating downstream sensitivity to protein kinase C. These effects are independent of changes in plasma catecholamine levels, adrenergic receptor expression or ligand binding affinity. Conversely, angiotensin II activity is augmented by increased angiotensinogen levels and increased receptor density. More generally, corticosteroids promote vasomotor tone by inhibiting endothelial nitric oxide release, stimulating endothelin release, and modulating vascular smooth muscle cell Na^+ and Ca^{2+} handling [71]. Outcome data regarding corticosteroid therapy in septic shock is conflicting and it is unclear whether steroid therapy in septic shock is associated with a mortality benefit. Nevertheless, administering 200 mg hydrocortisone daily to vasopressor dependent, mechanically ventilated patients with septic shock appears to be safe and associated with quicker resolution of shock [10, 72]. Evaluating adrenal function in septic shock (e.g. synacthen test, plasma cortisol assay) is difficult to interpret and not generally useful.

17.7.5 Adjunctive Therapies

Intensity of supportive therapies such as blood sugar control and dosing of renal replacement therapies are now informed by randomised control trial data [73, 74]. However, data supporting more specific adjunctive therapies for sepsis and septic shock have proven elusive. Strategies which enhance the activity of potentially beneficial endogenous pathways (activated protein C [75], thrombomodulin, intravenous immunoglobulins, granulocyte colony stimulating factor, anti-Enterobacteriaceae monoclonal antibody) or suppress activity of deleterious pathways (TNFα, IL-1, NO, arachidonic acid derivatives, TLR-4) have been evaluated in patients with septic shock. They have not shown convincing outcome benefit, and none are recommended for use in septic shock. Observational studies indicated that HMG CoA reductase inhibitors (statins) were associated with improved outcomes in sepsis, potentially through favourable modulation of the immune, endothelial and coagulation systems. However, prospective studies have not borne out this initial promise [76]. Blood purification techniques (such as haemofiltration) are effective at removing inflammatory mediators but do not appear to improve outcome from septic shock and a recent randomised controlled study indicates that polymyxin B haemoperfusion does not benefit patients with septic shock [77]. Biologically, perhaps this is not surprising—inflammatory and immune pathways have built in redundancy and mediators are pleiotropic. Targeting one mediator may not significantly redefine the immunological landscape and even if it does,

the effects may be unpredictable and dynamic. Furthermore, most of these adjunctive therapies, like steroids, are borne of the 'maladaptive inflammation' paradigm of sepsis and may be less rational in the context of subtler immune dysfunction.

17.8 Conclusion

Sepsis and septic shock are common and often fatal. Our understanding of the pathophysiology is maturing but remains incomplete. We are beginning to appreciate the tensions between adaptive and maladaptive changes, excessive inflammation and immunoparesis, but have not yet identified specific therapies which can resolve them. Patients are best served by a sound application of microbiological principles and physiologically rational approach to supportive therapy.

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Chapter 18
Pathophysiology of Reperfusion Injury

Prue Cowled and Robert Fitridge

Key Learning Points

- Ischaemia-Reperfusion Injury (IRI) is the paradoxical local and remote tissue damage which occurs when perfusion is returned to ischaemic tissue.
- Tissue ischaemia occurs when perfusion is less than what is required to provide tissues with adequate oxygen, glucose and other substances which are required for normal cellular function. This results in anaerobic glycolysis which inhibits ATP production and generates lactic acid. This results in tissue acidosis, failure of cellular ATP-dependent pumps and efflux of cellular potassium. A large number of pro-inflammatory genes and transcription factors are up-regulated during ischaemia.
- When oxygen returns to ischaemic tissue, xanthine oxidase catalyses the conversion of hypoxanthine to superoxide anions, which are subsequently converted to reactive oxygen and nitrogen species (such as superoxide anion, hydrogen peroxide, hydroxyl radical, nitric oxide and peroxynitrite).
- Reactive oxygen species (ROS) cause lipid peroxidation of cellular membranes and the generation of pro-inflammatory eicosanoids. ROS also activate endothelial cells which express adhesion molecules such as E-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), endothelial-leukocyte adhesion molecule-1 (ELAM-1), plasminogen activator inhibitor-1 (PAI-1), tissue factor and interleukin-8 (IL-8).
- Eicosanoids are straight-chain polyunsaturated fatty acids that are derived from arachidonic acid and include prostaglandins, thromboxanes and leukotrienes. They are signalling molecules that modulate inflammation, immune responses and tissue blood flow.

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- Nitric oxide is synthesised from L-arginine by nitric oxide synthases (NOS). There is an initial surge in NO levels in early ischaemia (first 15 min) due to transient activation of endothelial NOS (eNOS). During early reperfusion, the endothelial damage results in loss of eNOS function and a fall in NO production. This predisposes to vasoconstriction and potentially exacerbation of tissue ischaemia.
- Endothelins are potent vasoconstrictors which are produced by the vascular endothelium and are elevated in IRI.
- Hypoxia and IRI induce numerous cytokines such as tumour necrosis factor-α, interleukins-1, -6 and -8 and platelet activating factor, which are released systemically and play a key role in the development of systemic inflammatory response syndrome and multi-system organ failure associated with IRI.
- Neutrophils are activated during IRI and play a key role in tissue damage. Activated neutrophils generate ROS and a number of proteases, such as matrix metalloproteinases (MMPs).
- Selectins are transmembrane molecules expressed by activated neutrophils, activated endothelial cells and platelets. Selectins mediate the initial neutrophil-endothelial interaction/adhesion. Integrins and members of the immunoglobulin supergene family (e.g. ICAM-1, VCAM-1, platelet-endothelial cell adhesion molecule-1) mediate firm adhesion of activated neutrophils to the endothelium and allow their extravasation into the tissues. The ROS and proteases generated by infiltrating neutrophils contribute to tissue damage in IRI.
- Complement activation contributes to local and systemic IRI.
- Toll-like receptors (TLR) are proteins involved in the innate immune system. TLR signalling is a key mediator of inflammation during IRI.
- Matrix metalloproteinases (MMPs) are enzymes which can degrade extracellular matrix. Elevated MMP-2 and -9 have been detected in cerebral, skeletal muscle and pulmonary IRI, resulting in destruction of basement membrane collagen and laminin.
- The no-reflow phenomenon is the failure of microvascular perfusion following reperfusion due to plugging of post-capillary venules by activated leukocytes and increasing permeability of endothelium resulting in exudation of fluid and proteins with subsequent increase in interstitial pressure.
- The complexity of the pathophysiology of IRI has resulted in the failure of therapeutic interventions to have been adopted into clinical practice. Ischaemic preconditioning holds theoretical promise in reducing IRI in vascular surgery but further larger trials are needed. Ischaemic post-conditioning and the effects of volatile anaesthetic agents on ameliorating the severity of IRI also require further investigation.

18.1 Introduction

Ischaemia-Reperfusion Injury (IRI) is defined as the paradoxical exacerbation of cellular dysfunction and death, following restoration of blood flow to previously ischaemic tissues. Reestablishment of blood flow is essential to salvage ischaemic

tissues, however reperfusion itself paradoxically causes further damage, threatening function and viability of the organ. IRI occurs in a wide range of organs including the heart, lung, kidney, gut, skeletal muscle and brain and may involve not only the ischaemic organ itself but may also induce systemic damage to distant organs, potentially leading to multi-system organ failure. Reperfusion injury is a multifactorial process resulting in extensive tissue destruction. The aim of this review is to summarise these molecular and cellular mechanisms and thus provide an insight into possible windows for effective therapeutic intervention.

18.2 Ischaemia

18.2.1 ATP and Mitochondrial Function

Ischaemia occurs when the blood supply is less than the demand required for normal function, resulting in deficiencies in oxygen, glucose and other substances required for metabolism. Derangements in metabolic function begin during this ischaemic phase. Initially, glycogen breakdown by mitochondrial anaerobic glycolysis produces two molecules of adenosine triphosphate (ATP) along with lactic acid, resulting in a decrease in tissue pH, which then acts by negative feedback to inhibit further ATP production (Fig. 18.1). ATP is then sequentially broken down into adenosine diphosphate (ADP), adenosine monophosphate (AMP) and inosine monophosphate (IMP) and then further into adenosine, inosine, hypoxanthine and xanthine (Fig. 18.2, upper panel).

At the cellular level, a lack of ATP production causes ATP-dependent ionic pumps, including the Na^+/K^+ and Ca^{2+} pumps, to fail and the transmembrane ionic gradients are lost. Consequently, cytosolic sodium content rises, drawing with it, a volume of water to attempt to maintain the osmotic equilibrium and resulting in hydroponic swelling of the cells. To maintain the ionic balance, potassium ions escape from the cell into the interstitial space (reviewed in [1]). Calcium is released from the mitochondria into the cytoplasm and into extracellular spaces, thereby activating mitochondrial calcium-dependent cytosolic proteases including calpain, which then converts the cellular enzyme xanthine dehydrogenase to xanthine oxidase (Fig. 18.2, upper panel). Phospholipases are also activated during ischaemia, degrading membrane lipids and increasing the levels of circulating fatty acids.

18.2.2 Gene Expression During Ischaemia

As well as metabolic derangements, ischaemia induces expression of a large number of genes, which play a major role in the tissue's response to ischaemic damage. An RNA expression microarray analysis, using mouse soleus muscle rendered ischaemic by femoral artery ligation, found that expression of 962 genes was induced

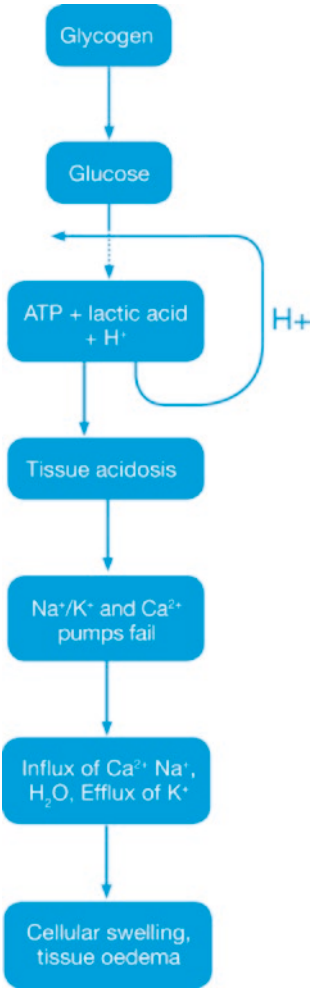

Fig. 18.1 Dysregulation of metabolic pathways during ischaemia. Anaerobic glycolysis during ischaemia results in negative feedback which inhibits ATP production, thereby inducing tissue acidosis, calcium influx and tissue oedema

and 327 genes were repressed [2]. The activated genes were largely clustered into mediators of inflammation, cytokine genes and genes associated with immune cell infiltration. The repressed genes were largely involved in energy production, including mitochondrial respiration and fatty acid oxidation. A similar study in rhesus monkeys confirmed that hindlimb ischaemia activated a range of pro-inflammatory genes, including interleukin-6 (IL-6), selectins and genes involved in immune responses to tissue damage [3].

Hypoxia itself activates a number of genes, particularly transcription factors, including activating protein-1 (AP-1), hypoxia-inducible factor-1 (HIF-1) and nuclear factor-kappaB (NF-κB). HIF-1 then activates transcription of other genes such as vascular endothelial growth factor (VEGF), erythropoietin and glucose transporter-1, which all play an important role in the cells' adaptive responses to

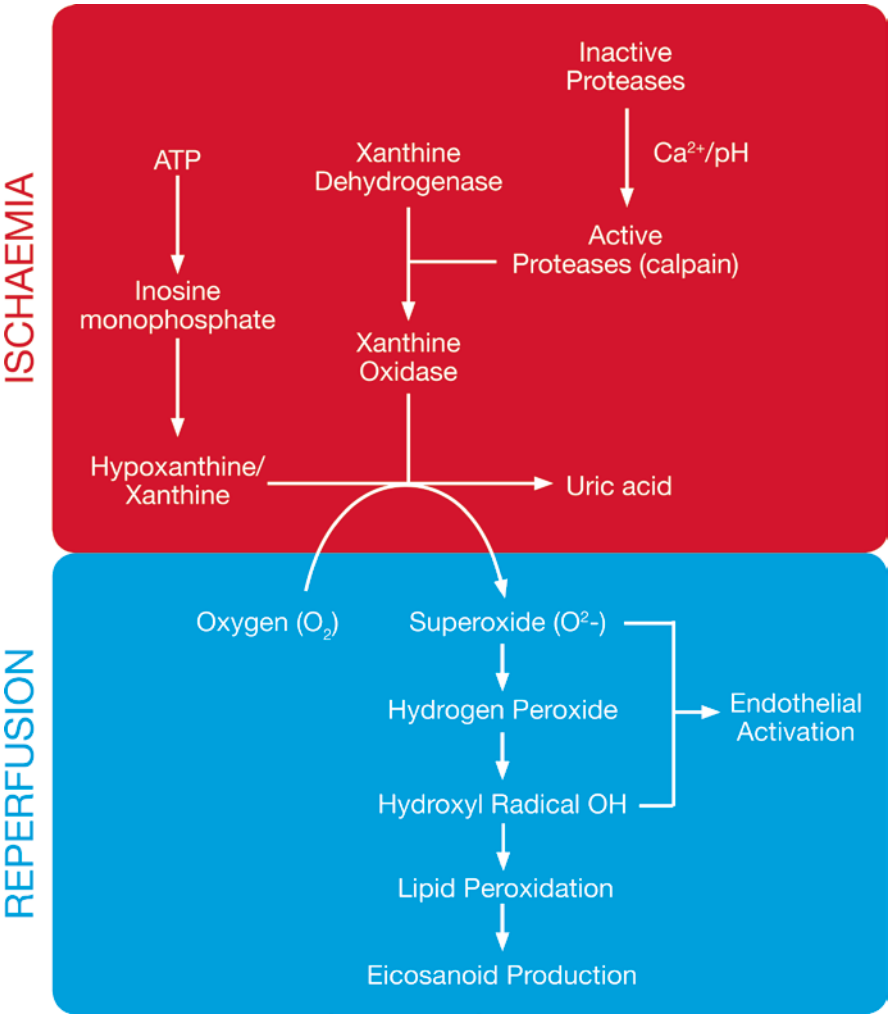

Fig. 18.2 Generation of reactive oxygen species during reperfusion. During ischaemia, ATP is degraded and xanthine dehydrogenase converted to xanthine oxidase. In the presence of fresh oxygenated blood, xanthine oxidase catalyses the conversion of hypoxathine to highly reactive and toxic superoxide anions with urea as a by-product. Superoxide then reacts with H^+ to initiate the production of both hydrogen peroxide and the hydroxyl radical, which ultimately mediate lipid peroxidation and tissue damage

hypoxia (reviewed in [4]). Expression of both HIF-1 and cyclo-oxygenase-2 (COX-2) was also induced in the lungs of rats subjected to haemorrhagic shock. COX-2 may promote the inflammatory response through the rapid and exaggerated production of nitric oxide and prostaglandins, thereby contributing to organ damage [5]. Activation of NF-κB occurs during both the ischaemic and reperfusion phases and will therefore be discussed below.

Table 18.1 Reactive oxygen species involved in IRI

Major ROS
Superoxide anion (O_2^-)
Hydrogen peroxide (H_2O_2)
Hydroxyl radical ($OH^{\bullet}$)
Nitric oxide (NO)
Peroxynitrite ($ONOO^-$)
Minor ROS
Lipid hydroperoxide
Lipid peroxyl radical
Lipid alkoxyl radical
Thiol radical
Sources of ROS during IRI
Xanthine oxidase system
Activated neutrophils
Mitochondrial electron transport chain
Arachidonic acid metabolism
Auto-oxidation of catecholamines

18.3 Reperfusion

18.3.1 Reactive Oxygen Species

Table 18.1 illustrates the major reactive oxygen species (ROS), which play a role in tissue damage during IRI and are the sources of generation of these species. Reactive oxygen species have a destructive role in mediating tissue damage during IRI. During ischaemia, the degradation of ATP produces hypoxanthine (Fig. 18.2, upper panel). Once the ischaemic tissue is reperfused, an influx of molecular oxygen catalyses xanthine oxidase to degrade hypoxanthine to uric acid and thereby liberating the highly reactive superoxide anion (O_2^-) (Fig. 18.2, lower panel). Superoxide is subsequently converted to hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$) (Fig. 18.2, lower panel). The major consequence of hydroxyl radical production is peroxidation of the lipid structures of the cell membranes resulting in the production and systemic release of proinflammatory eicosanoids, disruption of cell permeability and ultimately cell death. During IRI, ROS also activates endothelial cells, elevating the activity of the transcription factor, NF-κB. Once activated, the endothelial cell produces E-selectin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), endothelial-leukocyte adhesion molecule (ELAM-1) plasminogen activator inhibitor-1 (PAI-1), tissue factor and interleukin-8 (IL-8). These adhesion molecules contribute to important interactions between the neutrophil and the endothelium and will be discussed in more detail later.

Superoxide anions can be detected within ischaemic muscle and also in the venous effluent of reperfused limbs [6], suggesting an additional role for superoxide damage to distant organs during skeletal muscle reperfusion injury. Xanthine

oxidase is located within a spectrum of cell types and tissues to varying degrees, indicating widespread distribution and differing susceptibility to oxidant-mediated IRI. Inhibition of xanthine oxidase activity, by administration of allopurinol prior to ischaemia, reduces the production of superoxide and hence reduces the severity of reperfusion injury in animal models using a range of tissues including skeletal muscle, brain and gut. In an animal model of hindlimb IRI, allopurinol significantly reduced serum tumour necrosis factor-alpha (TNF-α) levels, indicating that systemic inflammation was inhibited [7]. Results in humans are also promising. A systematic review [8] provided evidence that allopurinol was effective in some studies in reducing the severity of post-operative cardiac dysfunction and arrhythmias after coronary artery bypass grafting, although larger trials are needed. Studies in other clinical settings of IRI remain limited.

18.3.2 Eicosanoids

As discussed above, ROS initiate the lipid peroxidation of cellular membranes, releasing arachidonic acid, the main substrate for the production of prostaglandins, thromboxanes and leukotrienes (Fig. 18.2, lower panel). These derivatives of arachidonic acid are collectively known as the eicosanoids which are signalling molecules that modulate inflammation, immune responses and tissue blood flow and play a major role in the pathophysiology of IRI.

Prostaglandins, synthesised from arachidonic acid via the cyclo-oxygenase pathway, have a protective vasodilatory effect in IRI. However, since prostaglandins are short-lived molecules, their rapid depletion subsequently leads to uninhibited vasoconstriction, reduced local blood flow and exacerbation of ischaemia. The potential of prostaglandins to ameliorate the degree of metabolic and tissue derangement following IRI has been demonstrated in various tissues. In an animal model of myocardial IRI, the prostacyclin analogue, iloprost, demonstrated protective effects against IRI by increasing myocardial contractility [9]. In a placebo-controlled trial of human liver transplantation, administration of prostacyclin was shown to improve postoperative liver graft function [10]. Patients who received prostacyclin demonstrated better post-operative myocardial oxygen consumption after coronary artery bypass surgery and also improved muscle blood flow following skeletal muscle IRI [11].

The ILAILL trial randomised 300 patients treated surgically with acute limb ischaemia to adjuvant iloprost bolus plus iloprost infusion for 4–7 days, versus placebo. At 90 day follow-up, the combined incidence of death and amputation was not significantly different between the groups. However, the overall incidence of fatal plus major cardiovascular events was 33% in the placebo cohort and 23% in the iloprost group [12]. In the cohort of patients over 70 years of age, those treated with adjuvant iloprost had a significantly reduced risk of death (6% versus 15%, $p = 0.03$) and combined death and major amputation (16% versus 27%, $p = 0.03$) [13]. Similarly, in a randomised trial, 204 patients who underwent open and endovascular procedures for acute limb ischaemia were randomised to receive adjuvant liposomal

prostaglandin E1 for 12–14 days or placebo. The combined incidence of perioperative mortality and any major adverse limb events (MALE) at 6 months was significantly lower in the prostaglandin-treated cohort (5.1% versus 13.2%, $p < 0.05$) [14]. Despite these positive studies, adjuvant prostaglandin analogues have not been adopted into routine practice as part of the management of acute limb ischaemia.

Plasma thromboxane A_2, also synthesised from arachidonic acid, increases within minutes following skeletal muscle IRI, thus promoting vasoconstriction and platelet aggregation. These events coincide with a rapid rise in pulmonary artery pressure and a subsequent increase in pulmonary microvascular permeability [15], which correlates with sequestration of polymorphonuclear cells in the lungs. In animal models of lower limb IRI, thromboxane synthase inhibitors and synthetic thromboxane A_2 receptor antagonists prevented pulmonary leuko-sequestration, thereby increasing blood flow to reperfused tissues and preserving tissue viability and function [16]. Together these studies suggest that administration of thromboxane A2 antagonists may improve limb salvage rates after surgery for acute ischaemia but clinical evidence is currently lacking.

Leukotrienes are also synthesised from arachidonic acid through the activation of 5-lipoxygenase and participate in the inflammatory cascade of IRI [17]. Leukotrienes lead to local and systemic injury by their direct proinflammatory action on endothelial and smooth muscle cells and indirectly by their effects on neutrophils. The leukotrienes C_4, D_4 and E_4 modify the endothelial cytoskeleton, leading to increased vascular permeability and also enhance smooth muscle contraction, resulting in vasoconstriction. The lung produces leukotrienes following remote IRI. The direct effects of leukotrienes on pulmonary microvessels leads to increased permeability, transient pulmonary hypertension and induction of the endothelium to produce thromboxane, resulting in additional vasoconstriction. The leukotriene B_4, released by activated neutrophils, leads to further pulmonary neutrophil accumulation.

The administration of 5-lipoxygenase synthesis inhibitors has been successfully used in animal studies to attenuate IRI. Such agents abolished the elevations in leukotrienes B_4 and C_4 and inhibited neutrophil infiltration normally induced by IRI, reducing mucosal permeability [18]. In a study of a mouse model of stroke, administration of a lipoxygenase inhibitor 2 h after induction of stroke, significantly reduced infarct volume and haemorrhage area [19]. However, there is currently no up to date information on their use in a clinical context.

18.3.3 Nitric Oxide

Nitric oxide (NO) is a signalling molecule synthesised from L-arginine by the nitric oxide synthase enzyme (NOS) of which there are three types, constitutive (cNOS), inducible (iNOS) and endothelial (eNOS). An initial surge in NO level in the first 15 min of the ischaemic phase is due to transient eNOS activation. This is followed during early reperfusion by a general decline in endothelial function and loss of

functional eNOS, so that NO production falls, along with an increased production of reactive oxygen species. eNOS-derived NO is also necessary for the maintenance of vascular tone. The reduction in eNOS levels that occurs in IRI may therefore predispose to vasoconstriction, a common response seen in IRI. The second surge in NO production is largely due to cytokine-mediated up-regulation of iNOS after about 3 h of reperfusion (reviewed in [20]).

The pathophysiological role of nitric oxide in reperfusion injury is variable, being dependent on the nature of its generation and appears to be tissue specific. In some instances, NO acts as an anti-oxidant and, in others, combines with the superoxide anion to form the peroxynitrite radical, a potent promoter of lipid peroxidation and hence cellular membrane disruption (reviewed in [20]). Manipulation of nitric oxide production during IRI, using a range of techniques, has recently provided considerable evidence for a principal role for nitric oxide in the aetiology of IRI. Myocardial IRI has been well studied, with paradoxical results, where low doses of NO were found to be protective and high doses harmful. The influence of NO in skeletal muscle IRI has been less well characterized, with some studies suggesting that NO may potentiate cytotoxicity and others suggesting a beneficial role for NO in extremity IRI. In skeletal muscle IRI, NO production may be deleterious and inhibition of NOS activity using a non-specific NOS inhibitor greatly reduced the severity of muscle damage [21].

The assessment of experimental data derived from pharmacological NOS inhibition is difficult due to the non-specificity of NOS inhibitors and administration of these inhibitors at differing times during the injury merely adds to the complexity. In essence, augmentation of NO delivery may be beneficial with respect to protection, particularly in the ischaemic and early reperfusion phase. Inhibition of the iNOS-induced surge in NO production at later times during reperfusion also mediates defense against IRI-induced tissue damage. However, in the clinical setting, systemic distortion of NO kinetics by administering NOS inhibitors would be likely to induce wide-ranging physiological disturbances. Further investigations will be needed to define a role for NOS inhibition in ameliorating the severity of IRI and local administration of these inhibitors may be required.

18.3.4 Endothelin

Endothelins are potent peptide vasoconstrictors produced by the vascular endothelium. Hypoxia, growth factors, angiotensin II and noradrenaline all stimulate endothelin production resulting in Ca^{2+}-mediated vasoconstriction. Endothelin-1 is elevated following skeletal muscle IRI during both the ischaemic and reperfusion phases and mediates capillary vasoconstriction, neutrophil aggregation and neutrophil-endothelial interactions. Endothelin-1 inhibitors, including bosentan and tezosentan, inhibit neutrophil infiltration, increase functional capillary density, microvascular perfusion and hence tissue viability and function following IRI [22]. In a rat model of spinal cord IRI, bosentan increased expression of VEGF and its

receptors. However, a reduction in IRI-induced damage to the spinal cord was not reported [23]. These inhibitors are not in clinical use and have not been tested in clinical trials.

18.3.5 Cytokines

Hypoxia and IRI both induce the expression of numerous cytokines, including tumour necrosis factor-alpha (TNF-α), interleukins -1 -6 and -8 and platelet activating factor (PAF), in association with elevations in activity of the transcription factor complex, NF-κB (reviewed in [24]). These cytokines are released systemically and are thus important in the development of systemic inflammatory response syndrome and ultimately multi-system organ failure.

Tumour necrosis factor-alpha (TNF-α) is a 17-kDa pro-inflammatory cytokine produced by activated macrophages, monocytes, T-lymphocytes, natural killer cells and fibroblasts. It is a potent chemoattractant and early response cytokine, which subsequently induces expression of IL-1, IL-6, IL-8, and PAF. Elevated serum levels of TNF-α have been detected during cerebral and skeletal muscle IRI and are known to increase neutrophil sequestration and permeability following pulmonary IRI. Serum TNF-α levels increased rapidly in an animal model of aortic clamping, thus inducing up-regulation of iNOS, which increased NO production in the lungs, leading to more severe lung damage [25]. In the same study, inhibition of TNF-α activity prior to limb ischaemia decreased pulmonary NO production and reduced the severity of IRI. TNF-α can also induce the generation of ROS and enhances the susceptibility of the vascular endothelium to neutrophil mediated injury, by inducing the expression of ICAM-1, which mediates binding of neutrophils to the activated endothelium.

Numerous studies in animal models attest to the potential of TNF-α blockade as a therapeutic modality to reduce the severity of IRI. Anti-TNF-α antibody protected against IRI-induced pulmonary injury in a rat model by preventing microvascular damage. The introduction of humanised antibodies including etanercept and infliximab, has provided encouraging results in the treatment of other TNF-α-mediated inflammatory diseases, including a number of forms of arthritis and inflammatory bowel disease (reviewed in [26]). However, clinical trials to test the efficacy of TNF-α blockade in human IRI have not yet been reported.

The cytokines interleukin -1 alpha and -beta (IL-1α and IL-1β) are produced during IRI by tissue macrophages, neutrophils and the vascular endothelium. IL-1α is a potent chemotactic agent and stimulates neutrophil infiltration during hepatic IRI. Both IL-1α and TNF-α also increase levels of expression of ICAM-1 on the vascular endothelium. Exposure of endothelial cells in culture to IL-1α and TNF-α induces synthesis of E-selectin, which then interacts with L-selectin on the neutrophil surface leading to rolling on the endothelial surface. Permanent adhesion of the neutrophil to the endothelium is then mediated by expression of ICAM-1, IL-8 and PAF in the endothelial membranes (Fig. 18.3).

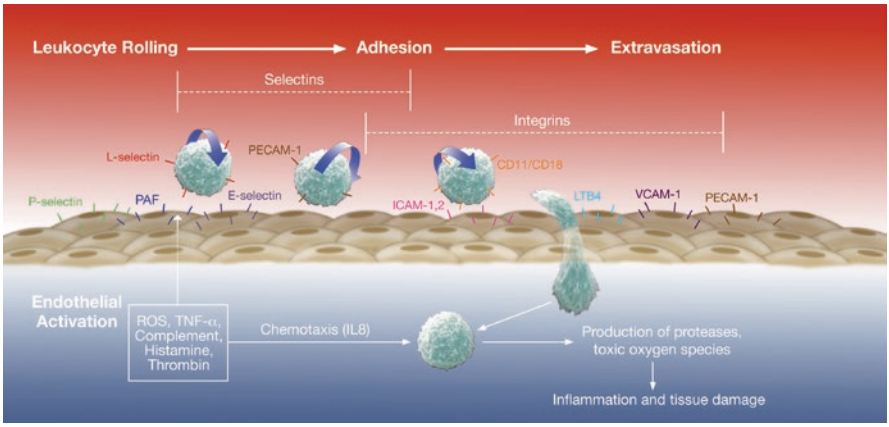

Fig. 18.3 Neutrophil rolling, adhesion to endothelium and extravasation. During reperfusion, activated neutrophils adhere to the activated endothelium and subsequently extravasate into surrounding tissue, resulting in proteolytic degradation of basement membranes. Activated neutrophils also generate toxic reactive oxygen species from molecular oxygen, contributing to tissue degradation during reperfusion

Numerous activating stimuli synthesised during IRI include H_2O_2, thrombin, leukotrienes C_4, and D_4, IL-1β, histamine, bradykinin and ATP; all of which induce the synthesis of PAF by monocytes, macrophages, neutrophils, eosinophils, basophils, platelets and endothelial cells. PAF functions as both an inter- and intra-cellular messenger, having three major effects, vasoconstriction, chemo-attraction, and increased microvascular permeability. PAF is rapidly produced following skeletal muscle and renal IRI with peak levels after 15 min of reperfusion. PAF enhances the binding of neutrophils to endothelial cells since a PAF-receptor antagonist has been shown to block adhesion of neutrophils to endothelial cells during IRI [27]. Similarly pre-treatment with the PAF inhibitor lexipafant reduced the severity of intestinal barrier dysfunction and pulmonary and liver permeability in a rat model of intestinal IRI [28]. However lexipafant is unlikely to be clinically useful as a pharmacotherapy for IRI since, alone, it failed to completely inhibit pulmonary endothelial damage after small bowel IRI in a rat model [29].

IL-6 is a proinflammatory 19-26 kDa protein produced by monocytes, fibroblasts, keratinocytes and endothelial cells in response to IL-1 and TNF-α. IL-6 primes and stimulates the respiratory burst in neutrophils, stimulates endothelial cell expression of ICAM-1 and increases endothelial permeability. IL-6 is produced in hypoperfused skeletal muscle in patients with peripheral arterial disease and is released from the gut into the systemic circulation during reperfusion in aortic aneurysm surgery [30]. In the setting of renal transplantation, IL-6 was released in large amounts from the reperfused transplanted kidney during the first 30 min of reperfusion [31].

IL-8 is a potent neutrophil chemotactic and activating factor. It is produced by monocytes, T cells, NK cells, fibroblasts, endothelial cells, eosinophils and neutrophils in response to IL-1, TNF-α, endotoxin, histamine and hypoxia.

The chemotactic activity of IL-8 induces diapedesis of activated neutrophils through the endothelium (Fig. 18.3). Elevated levels of serum IL-8 have been detected during early reperfusion following human lung transplantation and predict poor graft function [32]. An anti-IL-8 antibody prevented pulmonary neutrophil infiltration and tissue injury in a rabbit model of lung IRI [33].

18.3.6 Neutrophils and Endothelial Interactions

Neutrophils play a major role in tissue damage incurred during IRI. Activated neutrophils are a major source of ROS, which are generated through the activity of the membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex. Whilst oxidizing NADPH to NADP+, NADPH oxidase also reduces molecular oxygen to form the superoxide anion. Myeloperoxidase, stored in the azurophilic granules of neutrophils, converts hydrogen peroxide to toxic hypochlorous acid, which, in addition to its direct effects, is also capable of activating proteases. The activated neutrophils also secrete a number of proteases, including matrix metalloproteinases, which will degrade basement membrane and other tissue structures, contributing to the severity of tissue destruction.

Neutrophil infiltration is observed at sites of tissue damage [34, 35] and depletion of neutrophils before IRI reduces the severity of organ damage in a mouse model of liver IRI [36]. Depletion of neutrophils during cardiac surgery has been extensively investigated as a modality to reduce the severity of post-operative cardiac dysfunction with inconsistent results. Some studies have shown a reduction in markers of cardiac damage while others have been less successful in demonstrating a clinically relevant effect.

Selectins are a family of transmembrane molecules, expressed on the surface of leukocytes, activated endothelial cells and in platelets (reviewed in [37]). Selectins mediate the initial phase of neutrophil–endothelial cell interactions, often termed rolling (Fig. 18.3), which is essential for their subsequent adhesion and extravasation. L-selectin is expressed constitutively on the surface of neutrophils and initiates the reversible attachment of neutrophils to endothelial cells and platelets. Antibody-mediated blocking of L-selectin impairs the ability of neutrophils to roll on endothelial cells and reduces neutrophil infiltration following skeletal muscle and pulmonary IRI [38].

P-selectin is stored in the α-granules of platelets and the Weibel-Palade bodies of endothelial cells and is rapidly translocated to the cell surface along with PAF in response to thrombin, histamine, reactive oxygen species, complement and TNF-α. Typically, peak levels of endothelial P-selectin are detected 6 h after reperfusion. Endothelial P-selectin plays a vital role in the rolling of neutrophils along the activated endothelium. Activation of the endothelium by proinflammatory mediators also results in de novo transcription and synthesis of E-selectin. Expression of endothelial E-selectin is induced during both renal and cerebral IRI. The focal expression of E-selectin at sites of endothelial activation promotes neutrophil adhesion

and infiltration into adjacent tissues. In support of a vital role for E-selectin in mediating tissue damage during IRI, a study showed that antibodies against E-selectin reduced infarct size following cerebral IRI in mice [39].

Blocking the activity of selectins shows promise in ameliorating the severity of tissue damage in a number of animal models of IRI [40]. In support of their use in a clinical setting, a clinical trial (SELECT-ACS) tested the efficacy of administration of the P-Selectin antibody, inclacumab, administered before percutaneous coronary intervention, in reducing the severity of post-procedure myocardial damage. Blocking P-selectin with inclacumab significantly reduced the severity of myocardial damage and was most effective when given less than 3 h before the procedure [41].

The integrin and immunoglobulin supergene families of adhesion molecules mediate the strong adhesion of activated neutrophils to the endothelium and hence allow their subsequent extravasation during IRI. The integrins form a large family of cell surface adhesion molecules that mediate intercellular recognition and cellular binding to the extracellular matrix. The neutrophil β_2-integrin adhesion glycoprotein complex consists of a common polypeptide chain, CD18, which is non-covalently linked to three different α-polypeptide chains (CD11a, CD11b, CD11c). CD11a/CD18 is expressed on all leukocytes and mediates the attachment of stimulated neutrophils to the vascular endothelium through a specific interaction with ICAM-1 and ICAM-2. Chemotactic cytokines (IL-1, TNF-α) and ROS all induce neutrophil adherence to the endothelium by CD11/CD18-dependent mechanisms. The CD11b/18 complex on activated neutrophils interacts with ICAM-1 on the surface of the endothelial cell to mediate firm adhesion of neutrophils prior to their extravasation (reviewed in [42]). All of these molecules are required for the development of lung injury following skeletal muscle IRI. Using an anti-CD18 monoclonal antibody, inhibition of CD18-mediated leukocyte adhesion prevented vasoconstriction and increased microvascular permeability and vascular resistance in animal models of skeletal muscle IRI. However, despite encouraging animal studies, the clinical efficacy of blocking CD11/CD18-mediated interactions in IRI remains doubtful (reviewed in [43]). Clinical trials in humans failed to demonstrate any effect of CD11/CD18 in reducing infarct size following primary coronary angioplasty in the setting of acute myocardial infarction. A review published in 2005, [44] summarised the results from a number of clinical trials using antibodies to CD11/CD18, including for myocardial infarct and stroke, all of which failed to show any significant benefit to the patient and there have been no significant developments in the field since that date.

The immunoglobulin supergene family (ligands for integrins) contains a large number of molecules with multiple immunoglobulin-G-like domains. Several members of this family are involved in leukocyte-endothelial cell interactions including ICAM-1, VCAM-1 and platelet-endothelial cell adhesion molecule (PECAM-1). Levels of expression of ICAM-1 on endothelial cells are enhanced by exposure to circulating TNF-α that is generated in response to IRI. VCAM-1 was elevated during renal IRI in a mouse model but, unlike ICAM-1, was independent of TNF-α since renal IRI in TNF-α knockout mice also upregulated VCAM-1. PECAM-1 is expressed constitutively on platelets, leukocytes and endothelial cells. IRI induces

elevated PECAM-1 levels thereby enhancing activation of neutrophil-endothelial interactions mediated by β-integrins and exacerbating neutrophil extravasation and tissue damage.

The therapeutic potential of blocking the activity of adhesion molecules has been tested in a number of animal models with encouraging results. Using monoclonal antibodies, inhibition of ICAM-1 activity attenuated neutrophil adhesion in the liver, reduced pulmonary sequestration and oedema following skeletal muscle IRI and also reduced intestinal dysfunction following IRI [28]. Antisense oligonucleotides to ICAM-1 ameliorated renal IRI and prevented delayed graft dysfunction in a rat model of renal transplantation [45]. However, results obtained in clinical trials have not been as positive. A clinical trial which tested anti-ICAM-1 antibody therapy in ischaemic stroke (Enlimomab Acute Stroke Trial), concluded that this therapy was not an effective treatment and may significantly worsen stroke outcome, raising significant doubts regarding the efficacy of this therapeutic modality and there have been no further investigations since this date [46].

18.3.7 Complement Activation

Complement activation and deposition also contribute significantly to the pathogenesis of IRI. Rubin and colleagues have demonstrated reperfusion of skeletal muscle is associated with systemic depletion of the complement protein, factor B, indicative of activation of the alternative complement pathway [47]. The complex C5b-9 is also deposited into the endothelial cell membrane after IRI, leading to osmotic lysis [48]. Pulmonary damage following bilateral hind limb ischemia was significantly reduced when the soluble complement receptor was administered to rats, thus inhibiting complement activity [49]. In the clinical setting, a relationship has been demonstrated between the severity of multi-system organ dysfunction and degree of complement activation after aortic cross clamping [50].

Inhibition of the complement cascade has been demonstrated to improve outcomes following IRI in a number of different animal models. Complement depletion of circulating plasma improved the initial blood flow and decreased muscle necrosis and injury after ischaemia and prolonged reperfusion in dogs. Complement blockade also prevented leukocyte adhesion, leading to better capillary perfusion and muscle cell viability and attenuated the increase in permeability index in tissues [47]. Unequivocal evidence for the importance of complement activation during skeletal muscle IRI has been provided from experiments where limb ischaemia was induced in C5-deficient mice. These mice had approximately 50% less tissue damage than the wild-type animals [28]. An additive role of both complement and neutrophils in mediating skeletal muscle IRI has also been observed, with a greater reduction in histological damage in neutropenic C5-deficient animals than in neutropenic or C5-deficient mice alone [28]. These data continue to demonstrate the multifactorial nature of tissue damage induced during IRI since complement blockade alone failed to completely ameliorate tissue damage.

18.3.8 Toll-Like Receptors and the Innate Immune System

Toll-like receptors (TLRs) are a class of proteins that play a key role in the innate immune system and TLR signalling is an important mediator of the inflammatory response during IRI. In particular, TLR-4 has been increasingly recognized as playing a critical role in the pathogenesis of ischemia-reperfusion injury (IRI) [51]. TLR-4 recognises danger associated molecular patterns (DAMPs), protein complexes released from damaged and dying cells following IRI. DAMPS released from damaged cells include heat shock proteins (HSPs), some of which can enhance tissue damage during IRI while other HSPs may be protective [52]. Activation of TLR-4 by DAMPs also promotes the release of many of the proinflammatory cytokines described above, including IL-1β, IL-6 and TNF-α. TLR-4 also facilitates leukocyte migration and infiltration and activates the innate and adaptive immune system (reviewed in [53, 54]).

18.4 Tissue Destruction

18.4.1 Proteases and Metalloproteinases

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes that have the ability to degrade components of the extracellular matrix. Together with their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), they are the major physiological regulators of the extracellular matrix. MMPs are intimately involved in all processes that necessitate degradation or synthesis of the extracellular matrix and important roles for these enzymes have been identified in wound healing, periodontal disease, cancer metastasis and, of particular relevance, vascular disease including the development of aneurysms, atherosclerotic plaques and reperfusion injury.

Elevations of MMP-2 and MMP-9 have been detected following pulmonary, hepatic and cardiac IRI. MMPs are also elevated following cerebral IRI, corresponding with opening of the blood-brain barrier, degradation of the basal lamina, increased capillary permeability and cerebral oedema [55]. Definitive roles for MMP-9 in the pathophysiology of cerebral IRI have been demonstrated by using both selective MMP-9 inhibitors and MMP-9 knockout mice, which both significantly reduce cerebral infarct size. MMP-2 may have a late role in renal IRI with an elevation detected as late as 8 weeks after IRI. However the MMP inhibitor (Batimastat) did not alter the severity of IRI-induced renal dysfunction [56]. In contrast, the MMP inhibitor doxycycline, preserved renal function in a rat model of renal IRI [57].

Barr and co-workers [58] examined brain MRI scans from acute ischaemic stroke patients and correlated systemic plasma MMP-9 levels with a hyperintense acute reperfusion injury marker (HARM), measured by MRI 24 h later. Plasma MMP-9 was a significant predictor of elevated HARM measures, supporting the

hypothesis that elevated MMP-9 is associated with disruption of the blood-brain barrier after ischaemic stroke. These results raise the possibility that inhibition of MMP-9 may be a useful modality to reduce the severity of cerebral damage but further studies are required.

Studies in our laboratory have demonstrated both a local and systemic role for MMP-2 and MMP-9 in the degradation of type IV collagen in pulmonary tissues and in skeletal muscle following lower limb IRI [35]. Permanent ischaemia alone, without reperfusion, also resulted in elevation of MMP-2 and MMP-9, correlating with destruction of the basement membrane components, type IV collagen and laminin.

18.4.2 Apoptotic Cell Death During Ischaemia-Reperfusion Injury

Tissue destruction resulting from IRI can be due to either necrotic or apoptotic cell death. Apoptosis or programmed cell death is an active process characterized by a series of gene-directed events leading to a characteristic cell morphology, controlled DNA fragmentation and eventually death of the cell. The role of apoptosis in IRI-induced tissue damage has been widely investigated in recent years. Oxidative stress and the production of ROS will induce apoptosis, the characteristics of which can readily be recognised following cerebral IRI. Similarly, renal and cardiac IRI all result in detectable levels of apoptosis in the damaged tissue. Apoptosis therefore appears to play a fundamental role in cellular damage occurring during IRI in a number of tissues. However the role of apoptosis in skeletal muscle IRI remains controversial. Studies conducted in our laboratory, [34] in agreement with Knight and co-workers, [59] have failed to detect significant evidence of apoptosis in rat skeletal myocytes following IRI. This implicates a tissue-specific mechanism of cell death following IRI. Blocking the apoptotic cascade, using specific inhibitors directed against pro-apoptotic caspase enzymes, have been partially effective in animal models, reducing the severity and infarct size following hepatic and cardiac IRI.

18.4.3 No Reflow Phenomenon

No reflow is the failure of microvascular perfusion, following restoration of flow to previously ischaemic tissue. The cause of this phenomenon has not been fully elucidated (reviewed in [60, 61]) but is certainly multifactorial. Cytokines and activated neutrophils act synergistically to produce microvascular barrier dysfunction. The resultant increase in permeability leads to the exudation of fluids and proteins, increasing the interstitial pressure and decreasing the intravascular pressure. In addition, CD18-dependent leukocyte plugging produces partial occlusion of post-capillary venules, further contributing to no-reflow. Neutrophil depletion virtually abolishes the phenomenon in the myocardium, brain and skeletal muscle, confirming a vital role for neutrophils in no-reflow.

18.5 Therapeutic Approaches to IRI

18.5.1 Ischaemic Preconditioning

Ischaemic preconditioning consists of brief and repetitive episodes of IRI before the induction of sustained organ ischaemia and can be effective in reducing the severity of tissue damage. The preconditioning effect can be delivered remotely instead of to the target organ. This treatment could be useful in a number of operative settings including transplantation, coronary bypass grafting and elective major vascular surgical procedures where the onset of ischaemia can be tightly controlled. In these settings, brief extremity IRI (10 min) administered by tourniquet before surgery has been widely investigated and shows promise as a therapy to reduce the severity of IRI.

Animal models of a number of settings of IRI have been used to investigate mechanisms of ischaemic preconditioning but the basic molecular mechanisms remain unclear, probably due to the multiple signal transduction pathways involved in this phenomenon. However it is generally recognised that brief ischaemic preconditioning induces a cascade of intracellular kinases, which subsequently modify mitochondrial function. A recent study in a rat model of lower limb IRI illustrated clearly that two brief 10 min episodes of IRI before a full 60 min of ischaemia followed by reperfusion, was effective in reducing pro-inflammatory neutrophil-endothelium interactions. This effect was noted in both the lower limb itself and in remote tissues, illustrating the systemic nature of this phenomenon [62]. In a mouse model of hind limb IRI, preconditioning significantly reduced tissue damage in the limb itself and also in lung and small bowel. Preconditioned animals were also significantly protected against post-operative mortality [63].

A large number of clinical trials have also been reported which have investigated the efficacy of ischaemic preconditioning but with varying degrees of success (reviewed in [64, 65]). Remote preconditioning significantly protected against post-operative myocardial injury, myocardial infarction, and renal impairment [66]. An excellent ‘proof of concept” study of ischaemic preconditioning was reported in the setting of evolving ST-elevation acute myocardial infarction. Subjects were randomised while in the ambulance and received intermittent arm ischaemia during transport to hospital (four cycles of 5 min inflation and 5 min deflation of a blood-pressure cuff). The primary endpoint was the myocardial salvage index 30 days after primary percutaneous coronary intervention, measured by myocardial perfusion imaging. The data showed convincingly that remote ischaemic conditioning before hospital admission increased myocardial salvage [67].

A small randomised clinical trial aimed to determine if remote lower limb ischaemic preconditioning before endovascular aneurysm repair (EVAR) could reduce the severity of renal and cardiac damage [68]. A significant reduction in urinary biomarkers of renal injury was detected in the preconditioning cohort but this small pilot trial was unable to detect any effect on clinical endpoints. However, in the setting of open AAA repair where operative ischaemia is profound, promising results were obtained. In the context of vascular surgical procedures, a pilot randomised controlled trial reported on composite clinical endpoints for 200 patients

undergoing open AAA repair, EVAR, carotid endarterectomy or lower limb revascularisation. Patients were randomised to receive four cycles of 5 min of forearm ischaemia before surgery. The results, however, did not show any significant differences between outcomes in the groups and the authors suggest a larger trial is required [69]. A non-significant result was also recently reported in a similar patient cohort where no change in arterial stiffness could be detected as a result of preconditioning [70]. A recent systematic review and meta-analysis came to similar conclusions, stating that there was insufficient evidence that preconditioning improved outcomes after open AAA repair or EVAR, but the studies were underpowered and that there was considerable methodological heterogeneity [71]. Further studies are needed in larger trials to determine if remote preconditioning improves clinical outcomes despite this therapeutic modality appearing theoretically promising.

18.5.2 Ischaemic Post-conditioning

Ischaemic post-conditioning is defined as rapid sequential intermittent interruption of blood flow applied during the early moments of reperfusion. This interruption can be either local (to the ischaemic organ) or remote, typically where a limb is occluded for a short time. This technique is particularly relevant where the initial ischaemic insult could not have been predicted, thus a preconditioning approach to limiting tissue damage could not have been applied. Experimental animal models have been used to successfully show attenuation of organ injury, including the heart, spinal cord, brain, kidney, liver, muscle, lung and intestines (reviewed in [72]). The mechanisms of post-conditioning are not yet entirely clear but appear to involve multiple signalling pathways and molecules, including protein kinases, ROS, pro-inflammatory cytokines and NO, as well as alterations in mitochondrial function (reviewed in [73, 74]).

Animal models of particular relevance to vascular surgical procedures have been tested widely and results show promise as an effective therapy to reduce the severity of IRI. In a rat model of lower limb ischaemia induced by aortic clamping, rats underwent 180 min of ischaemia followed by post-conditioning consisting of six cycles of 10 s aortic occlusion followed by 10 s declamping at the beginning of reperfusion. Post-conditioning caused a significant reduction in both the severity of systemic inflammatory responses and degree of remote pulmonary and renal damage [75]. In a similar study in the rat, [76] 60 min infrarenal aortic cross-clamping followed by intermittent four times 15 s reperfusion-15 s ischaemic episodes before reperfusion, was effective in reducing production of ROS, leukocyte-endothelial activation and cytokine production. A more recent study in a rat model of myocardial IRI, demonstrated that remote postconditioning reduced the severity of myocardial apoptosis. Multiple signal transduction pathways were upregulated by remote postconditioning, including cytokine activity, Toll-like receptors and TNF-α [74].

Based on the experimental models, ischaemic postconditioning thus appears to show promise as an effective therapy in vascular surgery to reduce reperfusion injuries after aortic surgery and revascularization procedures (reviewed in [77]). Some clinical studies have verified these findings. Controlled limb reperfusion following

revascularisation for acute limb ischaemia resulted in lower levels of systemically circulating products resulting from muscle breakdown, including lactate and potassium levels [78]. However, this study did not determine if clinical outcomes were improved in these patients. In future studies, the duration of the occlusion and reperfusion periods will be critical to the degree of protection and further studies are needed to calculate useful algorithms to plan therapeutic strategies after a significant ischaemic insult.

18.5.3 Conditioning Effects of Volatile Anaesthetics

Anaesthetics have been widely demonstrated to reduce the severity of IRI-induced damage in the setting of myocardial ischaemia and reperfusion during cardiac surgery (reviewed in [79]). However, there is conflicting evidence regarding the relative contributions of preconditioning, conditioning during ischaemia and postconditioning to the significant cardioprotection provided by anaesthetics. The molecular mechanisms and signal transduction pathways involved in protection are an area of active investigation. A proteomic study demonstrated that volatile anaesthetics (isoflurane, sevoflurane or desflurane) induced long lasting changes in the expression of 106 proteins in the rat myocardium [80] and also in the hippocampus [81]. Mitochondrial protein expression after myocardial IRI was also significantly altered by sevoflurane preconditioning [82].

Anaesthetic-induced post-conditioning mechanisms are also multi-factorial. Volatile anaesthetics are known to inhibit neutrophil adhesion in the coronary arteries during the reperfusion phase, thereby inhibiting the inflammatory action of activated neutrophils in post-ischaemic tissues (Fig. 18.3). Evidence also suggests that inhibition by anaesthetics of the opening of the mitochondrial permeability pore may be a key mechanism of anaesthetic-induced preconditioning [83].

There is good clinical evidence for the cardioprotective effects of volatile anaesthetics during cardiac surgery. A meta-analysis examined randomized trials comparing volatile with non-volatile anaesthesia in coronary bypass surgery. There was no significant difference in myocardial ischaemia, myocardial infarct, intensive care unit length of stay or in-hospital mortality. However, patients receiving volatile anaesthetics had significantly higher cardiac indices, lower troponin I serum concentrations and a lower requirement for inotropic support [84]. A large multicentre study also provided excellent evidence that volatile anaesthesia significantly reduced mortality after coronary bypass grafting [85]. Good evidence for anaesthetic protection in vascular surgical settings other than in cardiac IRI is not currently available but is likely to be equally significant and should be actively investigated in the future.

18.5.4 Pharmacological Treatments

As discussed in many of the sections above, a wide range of pharmacological therapies have been tested in both animal models and in clinical practice. Although many

of the animal models show considerable promise in reducing the severity of IRI, results from clinical trials have uniformly been disappointing. A Cochrane Review reported on treatments to reduce IRI during liver resection under vascular control [86]. They identified 15 randomised trials, which examined 11 pharmacological interventions (methylprednisolone, multivitamin antioxidant infusion, vitamin E infusion, amrinone, prostaglandin E1, pentoxifylline, mannitol, trimetazidine, dextrose, allopurinol and a thromboxane A2 synthetase inhibitor). Although some therapies improved liver enzyme levels, there were no significant differences between the groups for mortality, liver failure, or perioperative morbidity. A second Cochrane review from the same authors [87] examined the effects of prostaglandin E1, pentoxifylline, dopexamine, dopamine, ulinastatin, gantaile, sevoflurane, and propofol during liver IRI and reached the same conclusion that there were no significant differences.

Statin therapies have been widely accepted into clinical practice and there is also considerable evidence, both experimental and clinical, that statins will reduce the severity of IRI in a range of settings. Statins inhibit a range of cellular responses to IRI-induced inflammation, including inhibition of NF-κB activity, which leads to decreased transcription of MMPs, adhesion molecules and cytokine genes. Binding of adhesion molecules on activated neutrophils to endothelial cell surface receptors is also blocked. Secretion of MMPs from activated neutrophils is also inhibited by statins. In the endothelium, levels of expression of eNOS mRNA are increased and the eNOS protein is activated, while expression of endothelin-1 is inhibited. All of these effects will ameliorate the severity of tissue damage during IRI (reviewed in [88]).

Trials of lower limb IRI in the rat were carried out in our laboratory and illustrated that pre-treatment for a week with simvastatin before IRI markedly protected both skeletal muscle and remote organs including the lungs and kidneys [21, 89]. A review [90] discussed the efficacy of statins to ameliorate IRI in patients undergoing a range of vascular surgical procedures. In the setting of infrainguinal bypass for peripheral arterial disease, the indications that statins may protect against IRI during surgery are not definitive with some conflicting results, although 1-year mortality was improved. Evidence for any effect of statin treatment on the severity of postoperative complications after AAA repair is lacking, although a retrospective observational study showed that all-cause mortality was reduced in those on long-term statin therapy [91]. However, since all vascular patients should be receiving statin treatment for secondary prevention of cardiovascular disease, prospective randomized trials to obtain definitive results can no longer ethically be performed.

Other pharmacological mediators have been tested as potential therapies to reduce IRI with varying results. N-acetyl cysteine, an anti-oxidant, was tested in a randomised controlled trial as an early intervention in STEMI patients who were about to undergo percutaneous coronary intervention. A significant reduction in infarct size was detected, supporting a potential therapeutic role of n-acetyl cysteine in reducing the severity of IRI [92]. Other drug therapies, including metoprolol [93] and cyclosporine [94], have been tested in clinical trials addressing myocardial IRI with conflicting results and further research is needed.

18.6 Summary

In summary, IRI is a highly complex series of interwoven pro-inflammatory and pathological events. The production, release and activation of cytokines, ROS, proteases and complement if left unchecked, leads to both local and systemic injury with potentially fatal consequences. The failure of therapeutic interventions to translate into routine clinical practice is a reflection of this complexity and redundancy within the system. New therapeutic agents directed towards multiple areas within this cascade may be required to overcome this difficult clinical challenge.

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Chapter 19
Abdominal Compartment Syndrome and Open Abdomen Treatment

Martin Björck

Key Learning Points

- Intra-abdominal hypertension (IAH) is the sustained or repeated pathological elevation of intra-abdominal pressure (IAP) > 12 mmHg.
- Abdominal perfusion pressure (APP) is the mean arterial pressure (MAP) minus IAP.
- Abdominal compartment syndrome (ACS) is the sustained IAP > 20 mmHg (with or without an APP <60 mmHg) which is associated with new organ dysfunction or failure.
- Intra-abdominal pressure is most commonly measured using the Foley manometer method, which can be performed in and out of the ITU environment.
- ACS occurs in up to 20% of cases of open and endovascular repair of ruptured AAA. A further cohort of patients managed with emergency ruptured AAA open repair will require prophylactic open abdomen treatment.
- Major risk factors for the development of ACS include fluid overload/generalised oedema, post-operative bleeding and bowel ischaemia.
- The duration of IAH before decompression laparotomy (DL) is associated with the frequency of development of acute renal failure and need for dialysis.
- Non-surgical management of IAH includes drainage of gastric contents, early enteral feeding, adequate pain relief, use of neuromuscular blockade, reducing fluid overload and the early use of a massive transfusion protocol in patients suspected to require 10 or more units of blood.
- A major problem associated with DL is lateralisation of the abdominal wall, which occurs when the musculature and fascia of the abdominal wall move laterally away from the midline with time. Vacuum-assisted wound closure and mesh-mediated fascial traction is widely used to manage ACS and is associated with high rates of primary delayed fascial closure.

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19.1 Introduction

The appreciation that a tense abdomen is life-threatening was first described in ancient Greece. In 1984, the vascular surgeon Irving Kron named the condition abdominal compartment syndrome (ACS). He described ACS following surgery for ruptured abdominal aortic aneurysm (RAAA) [1]. ACS is often a consequence of aggressive resuscitation after major bleeding, and thus it is partly an iatrogenic condition. The first international conference on Intra-Abdominal Hypertension (IAH) and the Abdominal Compartment Syndrome was held in 2004. The conference resulted in the publication of two important consensus documents describing the definitions [2], risk factors [2] and treatment guidelines [3]. These were later revised using the GRADE Methodology. The Updated Consensus Definitions and Clinical Practice Guidelines from the World Society of the Abdominal Compartment Syndrome were published in 2013 [4].

19.2 Definition of Intra-abdominal Hypertension (IAH)/ Abdominal Compartment Syndrome (ACS)

"*IAH is defined by a sustained or repeated pathological elevation in intra-abdominal pressure (IAP) >12 mmHg.*" This is the definition of IAH, as first stated in the 2006 consensus document [2], and was unaltered in the updated guidelines in 2013 [4]. It has been shown in both animal research and in clinical studies that an IAP above 12 mmHg negatively affects organ function, in particular renal function [5]. It is important to note that a single elevated value may be the result of the patient being in pain, or passing stools. This threshold for negative effects on organ function is important to consider in patients operated on for RAAA, since multiple prospective clinical studies have shown that it is uncommon that the IAP is <12 mmHg in the early postoperative period after open surgical repair (OSR) [6–8]. If hemodynamically unstable patients are treated with EVAR, the situation is quite similar [9].

Although the evidence based approach used in the revision of the Guidelines [4] did not find support for a sub-definition of low abdominal perfusion pressure (APP = MAP − IAP < 60 mmHg), it is a clinical observation that hypotensive patients are more sensitive to IAH. (APP = Abdominal Perfusion Pressure, MAP = Mean Arterial Pressure).

ACS is defined as a sustained IAP >20 mmHg (with or without an APP <60 mmHg) that is associated with new organ dysfunction/failure [4]". Again, the exact wording is important: "*a sustained IAP >20 mmHg*" means that the measurement has to be repeated at least once, and it needs to be associated with "*new organ dysfunction/failure*", with a temporally-associated deterioration of vital organ function. ACS is defined as the combination of this high IAP and its effect on vital organ function, never as a mere measurement of the pressure!

There are many ways to measure IAP. Most commonly IAP is measured in the bladder, intermittently or continuously. Our preferred method is the FoleyManometer method (Holtech Medical, Charlottenlund, Denmark) with the advantage that it can easily be applied outside of the ICU, a great advantage especially after EVAR for RAAA, since those patients seldom need to stay in the ICU after surgery (Fig. 19.1a, b).

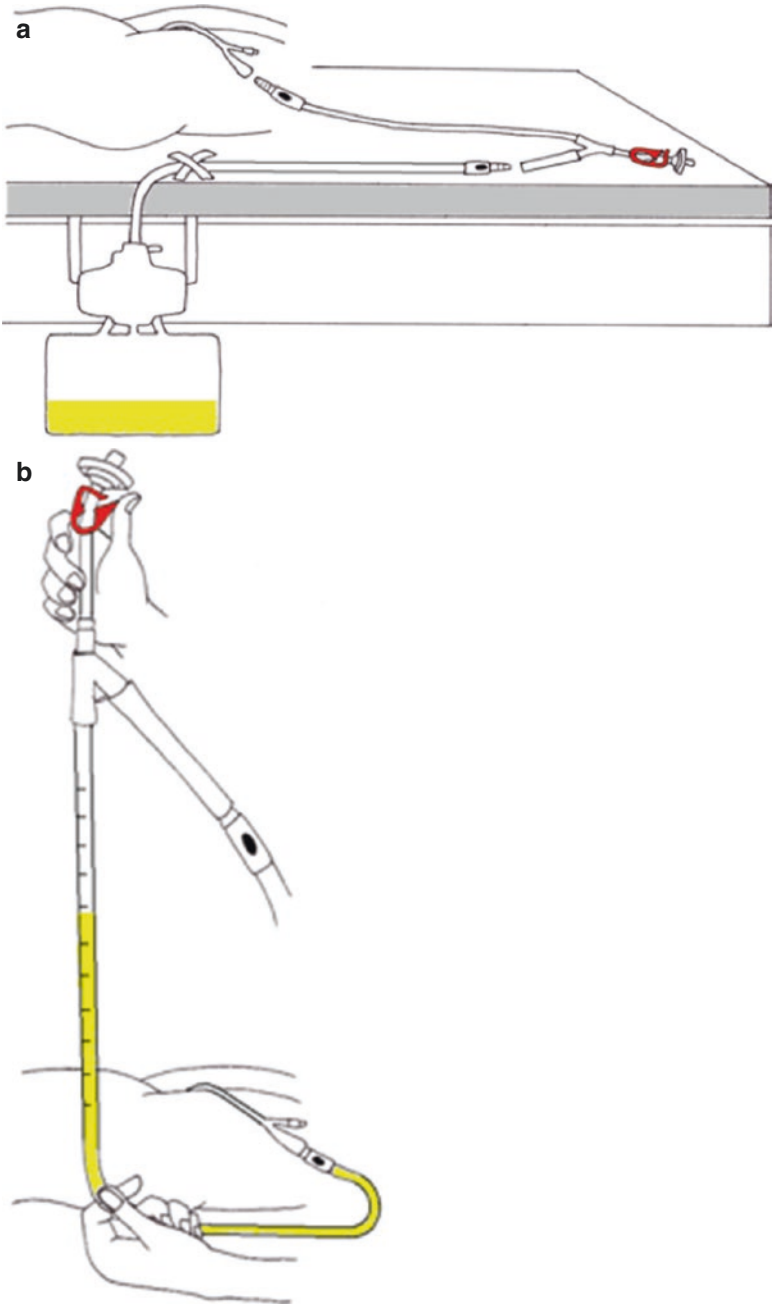

Fig. 19.1 (**a**) The Foley Manometer device is placed between the urinary catheter and the urine collecting bag. If the patient is anuric the system is filled with saline, otherwise the urine from the patient serves as measuring medium. (**b**) When the IAP is measured the "0 mmHg" mark of the manometer tube is placed at the mid-axillary line or at the level of the iliac crest (mark for future reference). The filter is elevated vertically above the patient, and the bio-filter clamp is opened. Read the pressure in end-expiration, the tube is graded in mmHg (13.6 mm between each mark). Finally the clamp is closed, and the system is replaced in its drainage position

19.3 How Common Is IAH/ACS After AAA Repair?

Although IAH and ACS occur in other clinical scenarios such as trauma, intestinal ischaemia and aortic dissection, the situation that the vascular surgeon most often has to consider the possibility of IAH/ACS is after AAA repair, in particular after rupture. The incidence of ACS will depend on several factors. The approach to resuscitation is of paramount importance. Balogh et al. showed that the administration of crystalloids is an independent risk factor for the development of ACS in abdominal trauma patients [10], and this is true in any bleeding patient. A policy of preoperative permissive hypotension in RAAA is likely to decrease the risk of developing post-operative IAH/ACS.

Mell et al. showed that patients who received less than one unit of plasma for every two units of red blood cells during RAAA repair, had a four times higher mortality than those given more plasma [11], highlighting the importance of "massive transfusion" protocols. These protocols were introduced in most modern hospitals during the last decade, reducing both mortality and fluid overload, thereby decreasing the risk of ACS.

The introduction of endovascular aneurysm repair (EVAR) [12, 13] by Volodos in 1985 transformed aortic surgery. The application of EVAR in patients with RAAA was first reported by Ohki and Veith in 2000 [14], and has become more frequently used over time worldwide [15]. In a contemporary nationwide study from the Swedish vascular registry (Swedvasc), ACS occurred as often after EVAR as after OSR (6.9% versus 6.8%), although 10.7% of those operated on with OSR had been primarily left with an open abdomen (OA), thus preventing ACS [16, 17].

If measured consistently, IAP >20 mmHg occurs in about half the patients after OSR of a RAAA, and 20% develop ACS [5, 6]. In many older series, patients operated on for RAAA with EVAR were more haemodynamically stable, resulting in a lower incidence of IAH/ACS after EVAR [7]. The Zürich group who treated virtually all ruptured AAA patients with EVAR and who monitored IAP, reported a high incidence (20%, 20/102) of ACS [9], similar to the incidence after OSR. In a prospective cohort study in four Swedish hospitals, the risk of requiring treatment with open abdomen (OA) after aortic repair was higher after rupture, but similar after EVAR and OR; in all, 28 of 1041 operations or 2.9% of cases [18].

19.4 Pathophysiology and Risk Factors for ACS After AAA Repair

Most risk factors for the development of ACS are associated with bleeding, shock and resuscitation, and they are well described in the guidelines [4]. In the largest study published so far on ACS after AAA repair, 120 out of 8765 patients (1.4%) developed the complication [17]. In these 120 patients, all the case records, including all the data from the ICU and reoperations, were scrutinized and *three main pathophysiological mechanisms* were identified.

1. The most common cause was *fluid overload/general oedema*, which was the main cause in 55 patients (47%).
2. *Postoperative bleeding* was the cause of IAH/ACS in 34 (29%). As expected, those patients developed ASC most rapidly after the first operation of AAA repair.
3. Finally, in 27 patients (23%) *bowel ischaemia* was identified as the main cause of IAH/ACS, although a vicious circle is established when in most cases a gangrenous colon dilates, resulting in further increase of IAP, and more pronounced ischaemia. This mechanism had previously been studied in a prospective study after RAAA, when a direct association between IAH and colonic ischaemia was verified [8].

This paper [17] also reported that ACS developed early in most cases (and in particular after EVAR). Decompression laparotomy (DL) was performed within 24 h after completion of AAA repair in 56 (49%), between 24 and 48 h in 30 (26%) and after 48 h in 29 (25%). The duration of IAH before DL was associated with with the likelihood of development of renal failure and need of renal replacement therapy (RRT). This association had previously been shown in a prospective multicentre study of patients treated with open abdomen (OA) for ACS, in a mixed ICU patient population which was dominated by trauma, but also included patients with RAAA [19].

19.5 Prevention of ACS and Medical Management

It is also possible to treat IAH in a proactive way, preventing further deterioration of the patient and development of ACS. This treatment is sometimes referred to as "medical management", or "conservative management", which is not an appropriate label since it can be quite aggressive. The aim is to prevent further increase of the IAP, as well as to support organ function.

There are two mechanisms through which the IAP can be reduced. One is volume reduction of the intra-abdominal cavity. Evacuation of the retroperitoneal hematoma after EVAR for RAAA has been attempted with lumpectomy (surgical approach through the lateral/dorsal part of the abdominal wall). Another alternative was described by Hörer et al., who inserted tissue plasminogen activator (tPA) through a 20F catheter placed in the hematoma with CT guidance in 13 patients [20]. None of these techniques are truly minimally invasive, and major (even fatal) bleeding complications, were reported. Decompression midline laparotomy seems both safer and more effective than these approaches.

Drainage of gastric content is important, but early enteral nutrition should not be halted [21], since bowel movements are of strategic importance. Enteral feeding can be initiated on the first postoperative day, even in the presence of IAH, but the gastric contents should be drained twice daily to avoid accumulation. However, enemas and other activities to stimulate the faecal flow are seldom effective after aortic repair. Early enteral nutrition and avoiding opioids are more effective and epidural anaesthesia is preferred (see below). It is common that the IAP increases hours before the first bowel action, after which the IAP drops substantially. Free drainable fluid in the abdominal cavity after AAA repair is uncommon.

Abdominal compliance (AC) measures the ease of abdominal expansion, expressed as a change (delta = Δ) in intra-abdominal volume (IAV) per change in intra-abdominal pressure (IAP): AC = ΔIAV/ΔIAP. This is a dynamic variable which is dependent on baseline IAV and IAP, as well as on reshaping and stretching capacity of the abdominal wall. The first phenomenon is that the abdomen transforms from an oval into a circular shape (reshaping), followed by stretching, and finally by a rapid increase in IAP. In a review of AC, the most important conclusion was that patients with high IAP have a reduced AC, making the IAP very sensitive to small changes in IAV [22]. This phenomenon explains the often dramatically fast increase of IAP before the ACS develops, and why proactive early and frequent monitoring of IAP, and preventive actions, are so important.

One of the most effective ways of decreasing the IAP is pain relief, but it is also strategically important to avoid opioids, to prevent obstipation. During RAAA repair there is seldom time to insert an epidural catheter prior to surgery, and after surgery the patient often has coagulopathy. We routinely discuss this with the anaesthesiologists, postpone the use of low molecular weight heparin (LMWH) medication, give platelets if necessary, and then use epidural analgesia whenever possible. This is quite effective in reducing IAP, can often increase urinary output, and become the turning point in the critical postoperative period.

Neuro-muscular blockade (NMB) is an effective way of immediately reducing IAP when the patient is on the ventilator, which is often the case, especially after OSR of RAAA. It reduces IAP by 30–50%, which is often sufficient to improve renal function, reduce fluid overload, and reverse the situation of increasing IAP before ACS develops. In a study on 191 trauma patients undergoing damage control laparotomy, 92 who were on NMB during the first 24 h had higher primary fascial closure rate [23]. A large French RCT showed that NMB used for 48 h in 340 mixed ICU patients was safe and improved survival in patients with acute respiratory failure [24]. There are no published specific data on the effect of NMB in RAAA patients, but in our experience it works well.

Reducing fluid overload acts through both mechanisms: reducing intra-abdominal volume and also making the abdominal wall more compliant, as the oedema decreases. Intensivists have different opinions how fluid overload can be prevented, and this issue is highly controversial. Many argue that colloids are beneficial in this situation, others that they only leak into the extra-cellular space, adding further to the fluid overload and affecting renal function negatively. In our practice, plasma tends to be used in the early post-operative phase, when the patient is often coagulopathic, and hypertonic 20% albumin combined with furosemide or renal replacement therapy is used later in the postoperative phase [25]. If the patient is on a ventilator, an increased PEEP may help to recruit fluid from the lungs.

Fluid overload is often more iatrogenic than is recognized. The Uppsala protocol is very restrictive with regard to the administration of crystalloids from early resuscitation. Not all are aware of the fact that when fractionated blood products (erythrocytes, plasma and thrombocytes, 1:1:1) are given to compensate for 1 L of blood loss, 4–500 mL of saline solution is also added. Thus, even if only blood products are given, the transfusion to compensate for 10 L of blood loss will automatically result in a fluid overload of 4–5 L of saline, making further administration of crystalloids dangerous [4, 11, 25].

19.6 Decompression Laparotomy (DL)

When ACS is developing or present, the only effective treatment is DL. It should preferably be performed in the midline, from the costal arch to the symphysis pubis. To not open the entire abdomen is a classical mistake. It is not only less effective, but also more difficult to close.

The timing of DL is important but a complex issue in clinical practice. Ideally the two strategies of early or delayed DL should be compared in a randomised trial. It does not make sense to wait until severe organ dysfunction/failure has developed before performing DL, but OA treatment itself is a morbid procedure associated with both morbidity and mortality. In the large Swedish cohort of 120 patients treated for ACS after AAA repair, timing of DL, i.e. the duration of IAP >15 mmHg, or >20 mmHg, was not associated with mortality, but was associated with need of RRT (dialysis) [17]. This lack of association with increased mortality is probably a result of confounding factors, since it makes sense to perform DL as soon as possible, once the decision has been made. This is another advantage of starting to monitor IAP and treat IAH early: if the patient fails to improve on intensive medical therapy the decision to perform DL can be taken without further delay, since we already know that the patient has not responded to non-surgical treatment.

When a decision to perform DL has been taken, often in the middle of the night, there may be a waiting list for the operating theatre. Other patients may have high priorities, in which case NMB can reduce the ischaemic injury to the abdominal organs whilst waiting. It is important to inform the anaesthesiologist that the patient needs to have an extra bolus of fluid prior to DL, to avoid hypotension, which is common when you open or reopen the abdomen during DL.

The effect of DL is often dramatic, reducing IAP, improving oxygenation and urinary output. Effects on multiple organ failure scores (SOFA, APACHE) are not as immediate, however, since multiple organ failure is not reversed quickly. In a multicentre study on 33 patients undergoing DL for overt ACS with different pathologies including RAAA, the IAP decreased from 23 mmHg (range 21–27) to 12 mmHg [9–15, 26] after 2 h [19].

19.7 Prophylactic Open Abdomen Treatment

Is it better to leave all patients open as a routine after OSR of a RAAA, or is it better to close most patients (who do not have an obvious tense abdomen), and follow them closely in the postoperative period? In their experience, the Mayo Clinic reported having left 19% open after RAAA repair (43/223) [27]. A similar experience was reported from Zürich [9], and in a Swedish national cohort study this proportion was 10.7% after OSR [16]. It is obvious from all reports that a proportion needs to be left open primarily, but what proportion remains controversial.

Based on a systematic EBM review of the literature, the Updated Consensus document favour primary closure and IAP measurement [4]. They recommend

"measuring IAP when any known risk factor for IAH/ACS is present in a critically ill or injured patient", and "*use of protocolized monitoring and management of IAP versus not."* Furthermore, they issued a strong negative recommendation: "*We could make **no** recommendation regarding the prophylactic use of the open abdomen*".

A recent paper compared 79 patients treated with a primarily OA after OSR for RAAA at a centre performing this as a routine, compared to a propensity score-matched control group of 148 patients treated at other centres in which 73% had the abdomen was closed at the end of the procedure [28]. There was no difference in mortality or post-operative complications, thus no benefit associated with a routine practice of leaving all patients open could be demonstrated.

Our policy in Uppsala, Sweden, is to leave the patient open primarily after OSR of a RAAA only if the abdomen is tense and difficult to close, which occurs in approximately 5–10% of cases. However, almost all patients with RAAA nowadays are treated with EVAR. We monitor IAP in the OR (which is of particular importance after EVAR), every hour for 4 h, followed by every 4 h for the first 48 h. Monitoring is performed more frequently when there is IAH, and DL is performed on demand. In a review article adapting the WSACS Guidelines to vascular surgery, this treatment algorithm was described in more detail [25].

19.8 Management of the Patient with Open Abdomen (OA)

How to care for the patient who needs a period of OA treatment is a complex issue. The first issue is how to manage the open abdomen itself: maintain a sterile environment, keep the intestines moist and protected from injury, protect the abdominal wall, and enable closure as soon as possible. A classification system of the open abdomen was developed in 2009, in order to facilitate training and research [29], and later updated in 2016 [30]. Preventing and controlling contamination, as well as lateralisation of the abdominal wall, are the key elements to enable closure of the abdomen as soon as possible [4, 29–31].

The problem of lateralization was defined and highlighted in the Updated Consensus document [4]: "*Lateralization of the abdominal wall is the phenomenon where the musculature and fascia of the abdominal wall, most exemplified by the rectus abdominis muscles and their enveloping fascia, move laterally away from the midline with time*." It is also included in the classification system of OA [29, 30].

The importance of closing the abdomen as soon as possible to maintain a sterile environment was illustrated by the results from Helsinki, Finland [32]. They used a temporary abdominal closure (TAC) device including continuous negative pressure (the VACM method, see below). The OA was progressively colonized and 80% of the patients had positive bacterial cultures after 2 weeks of OA treatment.

The choice of TAC has attracted much attention, and multiple solutions have been developed, ever since the first report in the scientific literature of leaving the abdomen open, in 1897 [33]. An important innovation was developed by paediatric surgeons, who started to repair omphalocele in the 1940s, using silastic coverage of

the intestines, a first generation of plastic. A similar system was later popularized in trauma surgery by the Colombian invention of the Bogotá bag, using the plastic bag from a drip that is sutured to the skin or the fascia. This system works well for a few days, but during a more prolonged treatment (which is often necessary after AAA repair) three major problems develop. Two of those were solved by the later development of the vacuum pack technique, developed in 1995 by Barker et al. in Philadelphia [34]. The active suction prevented leakage of fluids from the OA, and the surgical towels covered with plastic prevented adhesions forming between the intestines and the abdominal wall. This system was further refined by a commercially available ready-made system, (V.A.C.® Abdominal Dressing System; KCI, USA). Later other negative pressure systems were developed by different suppliers.

A third problem, the lateralization of the abdominal wall, remained however, making it difficult to close patients who had been treated with OA for at least 5 days. This was the reason why a novel method was developed in Uppsala and Malmö, Sweden; the Vacuum-assisted wound closure and mesh-mediated fascial traction (VACM) method. The pilot experience of VACM was published in 2007 [35]. This is a combination of the commercially available VAC system (later replaced by the ABThera system) with a prolene mesh that is sutured to the fascial edges to permit an active traction towards the midline (details shown in Fig. 19.2). A multicentre study of this technique (including only patients requiring OA for at least 4 days) showed an 89% primary delayed fascial closure rate after a median time of 15 days

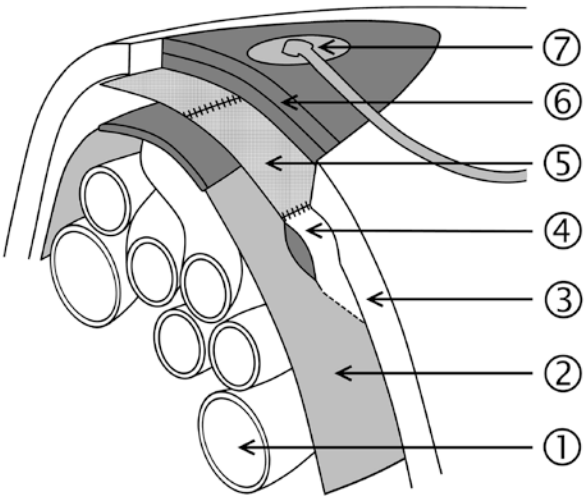

Fig. 19.2 A schematic drawing of the VACuum-assisted wound closure and Mesh-mediated fascial traction (VACM) system: (1) The bowel. (2) The plastic sheet covering and protecting the intestines. (3) The abdominal wall. (4) The rectus abdominis muscle that was divided in the midline at the primary laparotomy. (5) Prolene mesh sutured to the fascial edges, to be advanced toward the midline and removed progressively. (6) Black foam. (7) The track-pad transmitting the negative pressure into the system

with OA [36], and in a sub-group analysis of those treated for aortic disease this figure was 100% [18]. These results have been repeated independently at other major centres [37, 38], as well as in a large multicentre study on patients treated for ACS after AAA repair [39], and is now the preferred TAC method in many centres world-wide.

19.9 Prognosis

The overall mortality in patients who develop ACS after AAA repair is high. In the national Swedish study of 6612 patients, the 30 day mortality rate after RAAA was 42% among those who developed ACS compared to 24% without ACS. At 1 year mortality was 51% versus 32% [16]. After intact AAA repair, 30 day mortality was 12% with ACS versus 1.8% without. At 1 year mortality was 28% versus 6%. When ACS developed, renal failure, multiple organ failure, intestinal ischaemia, and prolonged intensive care stay were all very much more frequent. Interestingly, morbidity and mortality were similar, regardless of the primary surgical technique (OSR or EVAR), if the patient developed ACS.

Those results are actually rather encouraging, since untreated ACS has a mortality approaching 100%. Perhaps the fact that ACS has been recognized and treated for many years in Sweden is one of the explanations why survival after RAAA has increased over time [40].

19.10 Conclusion

In conclusion, IAH and ACS are common and life threatening complications after aortic surgery. Preventing and treating this complication in a timely way, when it occurs despite prevention, are important parts of any strategy aiming at improving outcomes after aortic surgery, whether by an open or endovascular approach.

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Chapter 20
Pathophysiology and Management of Limb Compartment Syndromes

David Lindström and Carl-Magnus Wahlgren

Key Learning Points

- Acute extremity compartment syndrome is a surgical emergency associated with significant morbidity if not expeditiously managed.
- The most important tool in diagnostics is to maintain a high level of clinical suspicion.
- Patients with classical clinical signs of compartment syndrome do not need any further investigation, and should undergo urgent fasciotomy.
- Incisions in skin and fascia need to be long enough to make tissues loose and allow for postoperative swelling.
- After compartment syndrome has resolved, delayed primary closure of the fasciotomy incisions is performed, with the intracutaneous suture method recommended.

20.1 Introduction

Acute extremity compartment syndrome is a surgical emergency associated with significant morbidity if not expeditiously managed. It requires prompt diagnosis and early treatment with compartment decompression for good clinical outcome.

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The definition of acute extremity compartment syndrome is an increased pressure within the compartmental space leading to decreased perfusion pressure and hypoxaemia of the tissues. This may lead to irreversible ischaemic necrosis of the muscles and nerves in the compartment causing functional impairment, limb amputation, multiple organ failure, or death [1, 2].

Acute limb compartment syndrome (ACS) in vascular surgery is mostly related to ischemia-reperfusion (I/R) injury associated with acute ischaemia, vascular trauma, and phlegmasia cerulea dolens [3–5]. Iatrogenic causes with accumulation of blood or contrast fluid within the compartment after catheter/wire perforation have also been described [3, 4]. Most extremity compartment syndromes result from internal compartment expansion due to fractures or crush injuries but also external compression from burns, tight plaster cast or bandage may occur [6]. The aim of this chapter is to summarise the pathophysiology and management of upper and lower limb compartment syndromes.

20.2 Epidemiology

No comprehensive review of the prevalence of extremity compartment syndrome has been published due to the variation of causes [7]. The incidence of lower extremity compartment syndrome after revascularization of acute ischaemic limbs is approximately 10–20% [5, 8, 9], but in contrast, the need for fasciotomy after elective vascular surgery is very low, from 0.15 to 0.45% [10]. Patman found that 32% of extremities with arterial injuries and only 2% with embolic occlusions underwent fasciotomy. In a retrospective series of vascular surgery patients (107 patients; 113 limbs) undergoing lower extremity fasciotomy, 72% of limbs underwent revascularization for acute limb ischaemia, 6.2% of limbs were related to acute aortic disease, and 20% of limbs had undergone elective vascular surgery [2]. In total, 57% of limbs had signs of ACS and a therapeutic fasciotomy was performed, while 43% fasciotomies were prophylactic. Data from the US National Trauma Data Bank showed that patients sustaining lower extremity arterial trauma required a fasciotomy in up to 42% of cases [11].

20.3 Pathophysiology

Skeletal muscles, nerves, and vessels in the upper and lower extremities are arranged into compartments surrounded by fascia. The upper arm contains two compartments (anterior and posterior) and the forearm has three (volar, lateral, and dorsal) compartments. In the thigh, there are three compartments (anterior, medial, and posterior), whereas there are four in the lower leg (anterior, lateral, deep posterior, and superficial posterior) [6, 12]. Compartments within the forearm and lower leg are especially limited in their ability to accommodate tissue oedema and are therefore

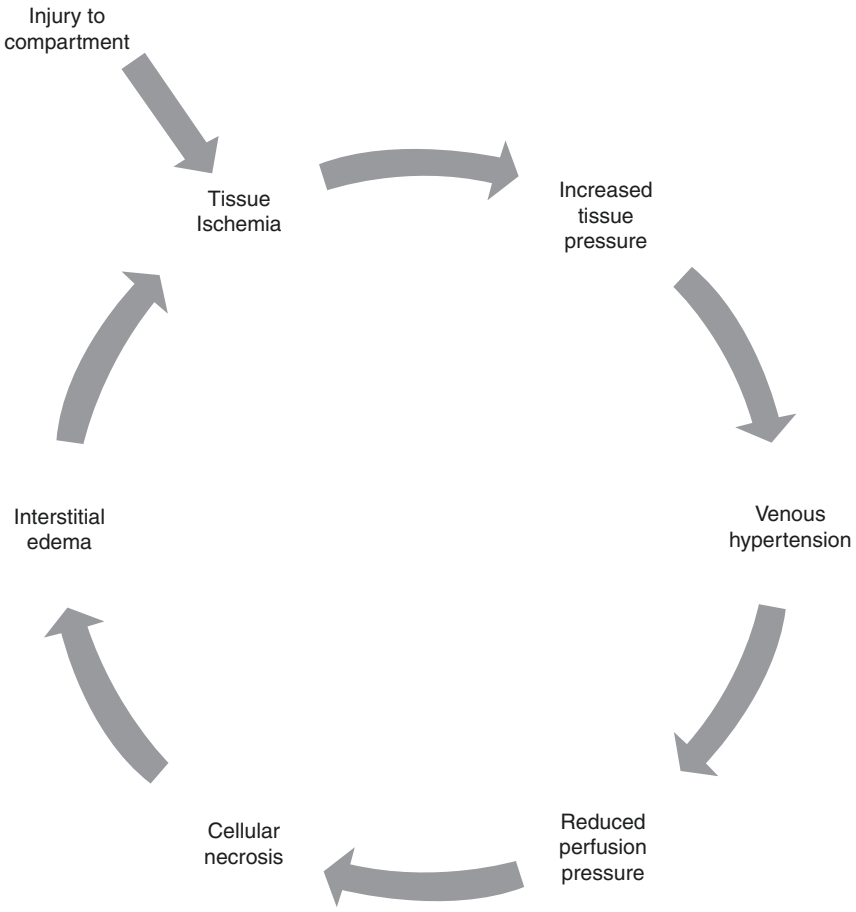

Fig. 20.1 Pathophysiology of acute extremity compartment syndrome [1, 14–16]

more prone to develop increased compartment pressures [13]. The lower leg is the most common location of acute extremity compartment syndrome, with the anterior and lateral compartments most frequently affected [12].

The pathophysiology of acute extremity compartment syndrome involves an external compression or an internal expansion within the compartment that leads to increased tissue pressure, reduced capillary blood flow, local tissue hypoxia and local tissue necrosis (Fig. 20.1) [1, 14]. Intrinsic causes of acute extremity compartment syndrome are tissue injury caused by a direct traumatic event or tissue ischaemia and reperfusion [1, 15]. The most common cause of compartment syndrome in vascular surgery is tissue oedema due to ischaemia-reperfusion injury caused by limb revascularisation. A traumatic vascular injury is often accompanied by fractures, so that ischaemia–reperfusion and haematoma formation may both contribute to the limb compartment syndrome [16]. The rising tissue pressure

impairs venous outflow by compressing the veins. This increase in venous pressure reduces the arteriovenous pressure gradient, resulting in diminished local perfusion [1, 15, 16].

The combination of increased pressure in the interstitial and intercellular fluid spaces shuts off transcapillary movement. The resultant cellular ischaemia leads to muscle and nerve damage. Interstitial oedema develops from tissue necrosis and further worsens hypoxaemia and compartmental swelling. During acute limb ischaemia, the combination of decreased oxygen supply and congestion of red blood cells within the capillaries triggers a complex cascade of metabolic, inflammatory, and prothrombotic pathways [17, 18]. The cell shifts energy metabolism from an aerobic to anaerobic mechanism, producing lactic acid. Continued ischaemia causes depletion of energy-rich adenosine triphosphate (ATP), leading to leakage of extracellular calcium into the muscle cells, which ultimately results in dysfunction and cell death [19].

Reperfusion injury represents the response to tissue injury when the blood flow is restored after ischaemia. This contributes to a systemic inflammatory, metabolic, and thrombotic response. Microvascular dysfunction mediates many of the local and systemic consequences of ischaemia-reperfusion injury with a plethora of changes specific to arterioles, capillaries, and venules [17, 20]. This includes impaired vasodilation, decreased perfusion and fluid leakage, and increased permeability.

Ischaemia-reperfusion injury leads to microcirculatory changes due to activation of inflammatory mediators. Increased arterial resistance leads to decrease perfusion in the capillaries. The permeability results in an increased rate of transcapillary fluid leakage. Impaired tissue perfusion and oedema will further raise the intracompartmental pressure [17, 20]. The no-reflow phenomenon is another factor in this response relating to vascular congestion in the arterioles and capillaries [13, 20]. Reactive oxygen species cause damage including injury to DNA, oxidation of fatty acids and lipids, and oxidation of proteins and co-factors necessary for enzymatic function [21]. This leads to capillary leakage, resulting in additional oedema and rising compartment pressure. (see Chap. 18, Pathophysiology of Ischaemia-Reperfusion injury).

The duration of ischaemia correlates with the onset of irreversible changes in various tissue types. The critical ischaemic time in different tissues at normal temperature can be defined as the maximum ischaemic time interval that a tissue can tolerate and still remain viable [22]. In the extremities, muscle tissue has the highest risk for ischaemia-reperfusion injury [17].

An ischaemic time of 4–6 h predisposes the patient to the development of a compartment syndrome [23]. Large-animal studies have demonstrated the neuromuscular ischaemic threshold of the limb to be less than 5 h, and similarly recommended restoration of flow within 3 h of injury for optimal functional recovery. Haemorrhagic shock also worsens the impact of ischaemia on the neuromuscular structures of the limb and reduces the ischaemic threshold to as little as 1 h [13].

20.4 Clinical Presentation

Acute limb compartment syndrome occurs after a precipitating causal event, most commonly trauma. In vascular surgery, compartment syndrome is also commonly seen following reperfusion after muscular ischaemia due to acute arterial occlusion or reduced distal perfusion during endovascular procedures or extracorporeal membrane oxygenation (ECMO) due to large catheters inserted into proximal arteries. Other contributing factors are fractures, haematomas, pressure injuries, and oedema. The time from insult to compartment syndrome development may vary from minutes to hours. The most sensitive tissues to ischaemia are unmyelinated nerve fibres followed by myelinated nerve fibres, skeletal muscle, skin and then bone. As an example, the first sign might be an altered sensation between digit 1 and 2 in the foot—corresponding to deep peroneal nerve ischaemia in the anterior compartment. Pulses will generally be preserved for a long time into the syndrome, well beyond the indication for acute surgery. Patients who are alert and awake normally complain of severe pain, often resistant to analgesics, sometimes pain "out of proportion"—meaning that it does not correspond to the severity of the injury sustained. Some of the other classical "P-signs" of compartment syndrome might also be present (pain, pain on passive stretch, paraesthesia, paralysis). Commonly foot drop occurs in compartment syndrome affecting the lower leg, due to the involvement of anterior and lateral calf compartments. In general, the sensitivity of each symptom is low but the specificity quite high. Absence of symptoms should therefore not be used to rule out compartment syndrome. But additionally, if three or more of the classical "4-P" symptoms are present, this increases the likelihood of the presence of a compartment syndrome [24].

Unfortunately many patients are not fully awake and alert when the syndrome occurs. This might be due to anaesthetics used in surgery, dementia, concomitant CNS or peripheral nerve injuries or drugs and alcohol. For this group of patients, firmness/tenderness of the compartments relative to the unaffected limb might be a helpful observation. The assessment of distended muscles alone is not sufficient for diagnosing increased compartment pressure [25]. In children, the adult symptoms described above are unreliable; signs of increasing need of analgesics, agitation and anxiety may be the clues to diagnosis and treatment [26, 27]. It should be noted that the syndrome usually evolves over some time, which is why repeated, serial clinical examinations over a short period of time sometimes makes it easier to diagnose. A sensory deficit is sometimes the first sign of compartment syndrome, but, in vascular surgery, the preceding ischaemic event may already have caused sensory loss, reinforcing why it is important not to rely only on a single clinical sign.

Clinical signs suggestive of irreversible ischaemic damage include fixed, non-blanching skin staining or gangrene. In these cases, urgent amputation should be considered to prevent systemic complications. Factors associated with post-ischaemic compartment syndrome in non-traumatic acute limb ischaemia are inadequate backflow, high serum creatine kinase (CK) level, positive fluid balance after

admission, and advanced-stage acute limb ischaemia [28]. In cases in which muscle swelling is highly likely, a prophylactic fasciotomy may be indicated. It may reasonable to perform a prophylactic fasciotomy in severe acute ischaemia exceeding 4–6 h, especially if there is inadequate collateral flow, and also in association with vascular trauma [9, 28].

20.5 Diagnostic Testing

Any suspicion of acute limb compartment syndrome (both in conscious and unconscious patients), must lead to either prompt treatment or immediate further diagnostic testing. Clinical assessment of suspected compartment syndrome is difficult; the majority of symptoms and signs are only reliably assessed in a fully conscious patient. Also, many patients may have some of these clinical signs present due to the injury that has caused the compartment syndrome such as the pulseless, painful, paraesthetic limb of acute ischaemia. The most important tool in diagnostics is to maintain a high level of clinical suspicion. A clinical situation with long-standing ischaemia, all the classical "P signs", and a firm and tense calf does not need any further investigations, and should undergo urgent fasciotomy. On the other hand, an unconscious patient in the intensive care unit with slightly firm calf after a short period of ischaemia should be worked-up more thoroughly before going ahead with immediate fascia release. The medicolegal and litigation situation in some areas of the world makes it wise to try to confirm the diagnosis if possible. The most commonly used method for confirmation of the diagnosis is pressure measurement in the respective compartment with multiple needle-sticks. All compartments in the affected limb should be measured. If any fracture is present, measurement should be done within five centimetres of the fracture. Some sedation or local anaesthetic might be helpful, especially in children. Most accurate results are reported with a side-port needle or a slit catheter connected to an arterial line or the Stryker manometer [29]. The normal compartment pressures in adults are around 8 mm Hg and in children 10–15 mmHg [1]. As a diagnostic cut-off, the perfusion pressure has been proven to be more accurate than an absolute value. The differential pressure (ΔP = diastolic blood pressure − intracompartmental pressure) of 30 mmHg is the most commonly used threshold [30, 31]. Collecting blood from the ipsilateral femoral vein and measuring lactate has also been shown to correlate with the risk of compartment syndrome but the clinical usefulness is not clear [32].

The dynamics of the syndrome is of importance; any measurement during surgery and anaesthesia should be repeated postoperatively if suspicion exists. Regional anaesthesia such as epidural catheters should be used with caution since they may mask symptoms and delay diagnosis [33]. Techniques with indwelling catheters allow continuous monitoring of limbs at risk, but these techniques are more complex and require prior training in their use [34].

Other methods to diagnose acute compartment syndrome have been proposed as well. Near-infrared spectroscopy (NIRS) has been useful in some case series [35]

but according to a recent review, the false-negative rate is too high to rely solely on NIRS in diagnosing a syndrome that needs timely treatment [36, 37]. In clinically advanced cases, the resulting inflammation may be associated with a leucocytosis or C-reactive protein (CRP) elevation. If tissue necrosis has developed, serum creatinine phosphokinase and myoglobin will be elevated, together with a metabolic acidosis. When rhabdomyolysis occurs, the urine will develop a brown discolouration, sometimes referred to as "tea-coloured". The colour stems from myoglobin in the urine which the kidneys are trying to excrete.

20.6 Management

20.6.1 Lower Extremity Operative Techniques

Acute limb compartment syndrome should be treated with fasciotomy of all affected compartments. In patients who present acutely (roughly within 12 h of onset of compartment syndrome), it should be performed immediately. Fasciotomy performed longer than 12 h after the onset of compartment syndrome results in a significantly poorer functional outcome [37, 38]. If the presentation or diagnosis is delayed, consideration must be given as to whether the limb is unsalvageable. If so, a fasciotomy may lead to significant morbidity without improving functional outcome. Beyond 36 h, rates of amputation, infection, neurological injury and death increase. Early amputation rather than futile attempts at limb salvage should be considered [39, 40].

Preoperative non-surgical measures such as removal of casts and dressings and elevation of the affected limb, should be performed during evaluation or waiting time for surgery. In the lower limb, the fasciotomy procedure should release any tissue that could constrict the respective compartment. The skin and fasciae should be released along the entire length of the compartments. The lower limb consists of the anterior, lateral (peroneal), superficial and deep posterior compartments. The easiest and most common way to decompress the compartments is through two incisions (Fig. 20.2). One posteromedial incision (to open the superficial and deep

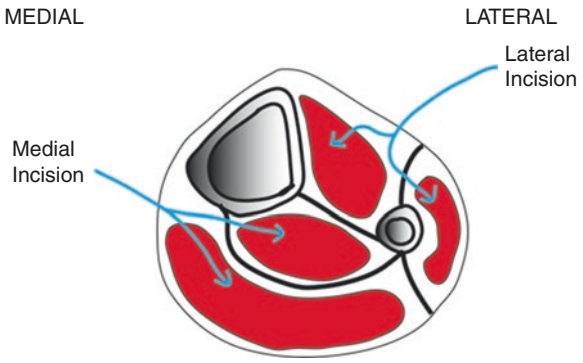

Fig. 20.2 Fasciotomy of the lower limb to decompress the four compartments (anterior; lateral; superficial and deep posterior) with two incisions (anterolateral and posteromedial)

posterior compartments) is performed two centimetres behind the medial border of the tibia; taking care not to injury the great saphenous vein. The superficial fascia is then opened along the length of the skin. Secondly, the soleus muscle attachment on the tibial border is sharply released to expose the deep posterior fascia which is then opened. The second anterolateral incision (to open the anterior and lateral compartments) is done one fingerbreadth anterior to the fibula from the fibular head to the ankle over the intermuscular septum between the anterior and lateral compartment, taking care not to injure the common and superficial peroneal nerves. The intermuscular septum can usually be seen as a white longitudinal line in the fascia. If any uncertainty exists, a short transverse incision can be used to confirm the location of the septum. The anterior and lateral (peroneal) compartments are then opened along the length of the incision. A single incision technique to treat lower limb compartments has also been used with similar outcomes but it is not recommended by the authors [41]. Irrespective of techniques used, incisions in skin and fascia need to be long enough to make the tissues loose and allow for postoperative swelling [42]. Any dressing material should be sterile and loosely attached. A simple and cheap way to prepare for later closure is to suture a running intracutaneous shoelace suture along the whole incision. The suture can be a 2/0 resorbable monofilament suture or a non-resorbable polypropelene suture. It is important to leave the sutures really loose; normally two sutures are needed to cover a lower limb incision.

Compartment syndrome in the thigh is rare, most commonly seen after blunt trauma. The thigh consists of three compartments (anterior, posterior and medial) and is usually decompressed medially and/or laterally (Fig. 20.3). No good data on the optimal method is available although a single-incision technique is reported more frequently [43].

20.6.2 Upper Extremity Operative Technique

In the upper extremity, the forearm is the most common site of development of compartment syndrome [1, 4]. Decompression of the volar and the lateral compartments is achieved through a curvilinear incision that begins proximal to the antecubital fossa, medial to the biceps tendon, extends to the radial side of the forearm,

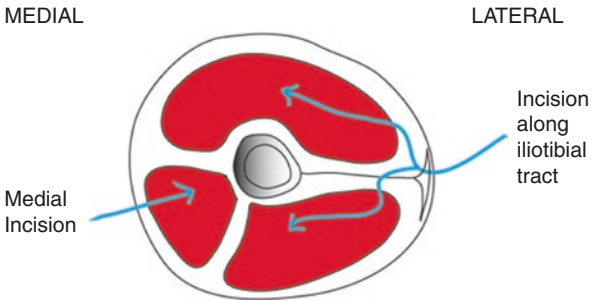

Fig. 20.3 Fasciotomy of the three compartments (anterior, posterior and medial) in the thigh with decompression from a medial and lateral approach

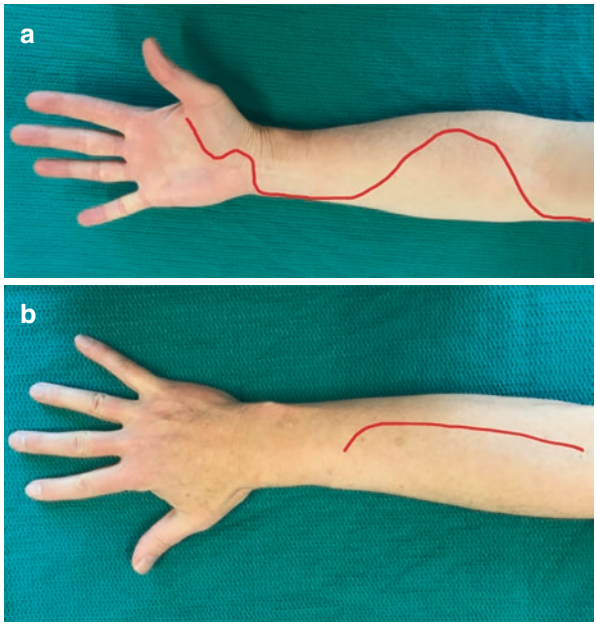

Fig. 20.4 Fasciotomy of the forearm with decompression of the volar and lateral compartments (**a**) and the dorsal compartment (**b**)

where it goes distally along the medial border of the brachioradial muscle, and finally across the carpal tunnel along the thenar crease (Fig. 20.4a) [3, 4, 6, 12]. The decompression of the dorsal compartment is achieved through an incision extending from just distal to the radial head to the midportion of the wrist (Fig. 20.4b). In the upper arm, the anterior compartment is incised just distal to the deltoid muscle to just above the elbow. The posterior compartment is incised over the triceps muscle in the midline to above the elbow [14].

20.7 Postoperative Care

The postoperative limb should be held elevated and a foot pump may be used if available. Physiotherapy should be started immediately to activate dorsiflexion of the ankle. Pain medication should be administered as needed. There are a few options for dealing with the fasciotomy wounds after the compartment syndrome has resolved. Delayed primary closure, vacuum-assisted closure, secondary closure, skin grafts, and healing by secondary intention are all options. Delayed primary closure with the vessel loop shoelace technique is often referred to and works well [44] but the authors would recommend the pre-positioned intracutaneous suture method described above which is easier, quicker and gives a better cosmetic outcome. Studies with vacuum-assisted closure have shown conflicting results, with low complication rates but the need for skin grafting has been quite frequent, as well

as high costs associated with the technique [45–47]. If the running sutures, described in the operative technique above, are used; the same sutures can be used to start closing and approximating the skin edges. If the fasciotomy was done prophylactically and no swelling is present, complete skin closure can be performed within 24–48 h. If a true compartment exists with muscular swelling, the successive closure process can usually start after 48–72 h. This can normally be done in the ward without the need to go back to the surgical theatre unless there is a need for surgical debridement or haemostasis. Most patients tolerate careful pulling of the sutures every 24–48 h. When the skin edges are closed, the suture is tied on the outside and left in situ for 3 weeks.

Some adjunctive non-operative management methods have been described but there are no standard methods in use. Free radical scavengers such as mannitol [48] and hypertonic saline [13] have been shown to be of some benefit in compartment syndrome caused by ischaemia-reperfusion injury. Hyperbaric oxygen therapy has also been shown to improve the outcome of reperfusion injuries [49]. The addition of sodium bicarbonate to the resuscitation fluids is beneficial, as it will correct metabolic acidosis, hyperkalaemia and also keeps the urine alkaline (urinary pH >6.5), thus decreasing the toxic effect of myoglobin on renal tubules. Forced diuresis with furosemide and mannitol may also help protect the kidneys [50]. If rhabdomyolysis occurs, continuous renal replacement therapy may be used, although a Cochrane analysis found that the supporting evidence is weak [51].

20.8 Outcome

Whilst fasciotomy wounds are associated with a moderate degree of morbidity [52, 53], fasciotomy does not appear to have any effect on long-term calf muscle pump function [54]. If fasciotomy is performed early for acute compartment syndrome, the outcome in terms of preventing limb loss, systemic complications and long-term functional disability is good [31]. Failure to prevent and promptly treat compartment syndrome risks the development of systemic complications such as multiorgan failure (including hyperkalaemia, hypocalcaemia, coagulopathy, myoglobinuria, and renal failure) with a correspondingly high risk of death. It is recommended that a fasciotomy should be performed as soon as possible once the diagnosis has been made, preferably within 6 h [55]. Fasciotomy performed early, less than 12 h after the onset of the compartment syndrome, resulted in normal function in 68% of the extremities. However, only 8% of those having late fasciotomy had normal function following decompression [38].

To study the outcome of compartment syndrome alone is difficult, since simultaneous vascular or traumatic injury may affect the outcome. The syndrome does have a high incidence of nerve deficit and foot drop (15–32%), [2, 56] and mortality rates of between 15 and 23% have been reported [2, 57]. In a retrospective study from 1980 to 1994, 53 patients had fasciotomies following surgical revascularization [5]. At discharge, 11 (21%) patients had undergone amputation and six (11%) patients

had died. In another retrospective review, lower extremity fasciotomies were performed for compartment syndrome after acute ischaemia and revascularization in patients with vascular trauma or arterial occlusive disease [53]. Fourteen (11%) amputations were required for refractory limb ischaemia and 18 (15%) patients died of cardiopulmonary or multisystem failure. After lower extremity trauma with compartment syndrome and fasciotomy, nearly 13% of patients required leg amputation [56]. In this study by Lollo et al., 67% of amputees had associated vascular injuries. At long-term follow-up, 10% of patients reported moderate lower extremity pain and 69% had returned to work. Neuromuscular sequelae, as well as long-term functional outcome, has been emphasized as important factors for patient expectation and satisfaction [13, 23]. Most children experience full recovery after fasciotomy but the literature is limited [58]. In a case series, persistent Volkmann's contracture as a post-traumatic complication occurred in one of 23 children (4.2%) [58].

20.9 Conclusion

Extremity compartment syndrome in vascular surgery is a limb-threatening condition requiring emergent fasciotomy. The management strategy is to expeditiously decrease tissue pressure, minimize tissue damage and functional loss. There are significant complications related to lower extremity compartment syndrome including neuromuscular dysfunction and amputation. The fasciotomy wounds per se cause minimal morbidity and can normally be closed with a variety of techniques. The role of inflammatory and metabolic biomarkers for compartment syndrome and whether they can guide therapy needs further evaluation. Future research should focus on early and improved diagnostics of compartment syndrome and refined treatment regimens for ischaemia-reperfusion injury.

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Chapter 21
Pathophysiology of Pain

Stephan A. Schug

Key Learning Points

- Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.
- Physiological pain is related to impending tissue damage (nociceptive pain) or actual tissue damage (inflammatory pain); it has a physiological function in preventing damage and promoting protection and thereby enabling healing.
- Pathological pain is not related to tissue damage but is the result of disease or a lesion of the nervous system (neuropathic pain) or central sensitisation processes (nociplastic pain). It is not a symptom of peripheral disease or injury, but is a disease in its own right.
- Peripheral sensitisation is the result of increased nociceptor activation due the inflammatory response and typical for most nociceptive-inflammatory pain states.
- Central sensitisation is the result of processes in the spinal cord and brain and the result of an imbalance of increased excitatory and diminished inhibitory processes.
- Central sensitisation occurs in response to any nociceptive input, but usually resolves with healing. Persistent central sensitisation beyond the period of healing is the hallmark of many chronic pain states.

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21.1 Introduction

Pain is classically defined by the International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage [1]. Currently there is an ongoing discussion on updating this definition, but consensus has not been reached [2].

The mechanism by which a damaging stimulus in the body is perceived as painful by the brain is a complex one. The complexity of the process results from the nervous system not being a 'hard wired' system, but exhibiting plasticity which enables it to modify its function under different conditions [3].

As per definition, pain serves the purpose of preventing tissue damage and protecting the body whilst it is healing. Under certain conditions, pain can become maladaptive and then persist as chronic pain. This pain serves no protective function and is described as pathological pain as opposed to physiological pain [4]. Applying these principles, nociceptive and inflammatory pain are physiological pain conditions, while neuropathic and CNS dysfunctional (now 'nociplastic') pain are pathological pain states [5]. The latter are then no longer a symptom of another disease, but diseases in their own right [6]. In order to adequately treat physiological, but even more so, pathological pain, an understanding of pain mechanisms is required.

21.2 Peripheral Mechanisms

21.2.1 Nociception/Transduction

Painful stimuli are detected by nociceptors, which are free nerve endings located in tissues and organs. They have high thresholds and, under normal circumstances, only respond to noxious stimuli [7].

There are two distinct types of nociceptor;

- High threshold mechanoreceptors which stimulate small myelinated Aδ-fibres and transmit a well-localised sharp or pricking sensation that lasts as long as the stimulus.
- Polymodal nociceptors that stimulate small unmyelinated slowly conducting C fibres. As well as responding to mechanical stimuli they are activated by thermal and chemical stimuli e.g.: hydrogen ions, potassium ions, bradykinin, serotonin, adenosine triphosphate and prostaglandins.

The ion channels for noxious stimuli have been partially identified; the transient receptor potential (TRP) family of these ion channels and here in particular the vanilloid-type TRP 1 (TRPV1) have been studied in most detail [8]. This receptor is sensitive to higher temperatures, acidity and capsaicin, an exogenous ligand (extract of chili pepper) and receptors like this one are currently being investigated as therapeutic targets for pain therapy.

Nerve growth factor (NGF) is also involved in the transduction process, as it binds to its receptor Tropomyosin receptor kinase A (TrKa) and thereby triggers increased transduction in pain states, in particular inflammatory pain. A monoclonal antibody against NGF, tanezumab, has shown very promising effects in current trials in osteoarthritis and chronic low back pain and is currently awaiting regulatory approval [9, 10].

21.2.2 Conduction

Voltage-gated sodium channels mediate conduction along primary sensory afferents. As for all other impulses throughout the body, action potential propagation is dependent on these channels. There are two types of sodium channels, differentiated by their sensitivity to tetrodotoxin. Both types are present in nociceptive neurons, with the tetrodotoxin-resistant type only present in nociceptors, which makes it a potential target for novel analgesics. Further research has identified three such voltage-gated sodium channels, (NaV1.7, NaV1.8 and NaV1.9), which seem to have specific roles in pain modulation [11, 12]. Mutations of these channels are linked to congenital insensitivity to pain and erythromelalgia [13], and attempts are currently being made to identify blockers or modulators of these channels as analgesics [14]. Research is also focusing on these sodium channels as a pharmacogenomic target [15].

Nociceptors also have voltage-gated calcium channels, which are found on the presynaptic membrane and are involved in neurotransmitter release at the dorsal horn. These are modulated by alpha-2-delta compounds such as gabapentin and pregabalin, now first-line treatments of neuropathic pain and central sensitisation [16].

Pain is transmitted by primary afferents, which have their cell bodies in the dorsal root ganglion (DRG). They terminate in the dorsal horn of the spinal cord. The dorsal horn cells are divided into specific regions or laminae called Rexed's laminae with I being the most superficial [17].

- Aδ-fibres are fast conducting and transmit the first sharp pain on initial stimulation. They terminate mainly in lamina I, but also send some fibres to lamina V of the dorsal horn where they synapse with second order neurones. They contain the neurotransmitter L-glutamate.
- C fibres are unmyelinated slow-conducting fibres which transmit a less well-localised persistent aching pain that lasts after the initial stimulus has gone. They terminate in lamina II of the dorsal horn. As well as glutamate, they contain several other neurotransmitters including neuropeptides, such as substance P, and calcitonin gene-related peptide (CGRP), cholecystokinin, brain derived neurotrophic factor and glial derived neurotrophic factor. C fibres express several presynaptic receptors that modulate transmitter release. These include cholecystokinin (CCK), opioid and gamma-aminobutyric acid subtype B (GABA B) receptors. Apart from the CCK receptor, they inhibit the release of transmitter.

- Aβ-fibres conduct low intensity mechanical stimuli which convey touch and not pain, however in chronic pain states they are involved in the transmission of pain (phenotypic switching) [18]. They terminate deeper in the dorsal horn in laminae III-VI

21.3 Spinal Cord Mechanisms

Primary sensory afferents terminate in the spinal cord where they synapse with cells of the dorsal horn. Nociceptive specific neurons are located mainly in laminae I and II but also lamina V and respond only to noxious inputs under normal conditions.

There are a number of different cells involved in the relay of painful stimuli including nociceptive specific cells and wide dynamic range neurons. Wide dynamic range neurons are located mainly in lamina V, but also in III and IV to a lesser extent, where they respond to stimuli from Aβ-, Aδ- and C-fibres [17].

The cells of the dorsal horn involved in nociception express a number of receptors;

- AMPA (a-amino-3 hydroxy-5-methylisoxazole) receptors which bind glutamate
- NMDA (N-methyl-D aspartate) receptors which also bind glutamate
- Neurokinin receptors NK-1 which bind substance P
- GABA-A receptors which are ligand-gated calcium channels that hyperpolarize the cell and reduce responsiveness to stimulation
- Voltage-gated calcium channels
- Glycine receptors that provide an inhibitory function

The ability to detect a potentially damaging noxious stimulus is mediated by glutamate acting on the AMPA receptor following stimulation of Aδ-fibres. The other receptors and neurotransmitters are involved in the modulation of the response.

When a high intensity noxious stimulus arrives at the dorsal horn via C-fibres, initially glutamate is released which acts via the AMPA receptor. As stimulus intensity increases, then other neurotransmitters are released such as Substance P. Slow post-synaptic currents are set up which are mediated by a number of receptors including the NMDA receptor. These are also involved in the modulation of the pain response [19].

21.3.1 Ascending Systems

Noxious information is conveyed from the dorsal horn to the brain via several ascending tracts in the spinal cord. The majority of the wide dynamic range neurons and nociceptive specific neurons are conveyed anterolaterally in three pathways [20]:

- The spinothalamic tract: Its fibres cross over to the contralateral side and pass through the brainstem to nuclei in the thalamus, finally terminating in the somatosensory cortex where pain is perceived and localised.
- The spinoreticular tract: It terminates in the reticular formation and has projections, which terminate in the pons, medulla and periaqueductal grey matter. It is involved in descending inhibition of pain.
- The spinomesencephalic tract: It is also involved in the modulation of descending control.

21.3.2 Descending Control

The dorsal horn receives inputs from higher centres that modulate the response to nociceptor input [21]. The descending control of output from the dorsal horn comes mainly from areas in the brainstem, namely the periaqueductal grey matter, the raphe nuclei and the locus coeruleus [22, 23]. Inhibitory tracts descend in the dorsolateral fasciculus and synapse in the dorsal horn. The key neurotransmitters involved are noradrenaline and serotonin. Noradrenaline acts via post synaptic α-2 receptors; the action of serotonin is less specific. Endogenous opioids are also involved in descending inhibition at a spinal and supraspinal level [22, 23]. These endorphins and enkephalins, acting via the descending system, are thought to be responsible for the analgesia induced by stress.

As well as descending control from the brainstem, nociceptive impulses are also attenuated by input via Aβ-fibres (transmitting information on touch), which is the basis for the use of Transcutaneous Electrical Nerve Stimulation (TENS) for analgesia, but also for simply rubbing a hurting body part. This observation formed the basis for the initial gate-control theory of pain [24].

21.4 Pain Modulation

The above description of pain explains the initial sensation of pain immediately following injury, however it does not explain the more complex phenomena associated with pathological pain due to neuroplasticity. These phenomena have a number of different causal mechanisms, which occur initially in the periphery, but later mainly in the dorsal horn as the main site modulation of painful stimuli.

21.4.1 Peripheral Sensitisation

Tissue injury results in release of inflammatory mediators, such as bradykinin, histamine, K+, H+, 5-Hydroxytryptamine (5-HT, also known as serotonin), ATP and nitric oxide, from damaged cells [25]. Breakdown of arachidonic acid by

cyclo-oxygenase produces leukotrienes and prostaglandins. Immune cell activation results in the release of further mediators including cytokines and growth factors. These mediators provide an 'inflammatory soup' which produces a painful area of primary hyperalgesia. These inflammatory mediators spread into the tissues surrounding the initial area of injury to produce an area of secondary hyperalgesia [26]. Therefore, most pain after injury of any kind is not purely nociceptive, but can be more correctly described as nociceptive-inflammatory.

The inflammatory mediators mentioned above act either by stimulating nociceptors themselves or by acting via inflammatory cells to stimulate release of additional pain-inducing agents. They also modify the response of primary afferents to subsequent stimuli, either by changing the sensitivity of the receptors, or by modulating the voltage-gated ion channels. For example, after tissue and nerve injury, N-type calcium channels become more active, resulting in greater release of glutamate in the spinal cord [27]. The magnitude of the current generated by sensory-neuron specific sodium channels is also increased.

Chronic inflammation and also nerve injury have an effect on the presence and distribution of voltage-gated sodium channels, which can become concentrated in areas of injury and produce ectopic discharges. Sensory neurone-specific sodium channels have a significant role in chronic pain states. Studies have shown them to become concentrated in neurones proximal to a site of nerve injury and this plays a role in hyperalgesia and allodynia (pain elicited by a normally non-noxious stimulus) [17]. In addition, NGF binding to TrKa receptors increases peripheral sensitivity as discussed before [28].

Not all sensory neurons are active all the time and this peripheral sensitisation will recruit "dormant" nociceptors, thus increasing the receptive fields of dorsal horn neurons and increasing the intensity and the area of pain [29].

21.4.2 Central Sensitisation in the Dorsal Horn

Central sensitisation is an increase in the excitability of the dorsal horn so that the dorsal horn cells have a lower threshold and respond to low intensity stimuli that are not usually painful. It also results in a greater response to supra-threshold stimuli thus producing the symptoms of allodynia and hyperalgesia. There are several mechanisms which occur at the dorsal horn and contribute to chronic pathological pain states by central sensitisation. These will be discussed in the context of neuropathic pain, as they are most relevant there.

21.5 Neuropathic Pain

Neuropathic pain is caused by disease or injury of the somatosensory nervous system and is related to a far-ranging number of aetiologies e.g., ischaemic, traumatic, infectious. Characteristics of neuropathic pain include spontaneous

stimulus-independent pain and pain that is stimulus-dependent and exhibits the features of allodynia and hyperalgesia. There are a variety of different mechanisms responsible for the generation of these symptoms, which may be quite different from patient to patient [30].

21.5.1 Mechanisms of Neuropathic Pain

The pathophysiology of neuropathic pain involves central and peripheral mechanisms and is in principle a '*maladaptive response of the nervous system to damage*' [31]. Usually more than one mechanism may be involved and producing a unifying hypothesis for all neuropathic pain states is inappropriate [32].

21.5.1.1 Peripheral Mechanisms

Spontaneous Ectopic Discharge

Normal primary afferent neurones require the input of a stimulus in order to reach firing potential. It has been shown that after a nerve injury spontaneous firing in the afferent neurone occurs. A and C fibres have been shown to demonstrate oscillatory activity resulting in ectopic firing [33]. Cross-excitation of other neurones increases this effect; in particular Aβ-fibres, usually not relevant for pain transmission, show ectopic discharge due to phenotypic switching [18].These phenomena are particularly relevant to the development of hyperalgesia, allodynia and chronic pain after nerve injuries.

Reorganisation of expression of ion channels in the peripheral nerves is responsible for these ectopic discharges [34]. Both sodium and calcium channels have been shown to be involved with their altered expression increasing the excitability of neurones. The afferent barrage provided by spontaneous discharge from neurones provides a constant input to the central nervous system that may induce central sensitisation [33].

Altered Gene Expression

Damaged peripheral sensory neurones undergo Wallerian degeneration and lose contact with peripheral targets and the supply of neurotrophic factors. The sensory neurones undergo altered gene expression, the result of which is a change in the type and level of neurotransmitters released in the spinal cord [35]. For example, some A-β fibres appear to release transmitters normally associated with nociceptors such as substance P. This seems to contribute to central sensitisation [36]. A change in gene expression also results in either up- or down-regulation of ion channels, in particular different types of sodium channels involved in ectopic spontaneous activity.

Spared Sensory Neurones

Changes have also been found in uninjured sensory fibres that are alongside those affected by a lesion. They frequently show the opposite gene expression changes from their damaged neighbours; possibly due to increased bioavailability of neurotrophic factors. This can result in increased activity in the spared afferents, although the exact mechanism is not understood [35].

Involvement of the Sympathetic Nervous System

Some patients exhibit neuropathic pain that is dependent on activity in the sympathetic nervous system. After a peripheral nerve injury, a coupling develops between the sympathetic nervous system and the sensory nervous system. Axons involved develop increased α-adrenoceptors and therefore have an exaggerated response to circulating catecholamines [37]. Morphological changes to the nerve follow with sympathetic axons sprouting into the dorsal root ganglion, forming baskets around the cell bodies of sensory neurones [38]. These changes lead to sympathetically maintained pain [39]. Evidence for a sympathetic component to a patient's pain include sympathetically maintained, often unilateral, limb pain, oedema, vasomotor and sudomotor asymmetries.

Effects of Bradykinin

This main plasma kinin, a vasodilator peptide, is involved in hyperalgesia associated with inflammatory pain, with a change in expression of its binding sites within the dorsal root ganglion after nerve injury [40]. Furthermore, there may be a role of the endogenous opioid dynorphin A as an agonist at the bradykinin receptor [41].

21.5.1.2 Central Mechanisms

The central mechanisms potentially involved in the generation of neuropathic pain are thought to result in neuroplastic changes in the CNS. A phenomenon termed central sensitisation occurs after peripheral nerve injury [42]. Central sensitisation changes the way the neurones respond to subsequent inputs [31]. This may result in spontaneous ongoing pain and abnormally evoked pain (allodynia and hyperalgesia) [29] .These mechanisms that are thought to be responsible occur primarily in the dorsal horn.

Wind-Up

The term wind-up describes the altered response of the dorsal horn neurones to repeated input from C-fibres [19, 29]. Following brief, repetitive C-fibre stimulation, the dorsal horn cells respond in a linear fashion. However if the stimulus continues, further C-fibre activation produces an amplified response in the dorsal horn to the same intensity of stimulus.

This phenomenon is mediated by the NMDA receptor [43]. Activation by sustained C fibre input leads to opening of the channel, an increased intracellular calcium concentration and an increased response to glutamate. Glutamate is the main excitatory neurotransmitter released from primary afferent neurones that acts at postsynaptic receptors. The NMDA receptor in its resting state is blocked by magnesium which is released when the cell is depolarised, thus opening the channel in the receptor and allowing an influx of sodium and calcium and further depolarisation. When a painful stimulus arrives at the dorsal horn, the cells are initially depolarised by glutamate acting at the AMPA receptor, thus allowing removal of the magnesium block. Once the stimulus is removed, the dorsal horn cells continue to fire for several seconds. There is potential for this to be modified pharmacologically; in particular NMDA antagonists such as ketamine prevent these phenomena including hyperalgesia [44]. Wind up is relatively short lived (seconds to minutes), whereas central sensitisation persists and thus the exact relationship remains unclear [45].

Central Sensitisation

Central sensitisation is also mediated by the NMDA receptor. Under conditions of prolonged C-fibre activation, depolarisation of the dorsal horn cells causes the NMDA receptor to lose its magnesium block [43]. Substance P, acting via its receptor, the neurokinin-1 receptor, prolongs this depolarisation and allows further influx of calcium. The increase of calcium in the dorsal horn activates calcium-dependent kinases such as protein kinases A and C, which are then able to phosphorylate amino acids within the NMDA receptor to produce a conformational change in the structure. This permanently removes the magnesium block in the receptor and allows it to be activated by glutamate. The process of central sensitisation differs from windup in that the changes remain long after the C-fibre input has ceased. Furthermore, the magnesium is removed by posttranslational changes in the NMDA receptor and is not just depolarisation induced [29, 36, 46].

Central Disinhibition

Central disinhibition results from loss of modulatory control mechanisms, which may lead to abnormal excitation of central neurones [47]. The main inhibitory neurotransmitter is γ-aminobutyric acid (GABA). It has been shown that suppression of this pathway results in allodynia [48]. Within 2 weeks after a peripheral nerve injury, GABA receptor levels are reduced. Down-regulation of GABA-mediated pathways may be, in part, responsible for central sensitisation [49].

Expansion in Receptive Field Size (Recruitment)

Receptive fields of dorsal horn neurones contain subliminal areas; these represent a reservoir of activity [50]. With ongoing activation after injury there is an expansion of receptive field size leading to increased perception of pain, resulting in secondary hyperalgesia. This expansion of receptive fields does not reflect peripheral nerve or

nerve root distribution, but spinal cord architecture. It might therefore be confusing from a diagnostic point of view, as it transgresses the boundaries imposed by a hard-wired model of the CNS [51].

Immediate Early Gene Expression

Immediate changes in gene expression in dorsal horn cells occur in response to Aδ- and C-fibre stimulation. These changes persist for a variable length of time and may contribute to central neuroplasticity. Noxious stimulation mediated by Aδ- and C-fibres produces an immediate change in the expression of certain genes within the dorsal horn cells [52]. These changes are detected within minutes of stimulation and may last for months or even years. The gene c-*fos* encodes for a protein, *fos*, which forms part of a transcription factor which may control the expression of other genes which produce long-term changes in the dorsal horn. *C-fos* activation occurs as a result of increases in intracellular calcium following release of neurotransmitters like substance P and glutamate, involved in relay of nociceptive information [53]. This is followed rapidly by the appearance of *fos* protein which can be detected in laminae I, II and V of the dorsal horn. The presence of *fos* protein can be used as a marker of noxious stimulation and thus also to determine the effect of agents to reduce noxious stimulation [54].

Anatomical Re-Organisation of the Spinal Cord

Primary afferent neurones synapse in the laminae of the dorsal horn with second-order ascending neurones. Under normal conditions, Aδ- and C-fibres terminate in laminae I and II, whereas Aβ-fibres terminate in laminae III and IV. Following C-fibre injury, the large unmyelinated Aβ-fibres sprout terminals into lamina II. Aβ-fibres, which are activated by low intensity non-painful stimuli can thus stimulate the dorsal horn neurons present in lamina II, usually associated with noxious sensation [55]. This observation could explain allodynia, as Aβ-fibres form synapses with second-order neurones and their low-threshold non-noxious inputs will be signalled as nociceptive in origin. However, doubt surrounds this theory as a main mechanism of allodynia because sprouting is not fully established until 2 weeks after the injury [56]. Furthermore it has been suggested that this sprouting only occurs in a small subgroup of Aβ neurones [35].

As well as sprouting fibres into lamina II, Aβ-fibres also undergo phenotypic switching and produce the neurotransmitter substance P and calcitonin gene-related peptide [18]. These neurotransmitters are usually produced only by C-fibres, but after nerve injury their expression by C-fibres is down-regulated. Aβ-fibres begin to release these neurotransmitters at the dorsal horn following low intensity stimulation. This release of substance P can maintain the central sensitisation changes in the dorsal horn at the NMDA receptor that is usually only maintained by continued C-fibre input [56].

Contribution of Glial Cells to Pain Conditions

The last years have seen an increasing understanding of the important role that activation of glial cells and neuro-glial interactions play in the maintenance of central sensitization and thereby chronic pain conditions [57]. Microglia, astrocytes in the CNS, and also satellite glial cells in the dorsal root ganglia (DRG) and the trigeminal ganglia are involved in these processes [58]. Glial activation is primarily mediated by activation of toll-like receptor 4 (TLR4) [59]. It results in a change of glial cell morphology, increase in glial cell numbers and release of powerful pro-nociceptive mediators including ATP, cytokines and chemokines. These processes lead via neuro-glial interactions to sensitisation of CNS neurons (through activation of their cognate receptors) and thereby contribute to the phenomenon of central sensitization. As μ-opioid agonists are also glial activators, opioid-induced hyperalgesia is partially mediated through the same processes [59]; this would explain the efficacy of low-dose naltrexone in central sensitization [60]. There is now significant support for the concept that chronic pain states could be a result of a "gliopathy" [57].

21.5.2 Symptoms of Neuropathic Pain

Patients with neuropathic pain usually experience persistent and/or paroxysmal pain [61]. The pain often has an abnormal quality, for example burning, electric-shock like, shooting, lancinating or numbing. Neuropathic pain can occur in an area of neurological deficit, but might also arise from areas still innervated normally [62]. Neuropathic pain exhibits often one or more of the following characteristic features;

- *Dysaesthesia*, an unpleasant abnormal sensation, whether spontaneous or evoked.
- *Hyperalgesia*, an increased response to a painful stimulus.
- *Allodynia*, pain elicited by a normally non-noxious stimulus
- *Hyperpathia,* a painful syndrome characterised by increased reaction to a stimulus, especially a repetitive stimulus, as well as an increased threshold.
- *Hypoalgesia,* diminished pain in response to a normally painful stimulus.

Clinical features of neuropathic pain are often summarised as stimulus-dependent, stimulus-independent and sympathetically-maintained pain [62].

21.5.2.1 Stimulus-Dependent Pain

Following nerve injury, increased C-fibre activity causes central sensitisation within the dorsal horn via activation of the NMDA receptor as described earlier.

Central sensitisation produces three main effects;

1. Enlargement of the sensory field of a dorsal horn neuron (secondary hyperalgesia)
2. Increase of the response to a suprathreshold stimulus (hyperalgesia)
3. Generation of a response to a subthreshold stimulus (allodynia)

These phenomena represent stimulus-dependent pain, although the relationship between stimulus and response may vary widely.

21.5.2.2 Stimulus-Independent Pain

As mentioned earlier, there are two types of sodium channels present on sensory neurons. The tetrodotoxin-resistant channels are implicated in the generation of the spontaneous pain of pathological pain states. Following injury there is reorganisation of the expression and location of the various types of sodium channel within the neuron. The tetrodotoxin-resistant channels relocate to the neuroma, where it produces areas of hyperexcitability and ectopic discharges. After nerve injury, both injured nerves and uninjured nerves close to the site of injury display spontaneous discharges. The alterations in expression of sodium channels are thought to be due to alterations in the supply of neurotrophins such as nerve growth factor and glial-derived neurotrophic factor [63].

21.5.2.3 Sympathetically Maintained Pain (SMP)

In a small but significant proportion of chronic pain sufferers, the pain has a definite sympathetic system element to it and is said to be sympathetically maintained. Following partial nerve injury in these patients, both injured and uninjured primary afferents express alpha-2 adrenoceptors on their membranes so they become sensitive to circulating catecholamines and noradrenaline release from sympathetic nerve terminals [29].

Direct coupling also occurs between the sympathetic and peripheral nervous systems with sympathetic nerves sprouting axons into the dorsal root ganglion to form baskets around the cell bodies of nociceptor neurons, where they form functional synapses. This sprouting is thought to occur under the influence of nerve growth factor. Other more central mechanisms of somatosensory-sympathetic coupling are also investigated [64].

21.6 Neuropathic Pain Syndromes

There are many causes of neuropathic pain including a number of disease states.

21.6.1 Peripheral Neuropathies

21.6.1.1 Metabolic/Endocrine

Diabetics can develop different types of neuropathies, which include polyneuropathies, autonomic neuropathy, compression neuropathy and focal neuropathies. Around 50% of diabetics have polyneuropathy and many of these present with

neuropathic pain [65]. Many diabetics, especially those with poor blood glucose control, develop a distal, symmetrical, proximally spreading and painful neuropathy [66]. Severe pain is often a feature and may be described as burning, aching or have lightning components to it. It seems that the main cause is demyelination and to a lesser extent axonal degeneration. The first stage in prevention and treatment of early neuropathies is good glycaemic control. Additionally, hyperglycaemia may have a direct effect on neuropathic pain by altering pain thresholds, tolerance and affecting opioid receptors.

Mononeuropathies, usually involve the motor supply to extraocular muscles and also nerve supply to the limbs. The third cranial nerve is most frequently affected. Pain is often a symptom. Additionally, an asymmetrical proximal, predominantly motor, neuropathy can occur, especially in older patients with poor glycaemic control [67]. Untreated hypothyroidism may result in neuropathic pain.

21.6.1.2 Toxic

Well-known neuropathies here include those caused by alcohol, chemotherapy (where the neuropathy maybe the dose limiting factor) and, more recently anti-AIDS drugs (e.g. isoniazid).

21.6.1.3 Post-infectious

The most common problem encountered is Post Herpetic Neuropathy (PHN), which increases in incidence, intensity and persistence with age [68]. The pain persists in the distribution of a peripheral nerve after herpes zoster infection (shingles). It is thought that chronic inflammatory changes result in damage to sensory nerves, resulting in deafferation of nociceptive fibres. The pain is persistent and can become intolerable with associated allodynia. Treatment is often very difficult, in particular in later stages.

21.6.1.4 Hereditary

Fabry disease, a rare lipid storage disorder, often presents with a painful neuropathy [69].

21.6.1.5 Malignant

Neuropathies can occur as a non-metastatic complication of malignant disease, usually a sensory neuropathy that can sometimes be painful. Neuropathic pain can also be caused as the result of direct tumour invasion involving nearby nerves.

21.6.1.6 Vascular

Vascular pain is a complex issue. Pain can be arterial, microvascular or venous in origin. Neuropathy can in particular follow venous insufficiency [70]. In every vascular disease, sympathetic changes may develop which contribute a neuropathic element to the ischaemic pain. The patient may develop skin hyperalgesia, dystrophic skin with a shiny appearance, muscle atrophy and vasomotor phenomena. Sympathetic blocks may be beneficial [71].

21.6.1.7 Posttraumatic

Posttraumatic neuropathies are common and can develop after any nerve injury. Even minor demyelination injuries without neurological sequelae can result in neuropathies. Examples are sciatica, neuroma or nerve entrapment after surgery or trauma, phantom limb pain, complex regional pain syndromes (CRPS) type I (without neurological deficit, previously called Reflex Sympathetic Dystrophy RSD) and Type II (with neurological deficit, previously called causalgia) and post-thoracotomy pain.

21.6.2 Central Neuropathies

Central neuropathic pain is due to a lesion or disease of the CNS [72]. These lesions may have associated symptoms that affect the patient and their pain e.g. ataxia, motor weakness and hearing/visual loss. Epilepsy and depression are also common with cerebral lesions. These aspects need to be addressed along with treatment of the pain. Central neuropathic pain is associated with spinothalamocortical dysfunction and may develop over a length of time and varies widely between individuals regardless of aetiology.

21.6.2.1 Vascular Lesions in the Brain and Spinal Cord

The aetiology here includes infarction, haemorrhage, and vascular malformation. Stroke is the most common cause of central pain due to its high incidence [73]. Around 8% of patients with acute stroke have been shown to suffer from central pain in the following 12 months.

21.6.2.2 Multiple Sclerosis

This demyelination process can result in neuropathic pain by a variety of mechanisms. Cranial nerve neuropathies, but also widespread central pain syndromes are common consequences and often difficult to treat [74].

21.6.2.3 Trauma, Tumours and Infections

Brain injury, but by far more commonly spinal cord injury, can result in a variety of central pain syndromes [75]. Syringomyelia and syringobulbia as a consequence of such injuries can cause further central pain. Tumours of the brain and spine as well as infections and abscesses can cause similar symptoms [72].

21.7 Nociplastic Pain

In 2011, the IASP changed the definition of neuropathic pain. The most relevant change was the removal of the concept of dysfunction of the nervous system from the definition [76]. This occurred in response to the increasing recognition, outlined in detail in this chapter, that most pain states change the function of the nervous system. Therefore, continued use of this definition was counterproductive, as many pain conditions fulfilled this definition, but were not the result of a lesion or disease (e.g. fibromyalgia, CRPS Type 1, nonspecific chronic back pain and many visceral and pelvic pain conditions).

This redefinition left many chronic pain conditions without an appropriate label; neurophysiologists used the term CNS dysfunctional pain states, but this is not a useful and acceptable term in clinical practice. The need for a third mechanistic descriptor besides nociceptive (inflammatory) and neuropathic pain became obvious [5]. In November 2017, the IASP decided to introduce such a third descriptor by coining the term nociplastic pain, defined as 'Pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion of the somatosensory system causing the pain.' In parallel, the new International Classification of Diseases (ICD-11) will have chronic pain as a new entity for the first time with a subgroup labelled primary chronic pain including such nociplastic pain states [77].

21.8 Conclusion

Pain perception is a complex process. Identifying the pathophysiology of the pain presentation is important in terms of giving an explanation to the patient and the choice of management strategies.

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Chapter 22
Post Amputation Pain Syndromes

Stephan A. Schug

Key Learning Points

- Post amputation pain syndromes are common and require prevention and appropriate therapy.
- Phantom sensation (the sensory perception of the amputated limb without pain) is ubiquitous, diminishes over time and cannot be treated.
- Stump pain (pain in the residual limb) has multifactorial causes and requires careful assessment and targeted therapy.
- Phantom limb pain (noxious sensory perceptions in the amputated limb) occurs in 60–80% of amputees and can be severely distressing.
- All above phenomena may coexist in one patient.
- A number of preventive and therapeutic approaches are described, although the evidence for most of these is limited
- As in all chronic pain management, non-pharmacological and pharmacological approaches should be combined.

22.1 Introduction

The phenomenon of pain in a missing limb has puzzled patients, doctors and the lay public for centuries. In the sixteenth century the French military surgeon Ambroise Paré published a medical description of the enigmatic affliction, while in the seventeenth century the great philosopher Rene Descartes looked at its potential pathophysiology. The most famous 'first' description of the condition is attributed to the neurologist Charles Bell [1], but it was only in the latter part of nineteenth Century,

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that the US military surgeon Silas Weir Mitchell introduced the term 'phantom limb': "*There is something almost tragical, something ghastly, in the notion of these thousands of spirit limbs, haunting as many good soldiers, and every now and then tormenting them.*"

We now know that post-amputation syndromes can occur with any amputated body part as well as from limbs e.g. breast, eye, tongue, teeth, genitalia and even inner organs such as the rectum [2–6].

22.2 Classification and Incidence

Following amputation (or deafferentiation injury such as brachial plexus avulsion) a number of phenomena can develop which require differentiation [7].

22.2.1 Stump Pain

Stump pain (or residual limb pain) is pain localized to the site of amputation. Stump pain can be acute (usually nociceptive) or chronic (usually neuropathic). Stump pain is most common in the immediate post-operative period [8]. The overall incidence of chronic stump pain is in the range of 45% [9]. The incidence of early stump pain is increased by the presence of severe pre-amputation pain [10] and severe acute stump pain [11]. The cause is unclear but probably multifactorial. Stump pain is problematic, in particular when interfering with prosthesis use.

22.2.2 Phantom Sensation

Phantom sensation is defined as any sensory perception of the missing body part with the exclusion of pain. Almost all patients who have undergone amputation experience such phantom sensations [3, 12]. These sensations range from a vague awareness of the presence of the organ via associated paraesthesia to complete sensation including size, shape, position, temperature and movement [13]. Phantom sensations usually diminish in intensity or size over time, but may persist for a long time. "Telescoping" of the phantom part can occur with time such that the phantom limb gradually shrinks proximally to approach the stump [14]. Eventually the phantom limb is felt to be within the stump itself.

22.2.3 Phantom Limb Pain

Phantom limb pain (PLP) is defined as any noxious sensory phenomenon of the missing limb or organ. The incidence of phantom limb pain is estimated to be 60–80% after limb amputation [8, 9]. The pain is independent of gender, level or

side of amputation [8]. There is, however, a lower incidence among children and congenital amputees [15].

Pain can be immediate or delayed in onset. It is typically intermittent and changes with time. Typical characteristics of phantom limb pain are burning, shooting, crushing, throbbing, cramping, aching, tingling, boring; often the limb is described as being in a hyperextended or otherwise unnatural position. The pain usually occurs in the distal portion of the missing limb [8]. The incidence of pain may be increased if pre-amputation pain was present and may then resemble the pre-amputation pain in character and localisation [11, 16]. However, the exact relationship between pre-amputation pain and PLP is not a simple one, especially as patients' pain perceptions alter and may be exaggerated with time [8]. The incidence of phantom pain diminishes with time after amputation, as does the frequency and intensity, being highest immediately following surgery [8].

It is important to realise, that the terms for the noxious syndromes, "stump pain" and "phantom limb pain", are subjective descriptive terms that do not make assumptions on differences in pathophysiology. There is, in fact, a strong correlation between phantom pain and stump pain and they may be inter-related phenomena [17]. All three phenomena can co-exist [10]; the incidence of combined phantom limb pain and stump pain is described as 36% [9].

22.3 Pathophysiology

The pathophysiology of post amputation pain syndromes is most likely based on a combination of peripheral and central factors, which interplay subsequent to the significant trauma of an amputation.

22.3.1 Peripheral Factors

The following changes occur after peripheral nerve injury such as cutting of a nerve [18];

1. Sensitization of peripheral nociceptors with a decreased threshold to noxious stimulation;
2. Increased response to supra-threshold stimulation;
3. Spontaneous activity of peripheral receptors due to sensitisation, including ectopic pacemaker sites (possibly as a result of the increase in sodium channels), α-adrenergic channels, calcium channels and stretch-activated channels following nerve injury;
4. Sensitization of non-nociceptive receptors to nociceptive impulses.

These changes contribute to hyperalgesia and allodynia in the stump; therefore stump manipulation and revision can worsen pain due to repeated deafferen-

tation injuries. The dorsal root ganglion (DRG) may also be the site of ectopic neuronal activity subsequent to deafferentiation and thereby contribute to pain syndromes.

Furthermore, regrowth of severed nerves often produces nodules called "neuromas". Neuronal activity originating from peripheral neuromas either spontaneously or in response to mechanical, chemical or electrical stimulation, may cause increased sensitivity of the stump to different stimuli [18].

Other peripheral factors include increased muscle tension in the stump correlated with cramping and spasmodic pain and decreased blood flow to the stump, correlating with descriptions of phantom pain such as burning or tingling. Low stump temperature correlates with burning pain.

There is currently a debate about the relevance of peripheral factors in the generation of phantom limb pain. In general, there is consensus that central factors described below play the most important role in a 'top-down' phenomenon. However, there is also some evidence for a 'bottom-up' component caused by ectopic activity in axotomized DRG [19]. This theory is supported by the beneficial effect of local anaesthetic block of the DRG on phantom limb pain and even phantom sensation.

22.3.2 Spinal Factors

The combination of increased afferent input from sensitised nerve endings and the dorsal root ganglion may contribute to central sensitisation. The following changes occur in the dorsal horn of the spinal cord after nerve injury [18];

1. Increased spontaneous activity of dorsal horn neurons
2. Increased response to afferent input
3. After-discharges following repetitive stimulation
4. Expansion of peripheral receptive fields
5. Wind-up (increased neuronal activity in dorsal horn neurons following repetitive C-fibre stimulation), mainly mediated by NMDA receptors

These factors play an important role in many chronic pain syndromes, but to which extent these factors are involved in perpetuation of phantom syndromes is currently unclear, although involvement is likely.

22.3.3 Supraspinal Factors

The presence of pain prior to amputation is thought to increase the likelihood of phantom pain [16]. In 1971, Melzack proposed that the painful extremity had created a painful central "engram". An engram is the schematic representation of body

parts in the CNS caused by consistent sensory input. This engram was thought to persist after amputation, causing phantom pain.

On the basis of these observations, the **neuromatrix theory** was proposed by Melzack in 1990 [20]. In this theory, the body's physical self is represented by a matrix, a complex network of neurons connecting somatosensory cortex, thalamus and limbic system. This *neuromatrix* is genetically determined and subsequently modulated by sensory input, thereby creating a *neurosignature* for each body part. This neurosignature determines how a body part is consciously perceived; phantom sensations are the result of persistence of the neurosignature after the loss of the limb. The genetic determination of the neurosignature is confirmed by the observation that children who are born with a missing limb may feel phantom sensations of the missing part.

In this theory, phantom limb pain is the result of abnormal reorganisation in the matrix, either due to a pre-existing pain state or the amputation process itself [20].

By analysing neuromagnetic fields, Flor's group has been able to show a close correlation between the degree of neuromatrix reorganisation and the development of phantom limb pain [21]. Reorganisation of somatosensory cortex occurs with neighbouring representation zones moving into the deafferented zone [22]. Here it is also of note that many of the sites of amputation that commonly lead to phantom sensation and pain are sites with a relatively large cortical somatosensory representation. However, a systematic review of publications on functional imaging in patients with phantom limb pain found limited evidence for the relationship between maladaptive cortical plasticity and severity of phantom limb pain [23]. In addition, recent findings link not only somatosensory, but also primary motor cortex changes, to phantom limb pain [24].

An alternative theory discussed in the literature is the **dynamic reverberation theory**. This originated from the observation that selective stereotactic cortectomies of the corona radiata or focal brain lesions in the parietal cortex, thalamus or corticothalamic fibres on the contralateral side have resulted in permanent relief of phantom pain. This led Canavero, in 1994, to the theory that phantom pain and sensation were a result of a localized dynamic reverberation loop between cortex and thalamus. He postulated that this loop could operate with or without sensory activation [25].

22.3.4 Current Pathophysiological Model

A comprehensive model incorporating the current state of knowledge has been proposed by Flor et al. It includes peripheral as well as central factors as relevant contributors to the development and perpetuation of phantom limb pain [22]. In principle, it suggests that somatosensory pain memories and a subsequently altered homuncular representation in the somatosensory cortex are the underlying factors of phantom limb pain, which can be sustained by peripheral factors. It assumes that memories of pain established before an amputation are powerful causative

contributors to phantom limb pain generation. Analogous to findings in other chronic pain patients, such pain memories increase the representation zone in the primary somatosensory cortex. The changes are then perpetuated after the amputation by selective C-fibre deafferentiation, random input from stump neuromas, abnormal changes in the dorsal root ganglia and the dorsal horn of the spinal cord and sympathetic activation [26].

22.4 Prevention

In view of the immense difficulties of treating phantom limb pain once it is established, considerable efforts have been made to identify techniques to prevent the syndrome. Regrettably, none of the methods tried have provided conclusive evidence of efficacy, although overall results on good perioperative analgesia, in particular epidural anaesthesia and possibly ketamine administration remain promising.

22.4.1 Risk Factors and Predictors

Attempts to identify predictors of postamputation pain syndromes have identified that pain before the amputation (chronic and acute) is the most significant predictor of these phenomena [27]. This may explain why, in contradiction to findings in other postoperative chronic pain syndromes, older patients have more phantom limb pain; the main indication for amputation in this age group is ischaemia, resulting in long-lasting pain states before amputation. Psychological risk factors are preoperative depression and anxiety. Last, but not least, subacute postoperative pain is a further risk factor. These predictors match those of chronic postsurgical pain in general [28]; this suggests the possibility of preventive measures by perioperative interventions.

22.4.2 Provision of Good Perioperative Analgesia

A study comparing provision of good perioperative analgesia preoperatively and into the postoperative period with conventional analgesia as a control resulted in reduced severity and incidence of phantom limb pain 6 months postoperatively [29]. The beneficial effects were achieved with patient-controlled analgesia and/or epidural analgesia independent of details of the technique. This supports the concept that optimised perioperative analgesia reduces perioperative pain severity and has a protective effect on peripheral and central sensitisation processes.

22.4.3 Perioperative Lumbar Epidural Blockade

In 1988, Bach et al. demonstrated that lumbar epidural blockade with bupivacaine plus morphine, started 72 h *prior to surgery*, reduced the incidence of phantom limb pain in the first year after surgery [30]. This study has been criticised for a number of reasons, but it initiated a number of similar studies with somewhat contradictory results [31]. However a meta-analysis showed that perioperative epidural analgesia reduced the incidence of severe phantom limb pain with an NNT of 5.8 [32]. Overall, the results are promising and a protective effect has again been confirmed in a recent unpublished audit at our institution. Furthermore, epidural techniques provided good early postoperative analgesia [33].

22.4.4 Peripheral Nerve Blockade

Infusions of local anaesthetics via peripheral nerve sheath catheters, usually inserted by the surgeon during the amputation, are a safe method of providing effective analgesia in the immediate post-operative period, although a recent meta-analysis describes the current evidence as indecisive [33]. The technique has no proven benefit in the prevention of phantom limb or stump pain [34].

22.4.5 NMDA Antagonists

The use of pre-incision ketamine as pre-emptive analgesia has been described previously in other settings. An observational study suggested that the incidence of severe phantom limb pain may be reduced with the use of ketamine as a bolus followed by an infusion started prior to skin incision and continued for 72 h postoperatively [35]. This promising study was small and used historical controls. A randomised controlled trial could not confirm these results, but was underpowered [36]. Epidural ketamine had no preventive effect in an RCT [37]. However, a trial of memantine in combination with regional analgesia showed a preventive effect [38]. Overall, further investigations are justified.

22.5 Evaluation of the Patient with Post Amputation Pain Syndromes

Phantom sensation requires pre- and post-operative counselling and education but it should not generally pose a clinical problem. Phantom limb pain, however, has a profound effect on the quality of life of amputees. Provision of information to amputees that such pain syndromes are not ‘mental or imaginary’ is important, as these misconceptions are still widespread [39].

Table 22.1 A systematic approach to assessment of postoperative pain

1.	*Prosthogenic pain*	
	(a)	Due to an improperly fitting prosthesis:
		• Poor socket fit, cushioning or alignment
	(b)	Inappropriate suspension resulting in pistoning
	(c)	Painful adductor roll in the above-knee amputee
	(d)	Distal residual limb weight bearing
	(e)	Poor trim line
2.	*Neuropathic pain*	
	(a)	Caused by neuroma formation
	(b)	Test for presence of wind-up by examining for Tinel's sign—shooting pains elicited by repeated tapping over the area
3.	*Arthrogenic pain*	
	(a)	Pain originating in neighbouring joint or surrounding soft tissue, ligaments or tendons.
4.	*Referred pain*	

22.5.1 Examination

Careful examination of the stump is important to exclude common causes of pain (Table 22.1). Excessive biomechanical stress in amputees may cause painful musculoskeletal conditions such as sacroiliac dysfunction, piriformis syndrome, facet syndrome or radiculopathy [7]. Therefore it is important to examine posture and gait.

Furthermore, it is of value to examine skin for areas of ulceration and infection, palpate for areas of tenderness, bony exostosis, heterotropic ossification and adherent scar tissue and evaluate muscle strength and range of movement of neighbouring joints to exclude contracture formation.

22.6 Therapy

A survey by Sherman et al. in 1980 identified over 50 different therapies currently in use for the treatment of phantom limb pain [40]. This suggests clearly that a treatment of phantom limb pain has not been established and that *'the results are poor and usually below the expected rate of cure of pain with placebo treatment alone.*'

Over the last 40 years, very little progress has been made. Even today, very few treatments have an evidence-base. However, as early treatment is more effective and often multidisciplinary approaches are needed, patients with severe postamputation pain should be urgently referred to a multidisciplinary pain clinic to ensure optimal and timely pain management.

22.6.1 Calcitonin

Calcitonin parenterally is a proven treatment for phantom limb pain and in our experience the most effective in early stages [41], while there is no benefit in chronic phantom limb pain [42]. After initial anecdotal reports [43], a randomised double-blind cross-over study by Jaeger and Maier showed excellent effectiveness [44]. 200 IU of salmon calcitonin was given as an intravenous infusion over 20 min and provided complete pain relief for 76% of patients. Seventy one percent did not experience recurrence of their phantom pain. Calcitonin may also be given subcutaneously or intranasally [41].

The mechanism of action of calcitonin in inhibition or modulation of pain perception is unknown, although a number of hypotheses have recently been published [45]. There is also evidence for an effect on pain in vertebral crush fractures and anecdotal descriptions of its effectiveness in a number of states of central sensitisation. Side effects including dysaesthesia, nausea and vomiting have been described, but most are transient and can be prevented by prophylactic use of anti-emetics [44]. The risk of an anaphylactic reaction is minimal.

22.6.2 NMDA Receptor Antagonists

Over-activity of NMDA receptors may be a factor in the maintenance of stump pain and phantom limb pain. Ketamine, an antagonist at the N-methyl D-aspartate (NMDA) receptor, is another treatment for stump and phantom limb pain with the best data on efficacy [46, 47], although the conclusions of a Cochrane review are more cautious [48]. With the increasing availability of oral and sublingual preparations of ketamine, this may offer future treatment options [49]. Memantine, another NMDA antagonist, may be another medication in this class for the treatment of early phantom limb pain, but with no effect on chronic states [50].

22.6.3 Opioids

Opioids are in principle effective in neuropathic pain, but only recommended as third-line treatment due to the considerable adverse effects and risks with their long-term use [51]. Morphine has been used successfully in patients with phantom limb [47], but these benefits may not be maintained long-term, which is also the problem with opioids in other chronic pain states [48]. The atypical opioid tramadol may be particularly useful in this setting [47].

22.6.4 Gabapentin

Overall, study results support a beneficial effect of gabapentin on phantom limb pain, although this is not a strong recommendation [47, 52].

22.6.5 Lidocaine

A randomised double blind study comparing the effects of intravenous lidocaine with intravenous morphine showed that lidocaine given as 1 mg/kg bolus followed by 4 mg/kg infusion, given on three consecutive days, significantly reduced stump pain, but had no effect on phantom pain [53].

22.6.6 Tricyclic Antidepressants (TCA)

Amitriptyline has been shown to be as effective as tramadol in the treatment of phantom limb pain [54] .There is no evidence to support the use of other TCAs in phantom limb pain.

22.6.7 Capsaicin

Capsaicin depletes the neurotransmitter substance P from sensory nerves and may give relief to some patients with stump pain when used topically [55]. Capsaicin 8% patches have been used for the treatment of stump and phantom limb pain successfully [56].

22.6.8 Symptomatic Treatment of Pain Components

The burning component of phantom limb pain alone can be decreased by pharmacological and behavioural therapies that increase the temperature of the stump such as sympathectomy, α- or β-blockade or biofeedback. Cramping can be relieved by treatments that reduce muscle tension, for example with the use of baclofen.

22.7 Nonpharmacological Therapies

With the development of theories on cortical reorganisation as a cause for phantom limb pain, there are now therapies available which are based on the concept of reversing such reorganisation [22]. Sensory discrimination training programs show promise here. This is a process during which patients have to discriminate the frequency or location of high intensity non-painful electrical stimuli applied through electrodes on their stump in an attempt to separate merged regions on their cortical somatosensory map.

A recent study using this technique showed that phantom limb pain was significantly decreased in the group who underwent the training process compared to controls. Cortical reorganisation, assessed by neuroelectric source imaging and structural magnetic resonance imaging, was also reduced in this group [57]. Mental imagery of limb movement [58, 59] and a combination of laterality recognition, mirror movements and imagined movements are other successful approaches based on this concept [60].

Overall, mirror therapy, motor imaginary, and virtual visual feedback reduce phantom limb pain, but the supportive data are not good enough to make a final decision on efficacy [61]. This is also the result of a separate systematic review of mirror therapy [62]. Similarly, virtual and augmented reality approaches are only supported by case studies and case report series [63]. The use of a myoelectric prosthesis may prevent cortical reorganisation and phantom limb pain [64].

22.7.1 Invasive Therapies

22.7.1.1 ECT

This psychiatric treatment is thought to interrupt the dynamic reverberations that maintain central and phantom pain in the thalamocortical pathway [25] and has been used in the treatment of refractory phantom pain [65]. There have been no trials in this area.

22.7.1.2 Transcutaneous Electrical Nerve Stimulation (TENS)

There are no data based on RCTs supporting the use of TENS in the indication of phantom limb or stump pain [66].

22.7.1.3 Peripheral Nerve Stimulation

There are some limited preliminary data suggesting a potential benefit of this technique for postamputation pain [67].

22.7.1.4 Spinal Cord and Brain Stimulation

Brain and spinal stimulation therapies for phantom limb pain were analysed in a systematic review, which could not offer robust results on the efficacy of these techniques [68]. This is confirmed by a specific review of spinal cord stimulation with similarly poor results [69].

22.7.1.5 Dorsal Root Entry Zone Lesions

DREZ lesioning has a limited effect for a limited time only in phantom limb pain [70]. This is in line with clinical experience with this neurodestructive approach. Other types of surgery and neuroablation often makes pain worse because of repeated stimulation and/or deafferentation of the affected nerves.

22.7.2 Psychological Therapy

Pre-amputation counselling is mandatory as amputees go through normal grieving processes. It is important to identify anxiety and depression early, as these can magnify pain perception. Behavioural, cognitive, group therapy and pain management programs are all useful methods of helping patients cope with their chronic pain. Hypnosis, biofeedback and muscular relaxation training to disrupt the pain-anxiety-tension cycle are important components of chronic pain therapy [71]. Overall, psychological interventions, in particular cognitive-behavioral therapies and hypnotherapy, show a reduction in phantom limb pain, but this statement is based on studies with poor quality [72].

22.8 Conclusion

Post-amputation pain syndromes remain a significant challenge in the management of limb amputees. A considerable number of preventive and therapeutic strategies are discussed, but the evidence for most of these is weak. As with all chronic pain states, a combination of pharmacological and non-pharmacological approaches to management should be utilised.

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Chapter 23
Treatment of Neuropathic Pain

Stephan A. Schug

Key Learning Points

- Chronic neuropathic pain requires a multidisciplinary approach combining non-pharmacological and pharmacological strategies.
- Neuropathic pain is unresponsive to many analgesics effective in nociceptive pain.
- In neuropathic pain, antidepressants (TCAs and SNRIs) and alpha-2-delta modulators are first-line treatments.
- Topical treatments of localized neuropathic pain with lidocaine and capsaicin patches offer new options with limited systemic adverse effects.
- Opioids are effective in neuropathic pain, but their risk profile limits use (except for the atypical opioids tramadol and possibly tapentadol).
- Combination therapy is promising, but not always the best option.

23.1 Introduction

Neuropathic pain is defined by The International Association for the Study of Pain (IASP) as pain following a lesion or disease of the somatosensory system [1]. It is caused either by peripheral lesions or diseases involving peripheral nerves, dorsal root ganglia and the dorsal roots (peripheral neuropathic pain) or by central lesions or diseases in the spinal cord or brain (central neuropathic pain). Neuropathic pain results in persistent pain syndromes that have no biological function, but are difficult to treat and cause great distress to the individual. Neuropathic pain is also referred to as neurogenic pain, deafferentation pain, neuralgia, neuralgic pain and nerve pain.

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Neuropathic pain may develop immediately after a nerve injury or after a variable interval. It may be maintained by factors different from the initial cause. It can persist for a long time and is frequently not explained by underlying pathology. Patients are frequently seen by many different specialists and their treatment often fails to resolve the pain. As the pain persists other factors such as environmental, psychological and social stressors become relevant contributors to the overall presentation.

23.2 Principles of Treatment

Treatment of neuropathic pain is not straightforward. The pain is commonly refractory to conventional analgesic regimens such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Opioids have only limited usefulness in neuropathic pain as outlined later in this chapter; therefore so called co-analgesics, medications which are not typically used as analgesics, are often the first-line treatment of neuropathic pain.

Data from randomised controlled trials (RCTs) and meta-analyses are leading to improvements in management and a more evidence-based approach. A number of recent evidence-based guidelines have been published by various societies and organisations. Of particular value are the guidelines published by the European Federation of Neurological Societies (EFNS) [2] and by the Special Interest Group Neuropathic Pain of the IASP (NeuPSIG) [3].

It is important that patients have realistic expectations regarding treatment efficacy and potential side effects in order to improve compliance with medication. A balance between these should be achieved on an individual basis. A single drug therapy should be tried before combinations of drugs are started. Non-pharmacological treatments are available that may be appropriate for certain cases. For optimal results, a multidisciplinary approach to treatment should be adopted that addresses affective and behavioural changes and disability.

23.3 Pharmacological Treatment

23.3.1 Conventional Opioids

Multiple RCTs have been conducted in neuropathic pain using morphine, fentanyl, oxycodone and other conventional opioids. The general consensus is that pain intensity is relieved by titration of opioids in neuropathic pain. A Cochrane Review of these RCTs confirmed a significant effect of opioids on neuropathic pain, but with limited results on long-term management [4]. This is also confirmed by the meta-analysis underlying the NeuPSIG recommendations [3].

However, all current guidelines recommend conventional opioids only as third-line treatment options for neuropathic pain due to the potential risks of dependence, abuse and diversion, which have fuelled the current opioid epidemic in many countries [5]. In addition, opioids show no effect or negative effects on function and disability in patients with neuropathic pain [6]. Last, but not least, long-term use of opioids, in particular in high doses, cause opioid-induced hyperalgesia, i.e., CNS hyperexcitability, and may thereby worsen neuropathic pain states [7].

23.3.1.1 Recommendations for Clinical Use of Conventional Opioids in Neuropathic Pain

There is now a general consensus that a small subset of patients with neuropathic pain may benefit from treatment with opioids. However, current guidelines for neuropathic pain treatment do not recommend opioids as first- or second-line options due to the potential adverse effects and risks of these drugs [2, 3]. If a decision for use of opioids is made, then the approach to identify these patients and manage their treatment should follow guidelines for the use of opioids in chronic noncancer pain (CNCP) [8–10].

Summarising such guidelines is beyond the scope of this chapter. In brief, they usually include a past/present history of drug addiction as a relative contraindication, the need for regular follow-up visits, and opioids to be prescribed and supervised by the same doctor. Legal issues with opioid prescriptions are associated with their controlled status, the risk of addiction and abuse, and the potential for diversion into illegal channels by selling or passing on to others. Methadone, due to its additional monoaminergic and NMDA receptor effects, might be a particularly useful opioid in the setting of neuropathic pain, although there is only poor evidence in favour of this statement [11].

23.3.2 Atypical Opioids

23.3.2.1 Tramadol

Tramadol is a centrally-acting synthetic analogue of codeine; however it is not a conventional opioid as it has relatively low affinity for μ-opioid receptors and is correctly called an atypical opioid. Tramadol together with its primary active metabolite has three synergistic mechanisms of action to provide analgesia. It combines weak effects on opioid receptors with monoaminergic mechanisms. Reuptake inhibition of 5-HT and noradrenaline contribute to the anti-nociceptive action of tramadol. Tramadol is a racemate, and opioid receptor activity and 5-HT reuptake inhibition are mainly associated with the (+)-tramadol enantiomer, whereas (−)-tramadol is a

reuptake inhibitor of noradrenaline. The monoaminergic effects suggest a higher analgesic potency of tramadol in neuropathic than in nociceptive pain states; this has been confirmed by our group [12].

A meta-analysis of these and other RCTS identified tramadol as an effective treatment for neuropathic pain (number needed to treat (NNT) for 50% pain relief of 4.4) with therapeutic efficacy on paraesthesia and allodynia and a number needed to harm (NNH) of 8.2 [13].

Tramadol causes less respiratory depression and constipation than conventional opioids. Phase IV clinical trials have reported the overall incidence of side effects from tramadol to be 15.3%. The majority of side effects were found to be dose-dependent; specific issues were serotonergic effects including the risks of seizures and serotonin syndrome in combination with other serotonergic medications such as antidepressants [14]. A review showed a low potential for misuse, abuse and dependency of tramadol in Germany, where tramadol is widely used [15]. For these reasons, tramadol is not under special regulatory control in most countries.

Experimental evidence and a meta-analysis have shown tramadol to be a particularly useful analgesic in neuropathic pain with a low incidence of adverse effects, mainly of a benign nature. It is therefore (in contrast to conventional opioids) listed as a second-line medication for the treatment of neuropathic pain in current guidelines [2, 3].

23.3.2.2 Tapentadol

Tapentadol is a newer atypical opioid; its analgesic effect relies on μ-opioid receptor agonism and noradrenaline reuptake inhibition [16]. The affinity of tapentadol for the human μ-receptor is around 18 times lower than morphine (but it is only three times less potent than morphine). The high analgesic efficacy is explained by the extensive synergy between the two mechanisms of action as shown in site-specific administration studies. Tapentadol differs from tramadol with regard to the nearly complete lack of a serotonergic effect and the fact that metabolites do not contribute to its analgesic effect. There is no causal relationship between tapentadol and serotonin syndrome and therefore no drug interactions between tapentadol and antidepressants. Tapentadol has shown efficacy in neuropathic pain due to diabetic polyneuropathy, as well as in back pain with a neuropathic component [17]. It is currently not yet listed in most guidelines for neuropathic pain due to its novelty and limited clinical data.

Tapentadol has a better adverse effect profile than conventional opioids and tramadol, and has therefore been rated top-ranking analgesic in a network meta-analysis of the tolerability of opioid analgesics for chronic pain [18]. It has also a very low risk of abuse and diversion.

23.3.3 Antidepressants

23.3.3.1 Tricyclic Antidepressants (TCAs)

In 1960, Paoli et al. made the incidental discovery that the tricyclic antidepressant (TCA) imipramine had an analgesic effect. Since then, other TCAs and other antidepressants have been evaluated and used for the treatment of neuropathic pain. TCAs were the first class of medication proven to be effective for neuropathic pain in a double blind placebo controlled trial. The role of TCAs in the treatment of neuropathic pain is now well established and has the best documented evidence [19]. The overall NNT for neuropathic pain is 3.6 with better efficacy in diabetic neuropathy than in postherpetic neuralgia (PHN). TCAs are listed as first-line treatment for neuropathic pain in all current guidelines [2, 3].

Amitriptyline is established as the "gold standard" as it has the most evidence available, especially for the treatment of painful diabetic neuropathy and PHN. However, amitriptyline and other TCAs have also been evaluated for the relief of pain in peripheral neuropathies and central post-stroke pain. In comparative trials, no single TCA has been found to be superior for neuropathic pain except in PHN, where amitriptyline was found to be superior to maprotiline.

Initially it was thought that the analgesic action of TCAs was related to their antidepressant activity. However, it is now clear that there is an independent specific analgesic effect, as the doses used to relieve neuropathic pain are smaller and the onset of analgesic efficacy is faster than an antidepressant effect and analgesia does not appear to depend upon mood improvement in depressed patients. In addition, pain relief was found to be independent of any sedative effect.

TCAs are inhibitors of the reuptake of monoaminergic transmitters and this mechanism mediates their analgesic effect by the following presumed mechanisms:

1. Central blockade of monoamine re-uptake, particularly serotonin and noradrenaline, leads to enhancement of the descending inhibitory monoaminergic pathways in the dorsal horn of the spinal cord.
2. Anticholinergic activity reduces firing of central neurones involved in pain, especially after deafferentation.

Additionally, there may be a number of other contributing mechanisms; moderation of NMDA receptor activity, opioid receptor activity, increase in dopamine or endorphin levels, blockade of central or peripheral histamine receptors, sodium channel blockade and blockade of adrenergic receptors on regenerating sprouts.

The optimum analgesic dose of TCAs can often not be reached due to unpleasant side effects. A systematic review of randomised controlled trials of TCAs used to treat neuropathic pain found that out of 100 patients, 30 had significant pain relief, 30 had minor side effects and 4 had to discontinue their therapy due to side effects. These include:

1. Anticholinergic: dry mouth, constipation, urinary retention and blurred vision
2. Antihistaminergic: confusion and sedation (the latter may be of benefit)
3. Anti α-adrenergic: postural hypotension and sexual dysfunction

Cardiac conduction abnormalities may also arise due to muscarinic anticholinergic actions. Patients at risk should have a pre-treatment ECG and cardiac conduction defects are a contraindication to treatment with TCAs.

Another potential problem is overdose in suicidal ideation, where TCAs are more dangerous than other groups of antidepressants and maybe fatal due to severe cardiac arrhythmias and convulsions. The use of TCAs in elderly patients should be avoided due to increased risk of cognitive impairment and mortality.

Desipramine, imipramine and nortriptyline are more specific to noradrenergic blockade and are associated with less anticholinergic and antihistamine side effects. They may be useful in patients who are not able to tolerate amitriptyline, before progressing to another class of drug. In PHN and painful diabetic neuropathy, they were both found to be as effective as amitriptyline [20–22], but associated with less side effects. Physical withdrawal reactions have been described for most antidepressants, but psychological addiction is not an issue.

23.3.3.2 Selective Serotonin Re-uptake Inhibitors (SSRIs)

These drugs such as fluoxetine and paroxetine have only limited efficacy in neuropathic pain [19]. They alter serotonergic far more than noradrenergic (NE) neurotransmission. However, due to their selectivity they do not interfere as much with adrenergic, histaminergic or muscarinic receptors and therefore have fewer side effects. There is currently insufficient evidence to make recommendations regarding their use for this indication [3, 23].

23.3.3.3 Serotonin/Noradrenaline Reuptake Inhibitors (SNRIs)

Venlafaxine and duloxetine are novel antidepressants, which belong to the class of SNRIs. They have similar mechanisms of action as TCAs, but no anticholinergic effects. Guidelines recommend them as a first line-treatment for neuropathic pain with an NNT of 6.4 [3]. Data on duloxetine are stronger and it is indicated for painful diabetic polyneuropathy in Australia. There are no direct comparisons to other antidepressants published, but SNRIs are thought to be better tolerated than TCAs.

23.3.3.4 Recommendations for Clinical Use of Antidepressants as Analgesics

It is difficult to generalise a dosage regimen for antidepressants in neuropathic pain, due to significant inter-individual variability. McQuay et al. have demonstrated a dose response relation for amitriptyline with a greater analgesic response at 75 mg/day than either 25 or 50 mg/day [24].

Current recommendations for prescribing TCAs are [25]:

1. Start with a low dose (5–10 mg/day given at night) especially in the elderly and increase this weekly to analgesic efficacy or unacceptable side effects.
2. Once the optimal dose is achieved, analgesic efficacy usually takes up to a week to achieve.
3. There have not been any trials conducted for longer than 6 weeks, so there is no evidence base for optimum duration of treatment. The current practice is to continue the same effective dose for several months and then try to reduce it.
4. If a therapeutic dose of a TCA fails to provide pain relief, other antidepressants are also likely to fail.
5. If a TCA provides pain relief at the expense of unacceptable side effects then other antidepressants (in particular SNRIs) with a lower side effect profile should be tried.
6. If due to contraindications or unacceptable side effects a patient is unable to be treated with TCAs, other antidepressants (in particular SNRIs) should be tried before excluding this drug category.

23.3.4 Anticonvulsants

In the 1960s phenytoin was found to have an analgesic effect in the treatment of painful diabetic neuropathy. Since then anticonvulsants have been evaluated and used in neuropathic pain states including old agents: cabamazepine, sodium valproate, phenytoin; and newer agents: gabapentin, lamotrigene, felbamate and pregabalin. Anticonvulsants have a specific indication in the treatment of trigeminal neuralgia with carbamazepine the first line therapy. They may prove effective in conditions that have proved intractable to other treatments.

23.3.4.1 Mechanism of Action

The neuronal hyperexcitability and corresponding molecular changes in neuropathic pain have many features in common with the cellular changes in certain forms of epilepsy [26]. The pain-relieving effect of anticonvulsants is thought to be due to dampening of abnormal central nervous activity that follows nerve damage. This may occur by;

1. Sodium channel blockade resulting in a reduction of ectopic firing in both peripheral nerves and the dorsal root ganglion
2. Indirect or direct enhancement of inhibitory GABAergic neurotransmission
3. Inhibition of excitatory glutaminergic neurotransmission

Overall effects may be due to a combination of these mechanisms and longer term neuroplastic effects. The process of ectopic impulse generation is so sensitive

to sodium channel blockade that these agents have an action at much lower concentrations than that required to block normal neuronal transmission.

23.3.4.2 Individual Medications

Clonazepam is a benzodiazepine anticonvulsant, acting as a GABA agonist. Lorazepam, nitrazepam and diazepam have also been used in chronic pain. They have anxiolytic and anticonvulsant properties. However, with the exception of clonazepam, benzodiazepines are not generally felt to have specific analgesic activity and their use is not encouraged for this purpose due to their addictive nature, tolerance and cognitive impairment.

However, for clonazepam several studies suggest a role in lancinating neuropathic pain. An old cross-over trial shows clonazepam to be superior to carbamazepine, phenytoin and sodium valproate with regard to efficacy in neuropathic pain and adverse effects.

This reflects our past clinical experience, where clonazepam was an easy to use agent with excellent efficacy and minimal side effects, in particular sedation. However, clonazepam is a benzodiazepine and thereby closely linked to risks of tolerance, dependence and addiction/abuse and should no longer be used as a first-line agent in neuropathic pain.

Gabapentin has been available in the USA since 1995, initially only in an anticonvulsant indication. It is a lipophilic gamma-amino butyric acid (GABA) analogue but does not interact with $GABA_A$ or $GABA_B$ receptors or directly affect GABA uptake. It is now clear that this drug has a modulating effect on the α2-δ subunit of voltage-gated calcium channels, an unexpected pharmacological target. By modulating the calcium influx into hyperexcitable primary afferent neurons, gabapentin reduces the release of excitatory amino acids, in particular glutamate, and thereby reduces the excitation of secondary neurons. This explains its effects in neuropathic pain, but also in other conditions presenting with hyperalgesia and allodynia including fibromyalgia, even postoperative and burns pain and its anxiolytic effect, with efficacy in generalised anxiety disorder. The efficacy in neuropathic pain has been well-demonstrated and an NNT of 7.2 was found in a meta-analysis [3]. Efficacy has also been shown in central neuropathic pain syndromes such as thalamic post-stroke pain. Gabapentin is also a first-line treatment for pain after spinal cord injury [27].

The most commonly reported side effects are somnolence, fatigue, ataxia and dizziness. A dose adjustment is required in renal failure, but not in hepatic disorders as gabapentin is excreted unchanged by the kidneys. The effective analgesic dose of gabapentin is variable, with some patients responding at low doses and others requiring high doses (more than 3600 mg/day) for the same benefit. This is partially due to uptake by an active carrier process, showing saturation kinetics. It has been suggested that treatment failure may be due to inadequate dosage, although rapid dose escalation can be responsible for the high incidence of CNS side effects [28].

The development of pregabalin with better kinetics and higher efficacy has reduced the usage of gabapentin.

Pregabalin, an analogue of gabapentin, has been developed with the indication for neuropathic pain. It has a similar pharmacodynamic effect to gabapentin, i.e. modulates the α2-δ subunit of voltage-gated calcium channels and thereby reduces excitatory amino acid release. It differs from gabapentin insofar as it has a higher potency, a better bioavailability, linear absorption kinetics and a longer half-life, permitting twice instead of three times daily dosing.

It is used successfully in a number of neuropathic pain states of peripheral and central origin including PHN, diabetic neuropathy and spinal cord injury pain. It has also been used successfully in fibromyalgia and generalised anxiety disorder and has these three conditions as an indication in many countries. It is a first-line medication for neuropathic pain with an overall NNT of 7.7 [3]. Pregabalin is not only superior to gabapentin from a pharmacokinetic point of view, but also in clinical practice, achieving better pain relief and quality of life.

Adverse effects include sedation, drowsiness, disturbance of balance and unexplained peripheral edema, as well as weight gain in some patients. However, these adverse effects are often mild and can be partially avoided by slow and careful titration of dose. Starting doses of 75 mg in ambulatory patients (with 25 mg in the frail), starting with an evening dose and higher evening than morning doses are useful recommendations for the titration process.

The efficacy of pregabalin and its mild adverse effects have made it a viable first-line alternative to antidepressants in the setting of neuropathic pain. An interesting aspect from a surgical perspective is its perioperative use, which leads to improved postoperative pain, reduced opioid consumption and opioid side effects [29]. While RCTs suggest further benefit from its perioperative use by improving recovery after laminectomy and reducing chronic neuropathic pain after knee joint replacement, it has not been shown to have a protective effect on chronic post-surgical pain in general.

Carbamazepine inhibits spontaneous and evoked responses of spinal neurones and increases brain serotonin. It has been the first-line treatment for trigeminal neuralgia for many years; it is still the best supported treatment for this condition in some guidelines [2]. However, a Cochrane Review found only poor evidence for a short-term benefit in neuropathic pain of various origins [30].

Side effects are the main limitation to its use and include sedation, ataxia, drug interactions and liver dysfunction. Serious but rare side effects are irreversible aplastic anaemia and Stevens-Johnson-Syndrome. With carbamazepine therapy, regular haematological, liver function and plasma concentration monitoring is recommended. Occasional monitoring of serum sodium is also recommended because hyponatraemia can occur. The sustained release preparations of carbamazepine may limit the side effects and oxcarbazepine may be better tolerated.

Sodium valproate is structurally unrelated to other anticonvulsants and does not block sodium channels. The exact mechanism of action is unknown but may be related to increased GABA synthesis and release, hence potentiating GABAergic inhibition. In addition, valproate attenuates the neuronal excitation caused by

glutamate activation of NMDA receptors. It is useful and well-tolerated for migraine prophylaxis. However, there is insufficient evidence to support the use of sodium valproate as a treatment for neuropathic pain; guidelines state a weak recommendation against its use in this indication [3].

Again side effects and the risk of serious toxicity limit its use. These include sedation, gastrointestinal disturbance, altered liver function with potentially fatal hepatotoxicity, decreased platelet aggregation and other haematological effects and drug interactions. Close follow-up is mandatory.

The use of phenytoin for the treatment of neuropathic pain is not supported by the evidence [31]. It has fallen from favour mainly due to its extensive side effect profile, and a lack of supportive studies. Side effects include sedation, gingival hypertrophy, hirsutism and coarsening of facial features. At high blood levels, neurotoxicity occurs and cardiac conduction is affected, therefore plasma level monitoring is required.

This anticonvulsant appears to act on voltage-gated cation channels (calcium and potassium) as well as inhibiting glutamate release. It has been extensively studied in high quality studies and no evidence for an effect in neuropathic pain was found [32].

23.3.4.3 Recommendations for Clinical Use of Anticonvulsants as Analgesics

Anticonvulsants are typically used for neuropathic pain that has a shooting, burning or lancinating character. Empirically they are often used in combination with a TCA or SNRI, although the evidence for using both classes of drug in combination is limited [3]. Good evidence supports only the use of pregabalin and gabapentin and their derivatives for the treatment of neuropathic pain, which are regarded as first-line options by all guidelines [2, 3].

For trigeminal neuralgia only, carabamazepine is suggested as the first-line treatment by some, but not all guidelines [2]. Although few trials exist for the treatment of central post-stroke pain, current guidance here proposes TCAs as first-line treatment, followed by gabapentin/pregabalin and then possibly lamotrigine [33]. There is a strong recommendation against the use of levetiracetam [3]. As with antidepressants, titration should start with low doses, gradually increasing to a dose that either produces analgesic efficacy or unacceptable side effects.

23.3.5 Local Anaesthetics and Antiarrhythmics

In 1948 systemic procaine was identified as beneficial in the treatment of neuropathic pain. This led to the evaluation of other local anaesthetics for the treatment of neuropathic pain.

The mechanism of analgesic action is thought to be due to membrane stabilising effects by blockade of voltage-dependent sodium channels and hence reduced

ectopic activity in damaged afferent nerves [34]. In addition, there may be a central action on sodium channels and at the spinal level blocking the actions of glutamate.

Over the last 35 years there have been reports of analgesic efficacy of intravenous lignocaine in a wide range of neuropathic pain states, including diabetic neuropathy, peripheral nerve lesions, PHN and central pain. The overall evidence for its usefulness in neuropathic pain is weak, however, and limited by the need for parenteral administration and for cardiac monitoring with higher dose use. In direct comparative trials, ketamine was significantly more effective than lignocaine. However, similar to ketamine, it may have a protective effect from the development of chronic postsurgical pain [35]. The main use is by topical administration as outlined below.

The antiarrhythmic Mexiletine is an oral analogue of lignocaine that has been used in neuropathic pain with mixed results. Current guidelines advise strongly against its use in neuropathic pain due to negative trials and safety concerns [3].

23.3.5.1 Recommendations for Clinical Use of Lignocaine in Neuropathic Pain

Side effects of lignocaine/lidocaine are CNS (dizziness, nausea, perioral numbness, convulsions & coma) and CVS effects (arrhythmias). Contraindications therefore include cardiac conduction abnormalities, left ventricular failure and ischaemic heart disease. An ECG should be obtained before and during treatment to monitor any cardiac effects. If there is a question regarding safety in a patient, a cardiology opinion should be sought, prior to starting treatment. For lignocaine the recommended starting dose is 1–1.5 mg/kg as a slow IV bolus; this is an ideal agent for a neuropathic pain emergency. Maintenance is by IV infusion of 1–3 mg/min with measurement of blood concentrations.

23.3.6 N-methyl-D-Aspartate-Receptor (NMDA) Antagonists

NMDA receptors are activated by the excitatory amino acid glutamate. They are thought to play an important role in the development of central sensitisation following a peripheral nerve lesion. NMDA antagonists may block this hyperactivity responsible for the maintenance of the pain. Drugs with NMDA receptor antagonist activity include ketamine, dextromethorphan, memantine and amantadine.

Ketamine is the most commonly used NMDA antagonist. Its original use was as an anaesthetic agent, particularly “in the field” and other difficult locations and situations. It has also been used for the treatment of severe asthma and for sedation. Besides its main action as an NMDA antagonist, it also interacts with nicotinic and muscarinic acetylcholine receptors, opioid receptors, monoaminergic receptors, and voltage-sensitive sodium channels. It also strengthens the descending inhibitory system and reduces glial activation [36].

Low-dose ketamine is widely used to treat acute postoperative and post-traumatic pain and, in this situation, improves pain control, reduces opioid requirements and thus reduces adverse effects of opioids [36]. This effect is the more obvious the more severe the pain is [37]. Ketamine in this setting is in particular helpful in opioid-tolerant patients, as it partially reverses opioid tolerance. There is also some evidence for a protective effect from chronic postsurgical pain, but this requires further study.

In chronic pain states, there is limited evidence for its usefulness as a third-line agent in refractory cancer pain [36]. In other chronic pain states, evidence is inconsistent and advice on its use contradictory. However, it might have a limited role in the treatment of refractory neuropathic pain and complex regional pain syndrome (CRPS) with severe allodynia. This might in particular be the case in acute neuropathic pain states after trauma and surgery; in peripheral and central neuropathic pain states low-dose IV ketamine was superior to IV lignocaine.

Ketamine is also useful for the treatment of procedural pain, eg for dressing changes in burns and surgical patients. There is increasing interest in oral and, in particular, sublingual preparations, which are currently undergoing clinical trials. They might play a role as add-on agents in patients who failed other approaches (fourth-line treatment) [38].

Unpleasant side effects limit its use, although they occur rarely with the low doses commonly used to treat postoperative and acute neuropathic pain. These are mostly psychomimetic: sedation, hallucinations, dysphoria, unpleasant sensations (dissociation) and paranoid feelings. It is important to warn patients in advance of these potential effects; they can be reduced by co-prescribing benzodiazepines such as midazolam if needed. With more extended use, there are concerns coming primarily from the addiction literature on neurotoxicity, hepatotoxicity, and urotoxicity. In this context, it is also important to consider that ketamine has an abuse potential and is a controlled medication in most countries.

Dextromethorphan, amantidine and memantine have been shown to have weaker actions than ketamine. Dextromethorphan had some benefits with regard to pain control and opioid-sparing in postoperative pain. In neuropathic pain, there are no convincing data to support its use. Memantine was also shown to be ineffective in phantom limb pain treatment; its routine use in neuropathic pain can currently not be recommended.

23.3.7 Miscellaneous Agents for Systemic Use

Clonidine is an α_2-agonist with analgesic activity. Its analgesic action is thought to occur centrally and at a spinal level, mediated by activation of α_2-adrenoceptors in the dorsal horn of the spinal cord. This results in direct inhibition of postsynaptic spinal dorsal horn neurones or by decreasing the release of noradrenaline from sympathetic nerve terminals.

Only a small number of studies have been conducted to look at a potential role of clonidine in the treatment of neuropathic pain. Significant improvement was reported in patients with PHN treated with clonidine. Transdermal clonidine (0.1–0.3 mg per day) may have some analgesic effect in neuropathic pain from diabetic polyneuropathy. It is registered in the USA as an adjuvant in combination with epidural local anaesthetics and opioids for resistant neuropathic pain and has supportive data for intrathecal and epidural administration. Side effects include drowsiness, dizziness and dry mouth.

Baclofen is a gamma-aminobutyric acid (GABA) receptor agonist, capable of crossing the blood-brain barrier. It is an agonist for GABAB receptors and has presynaptic action in the spinal cord preventing the release of excitatory neurotransmitters [39]. Baclofen causes muscle relaxation and is used to treat muscle spasticity. It has been shown to have an antinociceptive action and has been used to treat neuropathic pain. It was first used for this purpose to treat trigeminal neuralgia. Its efficacy has not however been confirmed in other neuropathic pain conditions. Baclofen has been administered intrathecally and may be useful for pain related to spinal cord injuries. Side effects include sedation, nausea, confusion, convulsions, hypotension, GI upset, visual disturbances and occasionally hepatic impairment. After prolonged use, baclofen requires a gradual dose reduction in order to minimise the risk of a withdrawal syndrome [39].

There has been increasing interest in the use of cannabis and cannabinoids as analgesics in chronic pain. Cannabinoid receptors are located in the central and peripheral nervous system. Animal models have shown that cannabinoid receptors do not undergo down-regulation after nerve lesions (unlike opioid receptors) and that cannabinoids may attenuate the associated sensory changes [40].

Cannabis has been used for thousands of years for medicinal and recreational purposes. There is much interest surrounding its legalisation and its potential role as an analgesic. Problems are that most currently used preparations are poorly pharmacologically defined with the exception of nabiximols, a 50:50 combination of THC and CBD as an oromucosal spray. It is registered in a number of countries for treatment of MS-related spasticity, but has shown no effect in neuropathic pain [41].

Similarly, a meta-analysis could identify only minimal efficacy of cannabinoids in chronic pain with an extremely high NNT of 24 for 30% pain reduction and no effect for 50% pain reduction [42]. However, there was a low NNH of 6 and no effect on physical or emotional functioning. Overall, use of cannabinoids in pain management has currently no scientific basis and guidelines recommend against their use [3].

23.3.8 Topical Treatments

Allodynia is frequently a feature of neuropathic pain especially in PHN, traumatic neuropathies and causalgia. It may therefore be helpful to consider the use of topical medications for the treatment of cutaneous hyperalgesia in these cases. There are a

few options in form of capsaicin, local anaesthetics, clonidine (discussed above) and NSAIDs, with some reports of good pain relief from PHN with topical aspirin preparations [43].

The mechanism of action of Lignocaine/Lidocaine 5% patches is by suppression of ectopic discharges from sensory afferents as well as providing mechanical protection to underlying allodynic skin. In 1999 the FDA approved the use of 5% topical lidocaine patches for treatment of PHN. The patches are indicated for localised neuropathic pain (LNP) such as PHN, diabetic polyneuropathy and nerve entrapment in scars. A meta-analysis showed superiority of the patch over capsaicin and pregabalin and similar efficacy to gabapentin, however with significantly fewer systemic side effects.

The advantages of this route of administration are its effectiveness, duration of analgesia, ease of application without dose titration and lack of systemic side effects. The safety profile is particularly advantageous in the elderly population whom are most affected by PHN; while guidelines are suggesting lidocaine patches in principle as second-line treatment, they become first-line treatments in frail and elderly patients [3]. The area of pain has, however, to be of limited size for practical reasons, therefore the specific indication in localised neuropathic pain (LNP) [44].

Capsaicin is the pungent component to chilli peppers. The chilli pepper has been recognised by various cultures for many years for its medicinal qualities. It is neurotoxic and has analgesic properties. When capsaicin is applied topically it initially causes a burning sensation and heat hyperalgesia that decreases with subsequent applications.

Capsaicin acts on transient receptor potential vanilloid type 1 ion channel (TRPV1) on the terminals of primary nociceptive afferents. When capsaicin binds to this receptor, it induces initial activation of the nociceptors, hence the burning sensation. However, over time it depletes substance P from the sensory nerve terminals of peripheral nociceptors. With repeat or prolonged application, this is followed by desensitisation and inactivation of the receptive terminals of the nociceptors. There is also evidence that it causes depletion of substance P in epidermal nerve fibres.

In a meta-analysis of RCTs, low-dose capsaicin cream (0.075%) repeatedly administered had an NNT of 6.6 for any pain relief [45]. A commercially available patch with high-dose capsaicin (8%) had an NNT of 9 to 11 for 30% pain relief, when used in PHN, HIV-neuropathy and painful diabetic neuropathy [46].

The use of capsaicin has been limited by this unpleasant burning sensation occurring in 60–70% patients, need for frequent applications and uncertain efficacy [25]. Co-administration of lignocaine gel has been used in order to improve compliance [25]. Capsaicin is regarded as a second-line therapy in localised neuropathic pain in the NeuPSIG guidelines [3].

23.3.9 Non Pharmacological Therapy

23.3.9.1 Transcutaneous Electrical Nerve Stimulation [47]

This technique applies cutaneous electrodes to stimulate peripheral nerves to relieve pain [48]. This is based on the gate control theory of pain transmission, so that by stimulating Aβ and Aδ fibres, pain transmission by C-fibres is inhibited. It utilises a

pulse generator that provides a range of currents, frequencies and pulse widths. The surface electrodes are placed either side of the painful area or alternatively the nerves supplying that area. The current is then increased until a tingling sensation is felt in the painful area. The timing and duration of pulses is a matter of titration to maximal response. In diabetic polyneuropathy, TENS may reduce analgesic requirements. A meta-analysis shows improvement in neuropathic symptoms in patients with diabetic polyneuropathy, but overall the evidence for efficacy of TENS in neuropathic pain is poor [49].

It has few side effects and complications; allergic dermatitis may occur at the contact sites and its use is contraindicated in patients with pacemakers. Its efficacy can be assessed quickly (there is a significant placebo effect) and can be therefore easily trialled for any potential benefit to an individual. Unfortunately tolerance may develop, resulting in loss of previously effective analgesia. Changing the stimulation variables can sometimes attenuate this.

23.3.9.2 Spinal Cord Stimulation (SCS)

This techniques requires an implantable device with electrodes positioned under direct vision at open laminectomy or via a needle in the epidural space percutaneously. The electrodes are placed above the level of the pain and connected to an inductance coil placed on the abdominal wall; an implantable power source can also be used. The mechanism of action is not yet clear, but it seems to be ineffective in nociceptive pain, but can be useful for a variety of neuropathic pain states. A systematic review describes efficacy in refractory neuropathic back and leg pain, failed back surgery syndrome and CRPS Type 1 [50]. According to guidelines, there is weak evidence for its use in CRPS Type 1 refractory to other treatments and for neuropathic pain in failed back surgery syndrome [51].

Complications include infection, bleeding, dural puncture and hardware failure; decisions on use of this invasive and expensive approach should be made ideally by a multidisciplinary team experienced in the use of such techniques. Deep Brain Stimulation and Motor Cortex Stimulation have also been used to treat neuropathic pain, but are currently more experimental.

23.3.9.3 Sympathetic Nerve Blocks

The diagnosis of sympathetically maintained pain can be confirmed by the response to a sympatholytic procedure. This may be helpful, if there is a significant sympathetic component to the patient's pain. A patient should receive sustained pain relief after administration of a sympathetic chain or sympathetic plexus local anaesthetic block or accumulative relief from a number of procedures. If the patient fails to respond, a systematic pharmacological approach is tried. However, a block may be incorrectly thought to be successful. This can happen for one of two reasons: either, the local anaesthetic is absorbed and provides a systemic analgesic effect or it diffuses locally and acts on nearby somatic nerves. In the case of effectiveness, progression to a sympatholytic procedure can be chosen. Techniques then involve the

use of neurolytic substances or radiofrequency ablation; regrettably the current scientific basis for this approach is poor.

23.3.9.4 Neurosurgical Destructive Techniques

There has been increasing awareness that destructive techniques may in fact increase pain in the long term due to the plasticity of the nervous system, sometimes resulting in incapacitating side effects. Therefore these techniques, which include neurectomy and dorsal root entry zone lesions, are now rarely used. The exception being in the treatment of trigeminal neuralgia, which has proved refractory to pharmacological treatments. In this situation a variety of surgical procedures can provide rapid pain relief, the most effective option being microvascular decompression. Recurrence is still a risk but appears to be more frequent after percutaneous radiofrequency rhizotomy or compression with a percutaneously positioned balloon than the more invasive microvascular decompression technique.

23.3.9.5 Cognitive-Behavioural Therapy

Chronic neuropathic pain is best managed in a multidisciplinary pain clinic [52]. This is because the patient often has cognitive, affective and behavioural factors influencing their pain; however new understandings of the physiology of cortical reorganisation in chronic pain might also lead to new psychological approaches. A multidisciplinary approach will address both the somatic and psychological aspects of the patient's condition. The main methods are cognitive-behavioural therapy, operant conditioning and mindfulness.

The cognitive-behavioural approach aims to identify and modify the patient's thoughts, feelings, beliefs and behaviour. Common problems are anxiety, depression and the development of fear-avoidance. Behavioural therapy procedures are utilised to bring about change. The aims are to enable patients to take a positive and active role in coping with their pain and to change maladaptive behaviour that may be aggravating the problem.

Operant conditioning uses firstly continuous re-enforcement to encourage positive behaviour from the patient that is then stepped down later on. This is based on the belief that the consequence of certain behaviour determines whether it is likely to recur. Mindfulness, often delivered in an Acceptance and Commitment (ACT) approach to chronic pain, improves quality of life and associated depression in chronic pain patients [53].

Psychotherapy may increase levels of activity and decrease medication requirements but an actual reporting in pain reduction may be more modest. In addition, the therapy may need to be extended to the carers of the patient in order to change their response to the patient's beliefs and behaviour. The current data situation on this approach to neuropathic pain is insufficient to draw any conclusions on efficacy [54].

23.4 Conclusion

The assessment and management of neuropathic pain is challenging for the vascular specialist. As it is not caused by nociceptive input, classical analgesic medications have limited efficacy in this condition. Management should rely on a combination of non-pharmacological and pharmacological approaches, ideally in a multidisciplinary treatment setting.

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Chapter 24
Pathophysiology of Varicose Veins, Chronic Venous Insufficiency and Venous Ulceration

Manj S. Gohel

Key Learning Points

- Chronic venous disease is common, increasing in prevalence and a major cause of both patient distress and healthcare expense.
- Venous disease is a wide spectrum of disorders, for which the Clinical, Etiological, Anatomical, Pathophysiological (CEAP) classification is a useful descriptive tool.
- Although the precise mechanisms are poorly understood, chronic venous hypertension is accepted to be the main driver of chronic inflammation, skin changes and ulceration in patients with advanced venous disease.
- Common causes of chronic venous hypertension include venous reflux (due to valve failure in superficial or deep veins) and venous outflow obstruction (usually post-thrombotic). However, other factors such as immobility, obesity and calf muscle pump failure are commonly present.
- Assessment of patients with venous disease should focus on the identification of treatable underlying causes of chronic venous hypertension.
- Intervention to address causes of venous hypertension (particularly superficial venous reflux and venous outflow obstruction) can result in significant improvements in patient quality of life.

24.1 Introduction

Venous disease represents a wide clinical spectrum of disorders, often with poor correlation between anatomical patterns of venous reflux or occlusion and clinical presentation. Recognised for thousands of years, venous disorders are common, disabling and an enormous cause of patient distress and health care expense [1–3].

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Moreover, with advanced age and obesity recognised as independent risk factors for advanced venous disease and ulceration, the prevalence of these conditions is highly likely to increase significantly in most western countries [4].

There have been significant advances in our understanding of the pathophysiological basis of venous disorders. However, many aspects of venous pathophysiology remain poorly understood, with different aetiological factors likely to be dominant in different patients. Patients with chronic venous insufficiency frequently experience poor pathways of care, with considerable inconsistency resulting in poor outcomes [5]. In this chapter, a summary of the potential mechanisms underlying chronic venous disease is presented.

24.2 Clinical Spectrum of Venous Disease

Patients with venous disease of the lower extremity present with a wide range of symptoms and clinical signs. A useful descriptive classification is provided by the Clinical, Etiologic, Anatomic and Pathophysiologic (CEAP) classification in which patients can be classified into 7 clinical grades of venous disease ranging from C0 (no venous disease) to C6 (active venous ulceration) (Table 24.1) [6].

Table 24.1 CEAP classification [6]

C: Clinical classification
C0: No visible or palpable signs of venous disease
C1: Telangiectasia or reticular veins
C2: Varicose veins
C3: Oedema
C4a: Skin pigmentation or eczema
C4b: Lipodermatosclerosis (or atrophie blanche)
C5: Healed venous ulcer
C6: Active venous ulcer
S: Symptomatic, including ache, pain, tightness, skin irritation, heaviness, muscle cramps
A: Asymptomatic
E: Aetiological classification
Ec: Congenital
Ep: Primary
Es: Secondary (e.g. post thrombotic)
En: No venous cause identified
A: Anatomical classification
As: Superficial veins
Ap: Perforator veins
Ad: Deep veins
An: No venous location identified
P: Pathophysiological classification
Pr: Reflux
Po: Obstruction
Pr,o: Reflux and obstruction
Pn: No venous pathophysiology identifiable

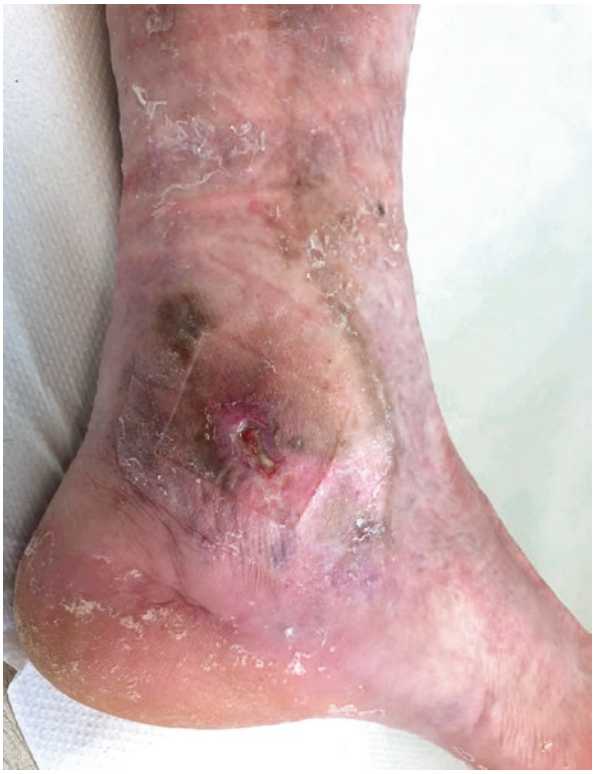


Fig. 24.1 Venous ulcer at ankle (CEAP classification C6)

The nomenclature can be confusing, but chronic venous insufficiency is a term to describe patients with disease ranging from C3 (oedema) to C6 (active ulceration) on the CEAP clinical grade [7] (Fig. 24.1). This represents the more severe end of the venous disease spectrum. Traditionally, superficial venous reflux was thought to cause varicose veins, whereas more advanced venous skin changes and ulcers were believed to be as a result of post-thrombotic deep venous disease. With the widespread availability and use of duplex ultrasound scanning (DUS) since the 1990s, it has become very clear that the association between anatomy of venous disease and clinical status is far more complex than previously appreciated. Most patients with chronic venous ulceration, for example have evidence of superficial reflux [8], not always with visible varicose veins.

24.3 Venous Anatomy

The lower extremity venous system consists of superficial, deep and perforating veins. In general, deep veins accompany the associated arteries, whereas superficial veins do not usually have named arteries in the same distribution. Frequent and variable connections exist between the deep and superficial venous systems of the leg through a complex network of tributaries.

24.3.1 Superficial Veins

The main superficial veins of clinical relevance are the great saphenous vein (GSV), small saphenous vein (SSV), anterior and posterior accessory saphenous veins (AASV, PASV) and associated tributaries. Saphenous veins are usually located within a fascial envelope outside the muscle compartments. Anatomy can vary between individuals, but the GSV drains into the CFV in the groin and the SSV usually enters the popliteal fossa between the heads of gastrocnemius and drains into the popliteal vein. The saphenopopliteal junction may be absent, in which case the SSV continues on the posterior aspect of the thigh (Giacomini vein) and may drain into the GSV.

24.3.2 Deep Veins

Located deep to the muscle fascia in the leg, deep veins usually accompany the associated artery and may exist in pairs, particularly below the knee. Below the knee, anterior and posterior tibial veins and the peroneal vein form the popliteal vein, which runs alongside the popliteal artery. Additionally, gastrocnemius and soleus veins are also considered below-knee deep veins. Frequently duplicated, the popliteal vein passes through the adductor hiatus to become the femoral vein (previously known as the superficial femoral vein). The common femoral vein is formed by the confluence of the femoral vein and the profunda vein (or deep femoral vein) in the groin. The common femoral vein terminates at the inferior margin of the inguinal ligament, where it becomes the external iliac vein, which in turn becomes the common iliac vein after the internal iliac vein confluence. Formed by the confluence of the right and left common iliac veins, the inferior vena cava (IVC) runs on the right of the aorta and drains into the right atrium. The IVC may be divided into infrarenal, suprarenal and suprahepatic segments.

24.3.3 Perforating Veins

A perforating vein (or perforator) in the lower extremity may be defined as a vein that pierces the muscle fascia to connect deep and superficial veins [9]. Although enormous variation is present, perforators commonly located along the medial lower leg (Boyd's & Cockett's perforators) or medial aspect of the thigh (Hunter's or Dodd's perforators). Incompetent perforator veins have been associated with chronic venous insufficiency [10, 11].

24.3.4 Valvular Anatomy

Infrainguinal superficial and deep veins have bicuspid, unidirectional valves directing flow towards the right heart. The valve leaflets open toward the vein wall to permit flow and shut towards the centre of the vein to prevent retrograde flow. Retrograde flow is known as reflux or incompetence, and can be evaluated using DUS. Iliac veins and the IVC are not thought to contain valves, whereas valves are present throughout deep and superficial veins below the inguinal ligament. In general, the frequency of vein valves increases down the leg. Cadaveric studies have described vein valves consistently located in the CFV and proximal femoral vein near the profound femoral vein confluence [12]. Perforating veins may or may not contain valves directing flow toward the deep system.

24.4 Normal Venous Function

The primary function of the lower extremity venous system is to return blood from the capillary bed to the right atrium. In comparison to arteries, veins are high capacitance, high volume, low resistance vessels. At any time, around 70% of the circulating blood is contained within veins. In healthy veins, the vast majority of the venous return is carried in the deep veins (>80%), with superficial veins draining only the skin and subcutaneous fat and tissues. However, the superficial veins also have an important role in thermoregulation and may play a much more dominant role in venous return in the presence of deep venous occlusive disease.

The primary mechanism for propelling venous blood toward the right heart is the calf muscle pump. This refers to the deep compartment of the posterior lower leg, where the deep veins are contained. In addition to the calf muscle pump, a smaller, but still significant foot muscle pump also exists. Calf and foot muscle contraction (during ambulation or exertion) results in extrinsic compression of the deep veins, thus emptying the venous blood in a cephalad direction [13]. At rest, the venous sinusoids within calf and foot muscles fill with blood so that muscle contraction during normal ambulation extrinsically compresses the veins and forces blood up the leg. Calf muscle contraction also acts to close perforating veins, therefore avoiding venous reflux through perforators into superficial veins. Venous return is also facilitated by a pressure gradient from the peripheral veins to the right heart. At rest, the venous pressure in the foot is 90–100 mmHg, dropping significantly (to 20–30 mmHg) on ambulation, but rising again to the baseline at rest (Fig. 24.2) [14]. The time taken for the leg to return to baseline venous pressure after calf muscle activity can be measured and is known as the venous refilling time (VRT). Very short VRTs (<20 s) indicate severe venous dysfunction. While several measures may be used to counter venous hypertension, elevation is the only method able

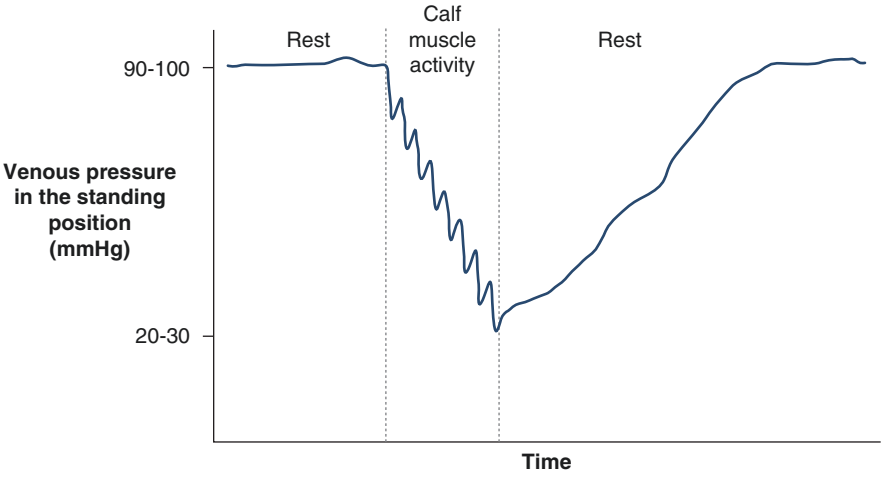
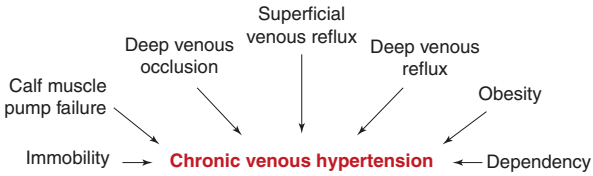


Fig. 24.2 Normal ambulatory venous pressure measurement

Fig. 24.3 Multifactorial nature of chronic venous hypertension



to completely reverse raised venous pressure. Therefore, functioning calf and foot muscle pumps may be considered as essential to reduce the venous pressure and avoid the symptoms and signs of chronic venous hypertension [15].

24.5 Pathophysiology of Chronic Venous Hypertension

Abnormally and persistently raised venous pressure in the leg as a result of venous dysfunction is termed 'chronic venous hypertension' and is widely accepted as the process leading to skin changes and eventually to venous ulceration. In most patients with chronic venous hypertension, there are pathological processes (most commonly venous reflux and/or outflow obstruction) that result in increased venous pressure and clinical symptoms and/or signs. However, any failure or dysfunction of the normal muscle pump mechanisms may lead to a deterioration in clinical status in patients with little superficial or deep venous disease on imaging. Factors such as poor mobility (with secondary calf muscle deconditioning), ankle stiffness (inhibiting effective calf muscle contraction), stroke or neuromuscular conditions may all inhibit calf muscle pump effectiveness [16]. Venous hypertension also be exacerbated by central causes of venous hypertension (such as right heart failure) or truncal obesity, which may act as a functional venous outflow obstruction [17] (Fig. 24.3).

24.5.1 Microcirculatory Changes in Venous Hypertension

The microcirculation refers to the smallest functional unit of the cardiovascular system, where there is a direct interface between the blood and organ or tissue. The association between chronic venous hypertension and microcirculatory changes leading to venous ulceration is generally undisputed, but poorly understood. In 1917, Homans postulated that stagnation of venous blood caused local tissue hypoxia, although the view that ulceration is due to simple hypoxia is probably too simplistic and has largely been abandoned. Venous hypertension causes increased capillary endothelial permeability, with resultant soft tissue oedema, haemosiderin deposition, white cell activation and the chronic inflammatory cascades that can culminate in skin ulceration. Based predominantly on observed microcirculatory changes in patients with chronic venous hypertension, multiple theories for venous ulceration have been proposed.

The presence of 'peri-capillary fibrin cuffs' around coiled capillaries in patients with venous ulceration led some researchers to hypothesize that these cuffs acted as a barrier to the diffusion of nutrients, resulting in skin breakdown [18]. However, the fact that ulcer healing was achieved despite these cuffs, led to the theory being challenged. A second proposed theory was the white cell trapping hypothesis [19], which focussed on the discovery that venous hypertension causes a reduced arteriovenous pressure gradient and relative 'trapping' of white blood cells (WBC) in the periphery. These 'trapped' white cells may become activated, fuelling the chronic inflammatory cascade that is observed in patients with chronic venous ulceration. However, inhibition of inflammation failed to demonstrate significant clinical benefits and it remains unclear whether the observed changes are primary, or simply secondary to the complex inflammatory processes in the ulcerated leg. Another proposed theory noted that extravasation of large proteins from capillaries could act as a 'trap' for important growth factors, thus restricting their availability for ulcer healing [20]. However, this theory was challenged by observations indicating high concentrations of growth factors in non-healing wound beds and a lack of clinical improvement despite extrinsic administration of growth factors.

Several other studies have attempted to measure concentrations of a range of cytokines and growth factors involved in inflammation, angiogenesis, proteolysis and fibrosis. Unfortunately, the patient group, sampling technique, assay type and specific cytokine have varied enormously between studies, making it difficult to draw firm conclusions. It is clear however, that there is enormous variation between patients making it difficult to utilise these measurements as markers of healing.

24.6 Causes of Chronic Venous Hypertension

24.6.1 Venous Reflux

Our understanding of the mechanisms that result in venous valvular incompetence remain far from complete. Several researchers have evaluated a range of biological and histological features in patients with varicose veins. Features such as disruption

of the extracellular matrix, imbalance of matrix metalloproteinases and tissue inhibitors of metalloproteinases (see Glossary), dysregulation of apoptosis (see Glossary) and reduction in venous tone have been observed in the vein wall [21]. It is debatable whether the vein wall changes precede valvular incompetence, or if the primary event is vein valvular reflux, leading to secondary vein wall changes. Usually, by the time patients present, multiple changes are present, making it impossible to separate the 'chicken' and the 'egg'. Venous stasis and hypertension may promote further vein wall stretch and hypoxia [22], suggesting that once vein wall and valvular function begins to fail, a vicious cycle of cellular damage is triggered [23, 24].

Interestingly, observational studies have evaluated patterns of venous reflux and showed that in some patients, the superficial reflux starts in the lower leg, before progressing up the leg (so called 'ascending' theory) whereas in other patients, there is primary reflux at the saphenofemoral junction, which progresses down the leg ('descending' theory) [25]. It is likely therefore that no single pathophysiological mechanism exists and in reality, the precise processes leading to venous hypertension probably vary between patients. The 'self-perpetuating' nature of venous dysfunction may encourage early intervention with for example, venoactive medications purported to reduce inflammation and increase venous tone. However, evidence showing that early treatment can prevent the development of varicose veins is lacking.

24.6.1.1 Secondary Valvular (Post-thrombotic) Damage

Damage to venous valves, either direct or indirect may result in incompetence and whether in isolation or in combination with venous outflow obstruction, may result in impaired venous return and venous hypertension. Direct damage to valves may occur when deep vein thrombosis (DVT) occurs where the valves are located and the subsequent fibrotic changes during thrombus resolution damage the normal valvular function. Indirect (or secondary) valvular damage may occur due to venous outflow obstruction because of the increased stress on valve leaflets. Impairment of valvular function caused by inflammatory mediators has also been proposed as an indirect mechanism. Particularly with direct post-thrombotic valvular damage, a challenging combination of reflux and obstruction may be present at the site of the valve.

24.7 Post Thrombotic Syndrome

Post thrombotic syndrome (PTS) is a complex and diverse clinical entity that occurs in 20–30% of patients after proximal DVT, usually occurring within 2 years of the DVT [26]. The diagnosis and assessment of PTS remains challenging and controversial, but the Villalta scale is the most widely accepted tool [27]. The score consists of 11 domains (each scoring 0, 1, 2 or 3) comprising of 5 patient reported

symptom domains (pain, cramps, leg heaviness, pruritis and paraesthesia), 6 clinician assessed domains based on clinical signs (oedema, induration, hyperpigmentation, venous ectasia, redness, calf tenderness) and assessment for the presence of a venous ulcer. A score ≥5 indicates the presence of PTS. The underlying cause of PTS is venous outflow obstruction, post-thrombotic valvular incompetence or a combination of the two, resulting in a spectrum of clinical presentations and severities. Interestingly, the pattern of symptoms and signs may reflect the dominant underlying pathology. Pain and swelling for example may be more indicative of outflow obstruction, whereas ulcers may be more commonly associated with reflux. In reality, clinical presentations and patterns of disease are enormously varied, preventing easy classification of patients.

24.7.1 Deep Vein Thrombosis (DVT) and Thrombus Resolution

DVT is common, with an incidence of 0.5–1 per 1000 adults per year in western countries [28]. The thrombus may cause complete or partial occlusion of the deep vein and commonly leads to the typical clinical features including pain, swelling, redness and tenderness [29]. For over a century, our understanding of venous thrombosis has focussed on Virchow's triad of venous stasis, vein wall changes and prothrombotic changes in the blood constituents [30]. Although precise factors causing DVT may vary from individual to individual, it is likely that multiple contributory factors combine to result in thrombus formation. Ordinarily, the venous endothelial cells maintain a non-thrombogenic environment by expressing fibrinolytic and anti-inflammatory factors [31]. Conversely, at times of endothelial disturbance (e.g. after trauma), a pro-thrombotic environment is created by vasoconstriction and release of factors such as platelet activating factor (PAF), von Willebrand factor (vWF) and others. The early phase of thrombosis (up to 2–3 days) is driven largely by neutrophils, whereas the later phase (to 14 days) is dominated by monocytes as the thrombus reduces in size with a progressive increase in fibrosis. The processes of inflammation and thrombosis are intimately related, with the presence of one, promoting the other. Another important group of mediators involved in thrombosis is the selectins. P- and E-selectin are cell-adhesion molecules that are integral in the white cell and endothelial wall interaction. Genetic 'knockout' studies in mice have shown reduced thrombosis when selectin genes were deleted and selectin-specific antibodies or receptor antagonists have also been shown to inhibit thrombosis [32].

As a counter to thrombosis, the fibrinolytic system is responsible for containing the extent of the thrombus. A proenzyme plasminogen is converted to the active enzyme, plasmin which is the primary enzyme responsible for breaking down fibrin. The fibrinolytic process is controlled by plasminogen activators (tissue-type and urokinase-type) which in turn are inhibited by plasminogen activator inhibitors (PAIs). PAI inhibitors have been proposed as a novel mechanism for reducing thrombosis [33]. There are several parallels between venous thrombus resolution

and wound healing, with eventual fibrosis. After DVT and subsequent inflammation and remodelling, one of three eventual scenarios may result:

1. Total thrombus resolution with no significant residual outflow obstruction.
2. Limited thrombus resolution with partial venous outflow obstruction.
3. No thrombus resolution with total obliteration of venous channel.

Most of the measures to prevent or treat PTS presume the 'open-vein hypothesis', which simply states that an open and widely patent deep vein after DVT is less likely to result in PTS [34]. However, the correlation between anatomical extent or severity of post-thrombotic deep venous outflow obstruction and clinical symptoms is notoriously poor. In some patients with very extensive and dramatic deep venous occlusive disease on imaging, there may be few clinical symptoms and signs or none. However, there remains a concern that patients with post-thrombotic deep venous occlusion may be at higher risk of developing symptomatic or advanced venous disease in the future. The anatomical disease may act as a 'permissive' pathology [35], remaining largely subclinical, but with patients becoming very symptomatic with only a small additional venous pathology. Reasons for variability in thrombus resolution after DVT between patients are poorly understood.

24.7.2 Impact of Venous Outflow Obstruction

Residual venous fibrosis after DVT results in increased outflow resistance and reduced compliance in a more rigid vein. Autologous resolution of thrombus is thought to be less effective for DVTs in the iliocaval segment, in comparison to infrainguinal DVT, which may explain the higher rate of PTS in this group. The resultant impedance to normal venous drainage, results in the symptoms and clinical signs associated with PTS. The impairment to effective venous drainage may be mitigated partially by the formation of venous collaterals as alternative draining channels. However, even florid venous collaterals are unlikely to ever match the venous drainage capacity and efficiency of an intact large deep vein. From Pouseille's law, flow is proportional to the fourth power of the radius of a tube. Therefore, it would require 16 1 cm diameter collateral veins to match the potential flow in a 2 cm deep vein.

24.8 Genetic and Metabolic Profiling in Venous Disease

The fact that a high proportion of patients with varicose veins and other venous disorders report a positive family history has led many to speculate that there may be a genetic predisposition. The more widespread availability of gene mapping techniques have opened a novel and exciting avenue for venous researchers [36]. It should be noted that some of the presumed heritability of venous disorders may be indirect, as other risk factors for venous disease (such as obesity) may have

significant genetic components. Several genetic disorders are known to be associated with varicose veins, including Klippel-Trenaunay syndrome, Ehlers-Danlos syndrome and specific gene mutations (such as thombomodulin). Studies assessing specific candidate genes have identified a number of interesting potential targets, although studies have been small. In the future, a genome wide association study (GWAS) approach may permit much larger scale evaluations and uncover as yet unsuspected genetic associations with venous disease.

Use of alternative technologies, such as metabolic profiling may allow direct evaluation of the phenotype, with indirect genotype assessment. In a recent study, several potentially significant metabolic markers were shown to differ between patients with and without varicose veins [37]. The authors were able to perform pathway analysis to provide interesting new insights into which metabolic pathways may be of pathophysiological relevance in patients with venous disease.

24.9 Principles of Management of Venous Disease

As the underlying cause of advanced venous disease and ulceration is chronic venous hypertension, the principles of management focus on the identification and correction of the factors causing venous hypertension. A detailed description of the clinical management of patients with venous disease is beyond the remit of this chapter, although principles of assessment and treatment are described.

24.9.1 Clinical Evaluation

A detailed clinical history should include the severity of symptoms and the impact on quality of life, as the decision to intervene may be largely driven by these considerations. Common symptoms of venous disease include pain, heaviness, swelling, restless legs, cramps and itching [38]. Details of previous venous thromboembolism, previous venous interventions and relevant medications (anticoagulation, immunosuppression, oestrogen-containing medications) should be recorded. In a clinical field renowned for a high level of litigation, careful documentation is essential and patient expectations from any interventions should be clearly established.

24.9.2 Investigations

The main goals of venous investigations are to identify potentially treatable superficial or deep venous disease and confirm the diagnosis. A wide variety of anatomical and haemodynamic venous assessments can be performed, but DUS is the

first-line investigation. DUS is non-invasive and can offer an excellent, detailed map of venous incompetence and obstruction. In many cases, this is the only investigation necessary to plan intervention. If venous outflow obstruction is suspected, additional evaluation using computerised tomography (CT), magnetic resonance (MR) or conventional venography may be required.

24.9.3 Treatment

24.9.3.1 General Principles

The primary goal of treatment is to reduce chronic venous hypertension. Treatable superficial reflux or significant venous outflow obstruction should be addressed where feasible. As chronic venous hypertension is usually multifactorial, there may be residual dysfunction despite superficial or deep venous intervention (or venous intervention may not be feasible or accepted by the patient). In such cases, compression and elevation are the mainstay of management.

24.9.3.2 Superficial Venous Interventions

The presence of incompetence in the GSV, SSV, associated tributaries or perforating veins is commonly seen in patients with varicose veins and chronic venous insufficiency. Traditional treatment of superficial reflux involved surgical ligation and 'stripping' of incompetent veins. In recent years however, superficial venous interventions have been revolutionised by minimally invasive endovenous ablation procedures. These treatments utilise heat (laser, radiofrequency) or chemical (sclerotherapy, cyanoacrylate) ablation to close the incompetent superficial vein(s). Often performed under local anaesthesia in an outpatient setting, endovenous ablation procedures offer a well-tolerated and effective approach to address the usual underlying cause of venous hypertension. Endovenous interventions have been shown to improve patient quality of life [39], accelerate venous ulcer healing and are cost effective [40, 41].

24.9.3.3 Deep Venous Interventions

The latest therapeutic developments have been interventions for venous outflow obstruction. Until recent years, patients with occluded deep veins (usually post-thrombotic) had no options to address the outflow obstruction. However, a range of customised deep venous stents are now available to maintain deep venous patency and reduce venous hypertension. Medium and long-term outcomes are awaited.

24.10 Conclusions

The venous system is a complex, highly dynamic component of the circulation. Low resistance, highly compliant venous channels effectively counter venous hypertension using intrinsic mechanisms such as calf and foot muscle pumps and vein valves. Venous dysfunction is often complex and multifactorial, resulting in a spectrum of venous disease. The underlying cause of chronic venous insufficiency and ulceration is venous hypertension. The precise mechanisms and pathways linking chronic venous hypertension to venous skin damage and ulceration are incompletely understood. The principles of managing chronic venous insufficiency are to identify treatable superficial venous reflux or venous outflow obstruction.

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Chapter 25
Pathophysiology of Wound Healing

Stuart J. Mills, Ben R. Hofma, and Allison J. Cowin

Key Learning Points

- Understanding of how signalling processes are key in how a wound heals as they control each stage of the wound healing process.
- Understand how important the role of infection is and how the methods of detection are far from optimal and need to be improved.

25.1 Introduction

Wounds that occur in the skin usually progress through a highly co-ordinated series of events that lead to the restoration of the skin barrier. The stages that occur can be split into four distinct but overlapping phases that include: haemostasis, inflammation, proliferation and remodelling. Specialised cells are required for each phase to progress including; platelets, immune cells, keratinocytes, fibroblasts and endothelial cells. Any compromise in cell function or disruptions in the stages of wound healing results in chronic wounds. These are particularly prevalent in diabetic populations, aged communities, wounds with underlying arterial and venous disease, and in people with obesity. This chapter will provide an overview of the healing process, the cells and pathways involved and the factors that contribute to impaired healing.

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25.2 The Wound Healing Process

25.2.1 Haemostasis

The wound healing process is usually initiated following damage to the skin, which results in extravasation of blood from disrupted vessels [1] and leads to platelets contacting fibrillar collagen, fibronectin and matrix proteins, that are not present within the lumen of the blood vessel. This activates the platelets and causes them to adhere, aggregate and degranulate, releasing the contents of their α-granules, which includes transforming growth factor alpha (TGF-α), TGF-β, platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), serotonin, thromboxane A_2 and cyclic AMP. Releasing the contents of the α-granules causes the vessels to constrict, reducing further blood loss. The intrinsic and extrinsic coagulation pathways are also activated at this time (Fig. 25.1). During this process, released thrombin converts fibrinogen to fibrin leading to the formation of a fibrin-platelet matrix/clot, which helps to prevent further blood loss and acts as a provisional matrix for cells to migrate through (Fig. 25.2) [2, 3]. Haemostasis allows the initiation of the other phases of wound healing to commence including: inflammation, angiogenesis, re-epithelialisation and matrix formation/remodelling.

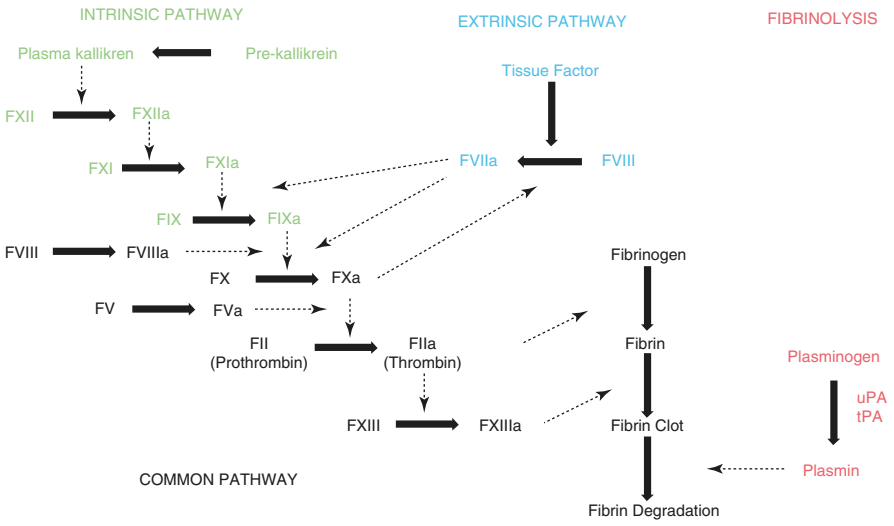

Fig. 25.1 Intrinsic and extrinsic pathways of the coagulation cascade

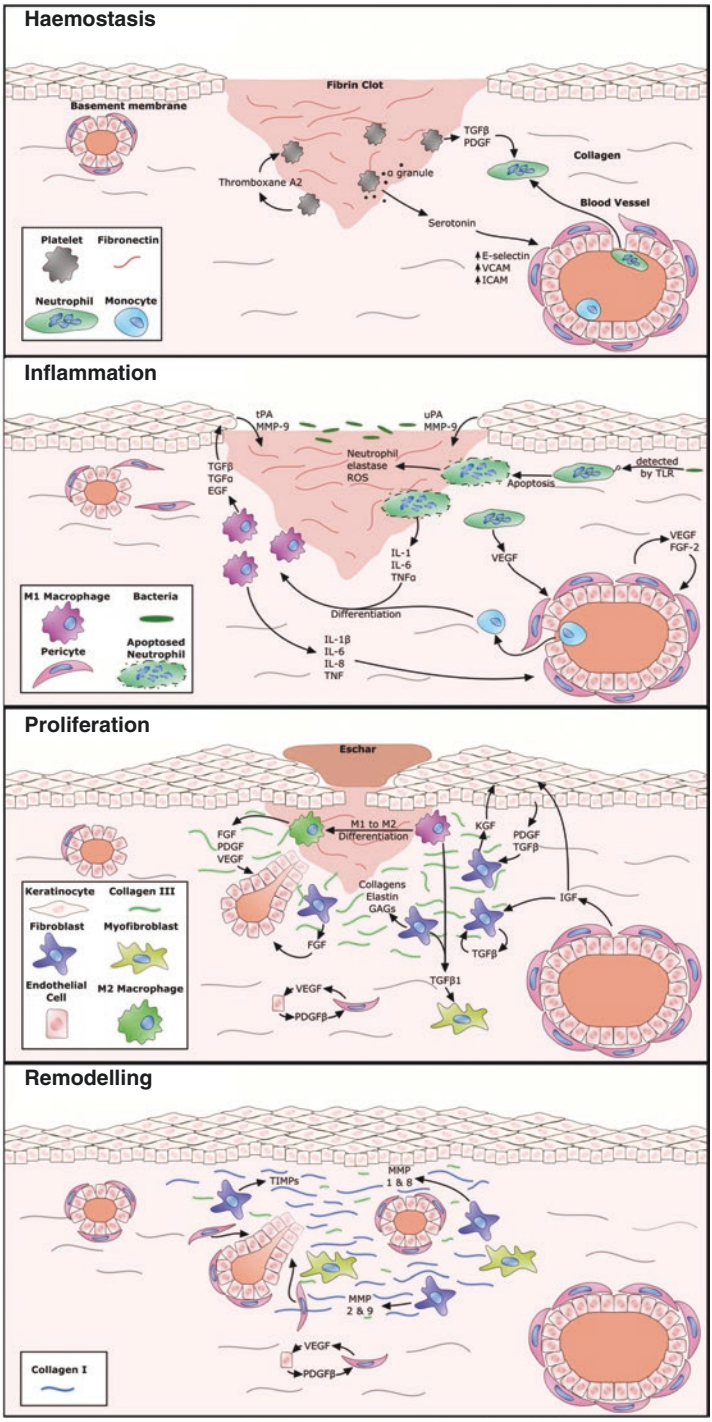

Fig. 25.2 The four phases of the wound healing process

25.2.2 Inflammation

The inflammatory phase initiates within 30–40 minutes post-injury and can persist for 2–3 weeks. During the early stages, there is an influx of neutrophils from the blood vessels to the wound, which are attracted by a variety of chemotactic factors [4]. Two of the main sources of the chemotactic factors are platelets, which release PDGF and TGF-β, and mast cells, which themselves release tumour necrosis factor (TNF), histamines, leukotrienes and interleukins (IL) [3]. Additional chemotactic signals from factors such as kallikrein, fibrinopeptides, cellular debris and invading pathogens are also present at this stage of healing [3]. Neutrophils detect the chemoattractant, TGF-β, and rapid chemotaxis through the cell surface is initiated, which activates TGF-β serine/threonine receptors and the Smad signalling pathway. This enables cells to detect a TGF-β concentration gradient enabling them to migrate towards the TGF-β source [5].

The migration of neutrophils from the blood vessels to the wound site is brought about by an increase in selectin expression on the luminal surface of the endothelial cells within the blood vessel (Fig. 25.2b). An increase in E-selectin expression, in response to TNF, enables a light adherence of the leukocytes to the blood vessel lumen reducing their velocity. In conjunction with this, and within an hour of wounding, there is around a 100 fold increase in expression of β2-integrins, intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1), which enables rolling or diapedisis of the leukocytes along the endothelial cell wall. A concurrent increase in bradykinin and serotonin increases the permeability of the vessel allowing leukocytes to leave the blood stream, enter the extravascular space and migrate to the wound site [6].

Neutrophils are the primary responders to a wound and generally persist for around 5 days. Once within the wound site, there is an increase in integrin expression on the cell surface of the neutrophils, which allows cell-matrix adherence and interaction with pathogens. Their primary function is to clear the wound of pathogens via uptake into phagolysosomes and by the release of reactive oxygen species (ROS) [7]. Neutrophils can also digest the matrix via secretion of proteases such as neutrophil elastase and collagenases, which act to remove any damaged matrix components [8]. Once the neutrophils have killed any invading pathogens they usually undergo apoptosis (programmed cell death). The signals released from the mast cells, platelets and apoptosed neutrophils then attract monocytes to the wound site, which usually arrive 2–3 days post-wounding.

Monocytes, when transitioning to the wound site, differentiate into macrophages and are often referred to as M1 macrophages. Monocyte migration towards the wound site is dependent on microtubules and their orientation. This was shown in a study where zebrafish were wounded and treated with nocodazole, which inhibited microtubule formation. This had the effect of altering the cell polarity and resulted in the macrophages migrating in random directions instead of directly towards the wound site [9]. Macrophages are key regulators of the inflammatory process and their main function is to clear the wound of cellular and matrix debris as well as any invading pathogens, which they detect through toll-like receptors (TLRs) [10].

Table 25.1 A list of DAMPs and PAMPs for TLR2, TLR3, TLR4 and TLR9

Toll-like Receptor	DAMPs	PAMPs
TLR2	High mobility group box 1 (HMGB1) Heat shock proteins Extracellular matrix proteins e.g. hyaluronan fragments ATP Uric acid Heparan sulphate	Glycosylphosphatidylinositol-linked proteins Zymosan Lipopeptides
TLR3		dsRNA
TLR4	Heat shock protein 60 HMGB1 Hyaulorinc acid fragments Fibrinogen	F protein LPS
TLR9	S100 proteins	Herpes virus DNA CpG DNA sequences

DAMPs damage-associated molecular patterns, *PAMPs* pathogen-associated molecular patterns, *LPS* lipopolysaccharide

TLRs are part of a family of pattern recognition receptors found on the cell surface of a wide variety of immune and non-immune cells. They initiate both innate and adaptive immune responses [10] and recognise both damage-associated molecular patterns (DAMPs) and pathogen-associated molecular pathogens (PAMPs). DAMPs or endogenous ligands are biomolecules that are released from damaged tissues or damaged/apoptosed cells, and include a variety of components both from the nucleus and the cytosol. PAMPs are conserved on a wide range of pathogens and are found on the surface or the interior of microbes (Table 25.1). TLRs all have highly conserved domains that span the extracellular space, across the membrane, and into the intracellular space. They are expressed either on the cell surface (TLR1, 2, 4, 5, 6, 10 and 11) or intracellularly in structures such as the endoplasmic reticulum, lysosomes, and endosomes (TLR3, 7, 8, 9, 12 and 13). Upon activation by a DAMP or PAMP, TLRs signal intracellularly through two distinct pathways. The first is the MyD88 dependent pathway, which signals through the MyD88 protein and leads to an increase in NF-κB expression. This results in the release of pro-inflammatory cytokines including Macrophage Inflammatory Protein 2 (MIP2), IL-1, IL-6, IL-8, IL-12 and TNF (Fig. 25.3). The secondary signalling pathway, referred to as the MyD88 independent pathway, or the TIR domain containing the adaptor-inducing interferon-β (TRIF)-dependent pathway, also leads to an increase in NF-κB production but results in an increase in interferon (IFN) factors such as type I IFN genes and pro-inflammatory cytokines [10].

The main TLR receptors involved in wound healing are TLR2, 3, 4 and 9 and interestingly have divergent roles depending on the wound environment. In acute wounds, depletion or knockout of TLR2 and 4 leads to a reduction in neutrophil and macrophage infiltration, a reduction in TGF-β and keratinocytes expressing CCL5, which significantly delays healing [10]. TLR3 deficient mice also have delayed healing and impaired immune cellular infiltration but in addition have reduced granulation

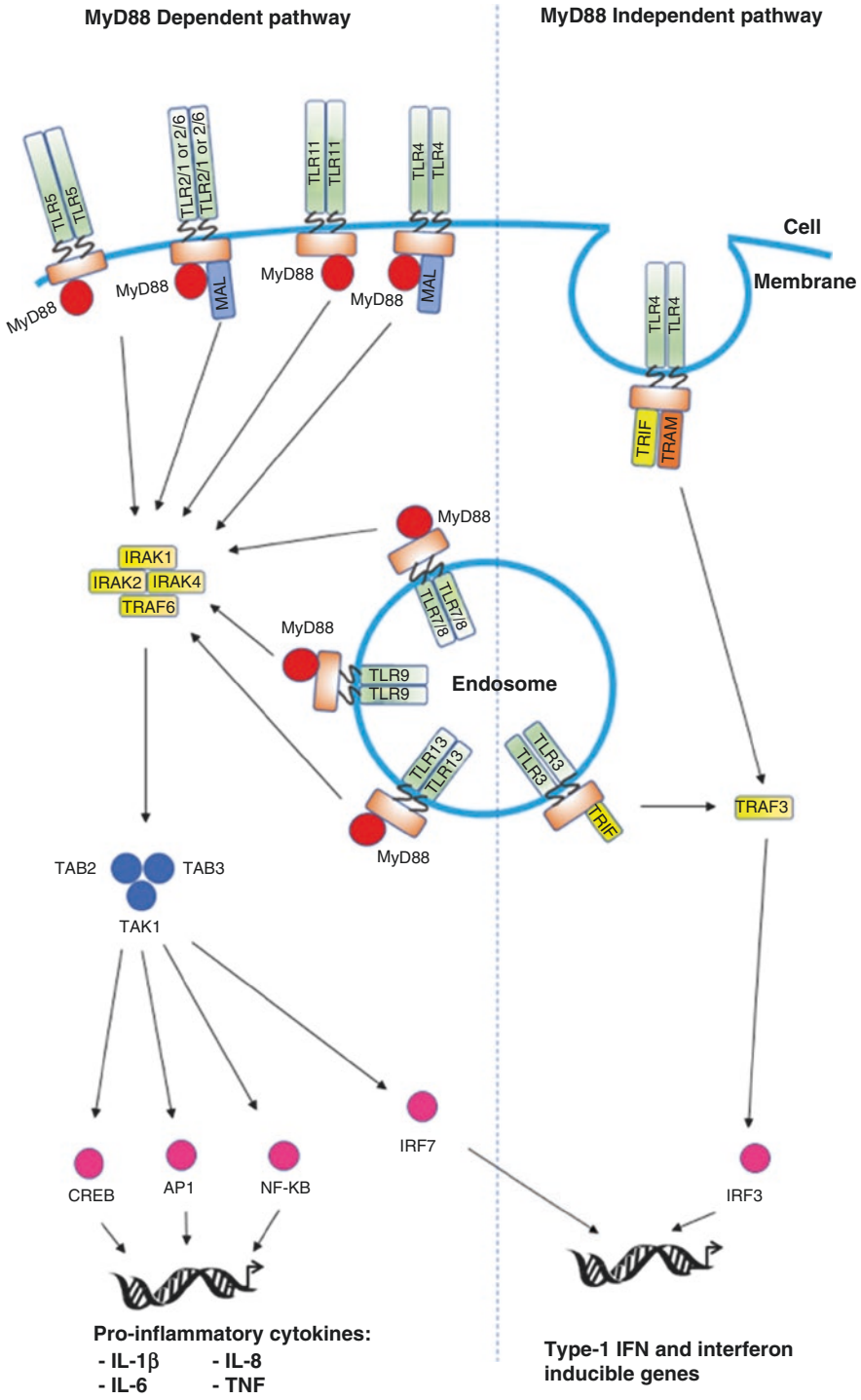

Fig. 25.3 TLR pathway signalling through the MyD88 dependent and independent pathways

tissue formation and neovascularisation [10]. In contrast to this, in diabetic wounds, there is an increase in TLR2 and 4 expression and a concurrent increase in pro-inflammatory cytokine production in mice [10]. When mice are made diabetic and TLR2 or TLR4 deficient, accelerated wound closure is observed as well as a reduced inflammatory cell infiltrate [10]. These studies suggest that when considering TLR function both the receptors involved and the wound environment should be considered.

Activation of TLRs helps to recruit more immune cells, in the form of monocytes, from the blood stream to the wound site in order to clear infection and cellular debris. Once within the wound they differentiate into M1 macrophages and begin to phagocytose dead cells, damaged tissue or infectious microbes. Once cleared of debris and pathogens, macrophages then undergo a switch in phenotype to become M2 macrophages, which are anti-inflammatory in nature. In this sense they have a bimodal function depending on the state of the wound. This switch is thought to be in response to adenosine created from the breakdown of adenosine triphosphate (ATP), which activates intracellular signalling through the A_2 adenosine receptors and TLR9. This results in a dampening of TNF, IL-12 and MIP-1α in macrophages and an increase in PDGF, VEGF, TGF-α and TGF-β [11, 12]. The expression of these factors prevents any further chemoattraction of macrophages to the wound site and initiates the proliferation phase of the wound healing process by stimulating keratinocytes to migrate and proliferate, fibroblasts to deposit matrix and endothelial cells to begin the process of angiogenesis.

25.2.3 The Inflammation Conundrum

The inflammatory response is an evolutionarily-conserved process to prevent infection by trying to heal the wound in as rapid a manner as possible. Many wound healing studies have shown that there is often a direct link between time to complete healing and the timing of the inflammatory response i.e. longer inflammatory processes lead to longer healing times. The inflammatory response is often considered excessive and so studies have been carried out to determine ways to dampen this inflammatory process. One such study knocked out the PU.1 transcription factor in mice, which resulted in an inhibition of the maturation of several haemopoetic lineages. This produced mice with no macrophages or neutrophils and when these mice were wounded they healed in a similar time frame to wild type mice. The wounds also displayed reduced scarring thought to be due to a reduction in TGF-β1 expression [13]. Interestingly, fibroblasts were found to phagocytose any cellular debris in place of the macrophages.

The phenomenon of other cells fulfilling the role of absent cells is often observed in the inflammatory process and is not limited to fibroblasts and macrophages. In a study where wounds were treated with platelet-specific antiserum to induce thrombocytopenia, reduced numbers of platelets were observed invading the wound site. This should have led to a reduction in TGF-β expression and a reduction in inflammation. In contrast it resulted in an increase in macrophages and T-cells, which were retained in the wound but with no effect on the rate of healing. Mice have also been

generated without any mast cells (SBB6F1/J-kit^{w}/Kit$^{w/v}$) and when wounded, these mice demonstrate a reduction in neutrophils. However, the wound healing process itself was completed in a similar timeframe to wild type controls [14]. These studies show that no single cell type is crucial for complete healing and that there are substantial compensatory mechanisms in place to ensure that wound healing proceeds to completion. These compensatory mechanisms make modulation of the inflammatory process difficult to completely control and therefore, successful treatments targeted at the inflammatory phase can be difficult to develop.

25.2.4 Proliferation

25.2.4.1 Re-epithelialisation

Within hours of wounding, proliferation commences at the leading edge of the epidermis to start the process of re-epithelialisation. This results in the restoration of the epidermal barrier, helping to prevent infection, reduce water loss and regulate temperature. For this process to occur, matrix metalloproteinases (MMPs) and plasmin are released at the leading edge of the epidermal tongue, which degrade the matrix, allowing the epithelial cells to migrate and proliferate into the wound space. MMP-9 is highly expressed by epithelial cells and acts to cleave basal lamina collagen (type IV) and anchoring fibril collagen (type VII). This enables keratinocytes to become detached from the basal lamina to initiate migration. MMP-1 is expressed by keratinocytes as they migrate past the leading edge, which degrades collagen I and III allowing continued migration [15]. Knockout studies have shown that plasmin from tissue type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) (Fig. 25.1), are both upregulated in keratinocytes and are crucial for wound healing [16]. To facilitate binding to and migration over the new matrix, which is predominantly made of fibrin, fibronectin and vitronectin, a shift in integrin expression occurs in keratinocytes, from collagen-laminin (α2β1) to fibronectin-tenacin (α5β1 and αvβ6) and vitronectin (αvβ1) [17]. This change in integrin expression enables keratinocytes to interact with the provisional matrix and enable migration. Keratinocytes also polarise their microtubules via Cdc42 signalling to activate GTPase Rac-1. This allows the cells to contract intracellular actin and myosin fibres, which then insert into new adhesion complexes, providing forward motion. There is also a shift in keratin expression from keratins 1 and 10 to 6, 16 and 17 [18]. Posterior to the migrating keratinocytes, the basal keratinocytes begin to proliferate in response to factors such as fibroblast growth factor-7 (FGF-7), TGF-α and epidermal growth factor (EGF). Interestingly, TGF-β has been shown to stimulate keratinocyte migration *in vitro* but to inhibit proliferation [19]. This mode of action was found to involve Smad-3, a downstream signalling molecule of TGF-β1. When utilising a mouse Smad-3 knockout acute wounding model, it was found that the wounds in the knockout mice re-epithelialised at a faster rate when compared to wild type controls [20]. To prevent excessive re-epithelialisation once the epidermis

is re-established, the filopodia at the leading edges interact with the opposing leading epidermal keratinocytes and this interaction leads to a "zippering up" of the wound [21].

25.2.4.2 Matrix Deposition

The principal cells involved in the formation of extracellular matrix (ECM) are fibroblasts, which migrate to the wound site approximately 72 hours post-wounding, in response to factors such as PDGF, TGF-β, fibrin and fibronectin [22]. Fibroblasts proliferate at the wound's edge then they change their integrin expression to α3 and α5 integrins in response to PDGF. This allows them to use the provisional matrix as a scaffold and to migrate through the wound [22]. The matrix deposited by fibroblasts is highly vascular and referred to as granulation tissue. It mainly consists of fibrin, fibronectin, hyaluron and collagen III. PDGF stimulates fibroblasts to release TGF-β, which in turn stimulates collagen, proteoglycan, glycosaminoglycans and elastin production. Initially, bundles of collagen III associated with fibronectin predominate but this is gradually replaced with collagen I.

25.2.4.3 Angiogenesis

Angiogenesis is the process of new blood vessel growth from already existing vessels and is distinct from vasculogenesis, which involves recruitment of endothelial progenitor cells (EPCs) from the bone marrow. Angiogenesis is vital for wound healing as it supplies the oxygen and nutrition that is required by cells to metabolise, divide and differentiate. Initially during angiogenesis, pericytes on the blood vessel migrate away from damaged vessels (Fig. 25.2) allowing endothelial cells to initiate proliferation. This is prompted by damaged keratinocytes, macrophages, platelets and endothelial cells releasing FGF-2 and VEGF [4]. This process is dependent on a provisional matrix for the endothelial cells to migrate into, which at this stage of the wound healing process is made up of fibronectin, vitronectin and laminins [4]. Angiogenesis is also reliant on other cells such as neutrophils and macrophages, which release proteases to remodel the ECM. In doing so, they uncover matricryptic sites, such as Arg-Gly-Asp (RGD). Matricryptic sites are areas of the matrix which store extracellular proteins and glycosaminoglycans, which can be cleaved to become biologically active. Some of these sites allow adhesion with endothelial cells allowing them to proliferate and migrate. Pericytes also play a key role by producing tissue inhibitors of matrix metalloproteinase-3 (TIMP-3), which not only regulate matrix turnover by MMPs, but also prevent capillary tube regression in response to MMP-1 and MMP-10 [4]. Once the endothelial cells have formed new blood vessels, the pericytes return in response to PDGFβ expression, which stabilises the vessels formed and prevents them degrading. The role of pericytes is clearly shown in PDGFβ knockout mice, which display endothelial hyperplasia and a lack of pericyte recruitment to newly formed capillaries [4].

25.2.4.4 Wound Contraction

The process of wound contraction acts to bring opposing undamaged epithelial edges into closer proximity to reduce the distance that the wound has to re-epithelialise. This process usually occurs in the second week of wound healing and wound areas can be reduced by up to 40% via contraction [3]. For this process to occur a percentage of fibroblasts convert to myofibroblasts, which express α-smooth muscle actin and generate contractile forces in response to TGF-β1 [23]. Once wound contraction is no longer required, fibroblasts release cyclic adenosine monophosphate (cAMP) and there is an influx of calcium into the cells, which desensitizes cell surface PDGF and EGF receptors resulting in quiescent cells [24].

25.2.5 Remodelling

The remodelling phase begins within the first week and can last up to 2 years. It involves the formation of collagen, its degradation, remodelling and its reorientation into bundles to form a mature scar. In general, collagen III is replaced by collagen I, hyaluronan is replaced by heparan sulphate in the basement membrane and by dermatan and chondroitin sulphate in the interstitium [25]. The turnover of collagen from type III to type I is tightly controlled by the expression of MMPs and TIMPs. These enzymes act by hydrolysing components of the extracellular matrix. MMP-1 and MMP-8 cleave triple helical collagens via hydrolysis of a Gly-Ile/Leu bond, creating fragments which are then further degraded by MMP-2 and MMP-9. TIMPs, as their name suggests, work by inhibiting MMPs by a direct 1:1 binding, masking the active site of the MMP [26]. This ratio of MMP:TIMP is tightly controlled, as an imbalance results in excessive matrix production or breakdown (Fig. 25.2). A clear example of this is seen in chronic wounds where there is overproduction of MMPs, which results in a breakdown of the ECM, and wound healing is delayed or in the worst cases remains incomplete.

Overall, wound healing is a multistage complex process that repairs the barrier of the skin to the external environment in as fast a manner as possible. There is a highly conserved inflammatory reaction that ensures that the wound is cleansed of pathogens and cellular debris. This response is often overly excessive and may delay rates of healing. A similar process is observed in the formation of the ECM which is deposited in as fast a manner as possible to repair the wound. This results in the formation of scars where the collagen bundles formed are weaker than the original skin. Interestingly, when a foetus is in the womb and protected from the outside environment, the rate of healing becomes secondary and different mechanisms to heal are employed. This type of healing has a reduced inflammatory response and forms a scar-free ECM, which is as strong as the original skin. This type of healing is referred to as regeneration.

25.3 The Effects of Ageing on Wound Repair

Significant changes occur in the structure and function of cells within the skin with increasing age. Ageing appears to reduce the number of cells that respond to a wound and also affects behaviour of those cells that do migrate into the wound site. These changes are caused by intrinsic (time, genetic factors and hormone levels) and extrinsic factors (UV exposure and pollution). They result in the epidermis, dermo-epidermal junction (DEJ) and the dermis becoming thinner over time. The epidermis thins due to a reduction in the proliferation of basal keratinocytes and epidermal stem cells [27]. The flattening of the DEJ is due to a retraction of the epidermal papillae and the microprojections of the basal cells into the dermis due to falling levels of chondroitin-6-sulphate resulting in changes in proteoglycan distribution [28]. Glycosaminoglycan, collagen and elastin levels in the dermis all decrease with age, which together alter the overall physical characteristics leading to a reduction in the resistance of the skin to shearing forces. Interestingly, this observed fall occurs in males with increasing chronological time whereas in females the decreases occur rapidly after menopause and are related to changing hormone levels [29].

25.3.1 Effects of Ageing on Inflammation

Ageing appears to exacerbate the inflammatory response through intrinsic changes in immune cells as well as resultant changes in the matrix. Leukocytes increase their binding capacity and express higher levels of cell surface receptors, which recognises the arginine-glycine-aspartic acid-serine (RGDS) peptide sequence conserved within fibronectin. This results in a delay in the immune cell influx into the wound site as well as their persistence within aged wounds when compared to younger individuals. An altered ratio of immature to mature macrophages is also observed within the wounds of elderly people when compared to younger individuals [30]. Investigations into the effects of ageing on neutrophils show that these cells display a reduction in neutrophil respiratory burst activity, phagocytosis and migration, which could again delay migration of neutrophils into the wound site and a decrease in pathogen and debris removal from the wound [31]. In another study where macrophages from a young donor were applied to wounds in aged mice, healing was restored to similar rates seen in younger mice [32]. Effects on ageing, therefore, prolong the inflammatory response and delay the overall rate of healing. It should be noted that although aged skin does take longer to heal there is often a reduction in scarring associated with these wounds.

25.3.2 Effects of Ageing on Re-epithelialization

Aged keratinocytes show decreased mitogenic responses, a reduced sensitivity to growth factors and an increase in sensitivity to toxic agents including; antibiotics, phorbol esters, radiation and heat shock proteins [33]. The secretome (secretion

profile) of keratinocytes alters during ageing with a decrease in the expression of EGF and its receptor resulting in slower rates of re-epithelialization. A study by Stanulis-Praeger and Gilchrest showed that culture media from aged keratinocytes inhibited the proliferation of younger keratinocytes grown in the aged media [34]. Hypoxia also has differing effects on keratinocytes from young and old donors. Keratinocytes from an older donor show decreased migration rates and reduced expressions of MMP-1 and -9 when compared to younger keratinocytes [35]. In addition when investigated *in vivo*, significantly increased rates of re-epithelialisation were observed in younger mice when compared to older mice. As with the immune response, ageing appears to fundamentally change the keratinocyte phenotype, which results in delayed healing.

25.3.3 Effects of Ageing on Matrix Deposition and Remodelling

Fibroblasts are significantly affected by ageing as they become smaller, fewer in number and have reduced function. Alterations in lipid membrane composition and mitochondrial membranes are observed [36]. Fibroblasts become quiescent with increasing age and form a poorly developed endoplasmic reticulum and numerous dense bodies [36]. When fibroblasts from a 66 year old donor and a 20 year old donor were compared, the former displayed reduced motility and reduction in response to chemotactic signals [37]. The reduction in motility with increasing age, was found to be due to a lack of responsiveness to growth factors such as insulin, EGF and PDGF [33]. Whilst the production of collagen appears to remain consistent between younger and older fibroblasts there is an apparent alteration in fibronectin production in older fibroblasts. There is also an increase in MMP-2 and -9 production in older fibroblasts, seen in both human acute and chronic wounds, which promotes the breakdown of the ECM in these wounds [38]. As fibroblasts display an increase in MMP production with increasing age it has also been shown that human skin and wounds shown a decrease in TIMP production with increasing age. This disrupts the MMP:TIMP ratio making the microenvironment more favourable for ECM breakdown [39]. The shift in the composition of the ECM not only affects the mechanical properties of the skin but it also has further effects on cells residing within the matrix. As mentioned above leukocytes have an increased capacity for binding fibronectin due to changes in surface receptor expression with age, which increases the inflammatory response. This response is not only due to changes in the leukocytes but also by the makeup of the ECM, which becomes richer in fibronectin exacerbating this response.

25.3.4 Effects of Ageing on Angiogenesis

Ageing delays the rate of blood vessel formation after wounding. However, the overall degree of angiogenesis appears to remain unchanged in ageing skin [19]. As seen with other cell types, the secretome of endothelial cells alters with

increasing age [40]. Upon senescence, an increase in the expression of IL-1α mRNA in human endothelial cells is observed in addition to increasing levels of TNF released. Both these factors block the proliferation of endothelial cells and reduce their lifespan. Human umbilical vein endothelial cells have an impaired response to FGF due to tyrosine phosphorylation of their FGF receptors with increasing age [40]. In addition, ageing leads to a decreased production and an overall reduced bioavailability of nitric oxide (NO) for endothelial cells. This reduces the vasodilatory effects of NO on blood vessels but it also increases the susceptibility of the endothelial cells to pro-apoptotic signals [41, 42]. Production of factors, from other cells, that stimulate angiogenesis is also altered. Macrophage production of VEGF reduces with age, potentially due to reduced levels of hypoxia inducible factor-1α (HIF-1α), a transcription factor for VEGF [43].

Endothelial progenitor cells (EPCs) are cells recruited from the bone marrow that migrate to areas of damage and help to promote neovascularization by differentiating into endothelial cells. Ageing affects EPCs in a similar manner to differentiated cells in that there is a reduction in their population numbers, lifespan, proliferation and migration, due to a larger percentage of these cells entering senescence [44]. Senescence is one of the major contributors for vascular ageing and is a process that results in the cells no longer being able to proliferate. One of the main causes of this is a shortening of telomere length, which is consistent in most cell populations. Factors such as angiotensin II and its activation of NF-κB to promote pro-inflammatory cytokine expression can also induce senescence. Whilst this process is advantageous when considering diseases such as cancers, which require a blood supply to grow, it has been shown to adversely affect wound healing.

Ageing decreases the proliferation and migration of cells involved in wound healing. This is in response to intrinsic changes within the cells that alters their secretome and receptor expression. This in turn alters their overall function and decreases their responses to stimulatory cues from surrounding cells and ECM. In addition to this, a greater proportion of the cell populations reach senescence, which diminishes the responses further and prevents adequate responses when wound healing is initiated. Overall, the changes brought about by ageing prolongs and delays the phases of wound healing.

25.4 Chronic Wound Healing

In humans, following wound closure, the dermis continues to be remodelled. If there are perturbations in the wound healing process this timeline can be greatly delayed and any wound that fails to heal within 6 weeks is considered a chronic wound. Chronic wounds occur when insufficient healing occurs, the wounds fail to re-epithelialise (ulcers) or when there is persistent infection.

25.4.1 Chronic Wounds

Inadequate healing and a breakdown of the extracellular matrix (ECM) often leads to the formation of ulcers. There are various types of ulcer including pressure ulcers, venous ulcers, arterial ulcers and diabetic ulcers. These have varying pathologies but at the same time, share similar characteristics. One of the main causes for these non-healing wounds is that the inflammatory phase of healing fails to resolve and results in an escalation of inflammatory cells invading the wound with a subsequent increase in ECM destruction. Neutrophil activity in these wounds is elevated with increased production of ROS, leading to the breakdown of the provisional matrix as well as cellular membranes, resulting in senescence [45]. In addition, neutrophils also secrete neutrophil elastase and MMP-8, which result in the degradation of growth factors (PDGF and TGF-β) and ECM respectively. The effect of this is two-fold; not only is there destruction of the ECM but the cells present in the wound cannot respond to make more ECM due to the concurrent breakdown in growth factor signalling. This results in excessive damage to the ECM [46]. In addition to these effects, neutrophils and macrophages within the wound also express pro-inflammatory cytokines similar to the normal healing response. However, due to decreased availability of key growth factors, there is reduced regulation of their expression leading to an increase in cytokines such as; IL-1β, IL-6, IL-8 and TNF. This exacerbates the inflammatory response by recruiting more activated neutrophils and macrophages, which in turn produce more pro-inflammatory cytokines. This also has a direct effect on proteinase production within the wound by unbalancing the MMP:TIMP ratio, leading to increased MMP and decreased TIMP expression [47]. Consequently, further degradation of the ECM occurs, which impairs cell migration and reduces collagen deposition, leading to a self-perpetuating cycle of ECM degradation and inflammation [48].

25.4.2 Diabetic Ulcers

Diabetic ulcers are of growing concern with the increasing age of populations and the increasing occurrence of diabetes, which now stands at 21 million diabetic and 54 million pre-diabetic patients worldwide [49]. Diabetes will be the leading cause of disease burden by 2023 and approximately 15% of diabetic patients will develop non-healing ulcers during their lifetime. This ultimately leads to an amputation every 30 seconds and is placing a huge burden on Health Services worldwide [49]. Diabetic ulcers can be classified as either neuropathic, neuroischaemic or ischaemic and develop in response to a variety of host factors including hyperglycaemia, peripheral neuropathy and arterial occlusive disease. Hyperglycaemia can affect the immune function of the host in a variety of ways. One effect is on neutrophil function, where it acts to lower superoxide and myeloperoxidase production in the cells resulting in poor chemotaxis and phagocytosis of pathogens within the wound.

Unlike hyperglycaemia, peripheral neuropathy does not act directly on the immune response within the wound but is a cause of ulcer development. The reduction in sensation of the lower limbs leads to minor trauma going undetected and combined with muscle weakness, can lead to increased forefoot pressure and a breakdown of tissue. Combined, these factors can result in small fissures which can easily become infected. Peripheral artery disease (PAD) can reduce blood flow in the lower limbs, which also delays the delivery of immune cells to the local area to combat infection. In some cases this can cause blood stasis, which initiates a host immune response and leads to the breakdown of local tissues [50]. When this occurs, nitric oxide synthesis is blocked, leading to increased production of ROS and cellular damage. There is also the Maillard reaction between sugars and amino groups, which lead to the formation of advanced glycation endproducts (AGEs) [51]. These products reduce nicotinamide adenine dinucleotide phosphate (NADPH) and antioxidant production, which protect the cells from ROS. The cellular damage leads to increased leukocyte infiltrate and an exacerbation of the inflammatory response as well as an increase in ECM breakdown.

25.4.3 Pressure Ulcers

Pressure ulcers occur over bony prominences such as the heels, ankles and toes and are caused by pressure on the soft tissue, which restricts local blood flow. This is also accompanied by shearing forces, which together results in an increase in hypoxia and tissue necrosis. Pressure ulcers are prevalent in patients with reduced mobility, neuropathy or in conditions with compromised blood flow such as venous and arterial ulcers [46]. This type of wound has elevated levels of MMP-1, MMP-2, MMP-8 and MMP-9 and decreased levels of TIMPs due to breakdown from neutrophil elastase. This imbalance of proteinases and their inhibitors leads to an increase in ECM breakdown and incomplete healing.

25.4.4 Arterial and Venous Ulcers

Venous ulcers occur due to venous incompetence in the deep and/or superficial veins, leading to high pressures within these vessels. This increase in luminal pressure results in the vessels becoming leaky. ECM proteins, such as fibrinogen, build up around the static blood, forming a build-up of fibrin around the vessel. This build-up prevents optimal exchange of nutrients and oxygen to the surrounding cells, leading to necrosis. Leukocytes are able to leave the vessels and release ROS into the local microenvironment resulting in a breakdown in the local ECM which can contribute to the formation of the chronic ulcers.

Causes of arterial ulcers include atherosclerosis, thromboangiitis obliterans, vasculitis, pyoderma gangrenosum, thalassaemia, sickle cell anaemia, and embolism.

While venous ulcers generally arise between the knee and the ankle, arterial ulcers most commonly occur distally such as at the tip of a toe. Unlike venous ulcers, which can often be treated successfully with compression of the limb, arterial ulcers require revascularisation of the tissue [52].

25.5 Wound Infection

Chronic wounds often exhibit persistent infection once the ulcer has developed, which promotes chronicity, morbidity and potentially, mortality [46]. Some of the most common bacteria that infect wounds and are known to delay the wound healing process when present in excess of 10^5 per gram of wound tissue, include; *Staphylococcus aureus, Pseudomonas aeruginosa* and β-haemolytic *Streptococcus* [45]. Infection may be clinically obvious, but biofilm formation can occur in the absence of these signs. Optimal treatment ideally should occur before the onset of clinical infection. If this opportunity is missed (Fig. 25.4), it becomes much more difficult to treat. The problems associated with severe infection are exacerbated in patients with diabetic foot ulcers, of whom up to 50% will require local or major amputation if this level of infection is reached [50].

Within infected wounds there is an increase in inflammatory infiltrate due to activation of TLRs by infectious agents such as bacteria, viruses and fungi. In an infected wound, there is also the production of ROS and proteases from bacteria, which again break down the ECM and initiate an immune response. Therefore, the exacerbation of the inflammatory reaction is two-fold from both the host and the invading bacteria. In a chronic infection the immune cells infiltrating the wound are unable to sufficiently clear the infection and debris from the wound, and there is a

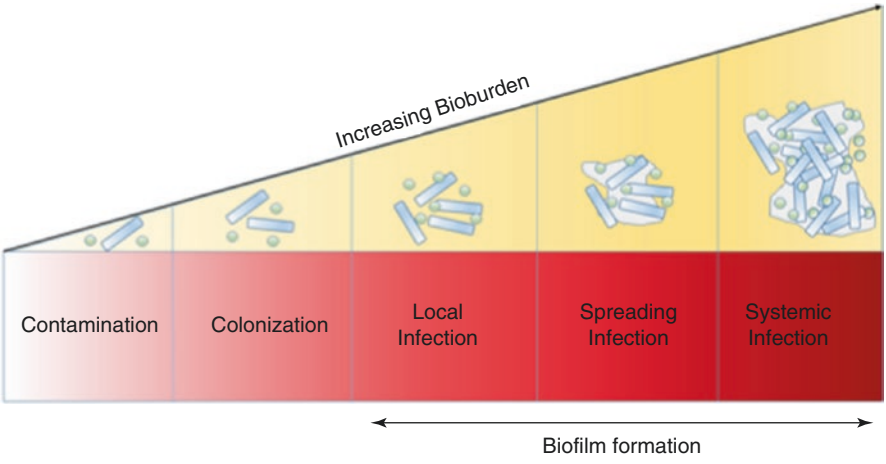

Fig. 25.4 Increasing bioburden seen in diabetic foot ulcers

continuing escalation of the inflammatory response and breakdown of the ECM, which prevents the resolution of the inflammatory phase. Interestingly, it is still unclear whether these changes are due to the infection or because of the chronicity of the wound and, therefore, the full influence of infection on the wound healing process still remains unclear.

25.5.1 Biofilms

Microorganisms in wounds can form polymicrobial communities, which encase themselves in polysaccharide and lipid envelopes, called biofilms. These envelopes enable them to mask themselves from immune surveillance [46]. Biofilms are resistant to penetration by large protein antibodies, antibiotics, silver ions and even neutralisation of highly oxidising solutions such as bleach. This makes the bacteria more resistant to immunological responses, antibiotics, antiseptics and chemical treatments [51]. Due to these factors, biofilms can lead to a range of pathologies including peridontal disease, prosthetic/graft infections and osteomyelitis.

Biofilms are more than just an envelope protecting the bacteria inside from host immune attack. They are highly organised structures, more akin to a microcolony, with channels for nutrient transfer and exchange of signalling molecules for communication between bacteria. There can be a range of pH and oxygen concentrations throughout the biofilm, which make it an ideal environment to host a wider range of bacteria, with differing metabolic states, including both aerobic and anaerobic bacteria [50]. The variations in the microenvironment also affects the bacteria directly. For example, aerobic bacteria which are found in low oxygen areas of the biofilm become dormant and are called “persister cells”. The presence of a biofilm can severely impede wound re-epithelialisation but it also provides a constant stimulus to the immune system, which promotes an excessive influx of immune cells in an attempt to remove the biofilm. This floods the wound with further ROS and proteases, which act to breakdown the ECM and degrade the wound further. The presence of the biofilm also leads to higher rates of chronic, recurring infection. Identification of biofilm formation, particularly in its early stages is difficult due to a lack of routine diagnostic tests. However, microscopy can be used to detect biofilm formation. Interestingly, a recent study by Johani et al. investigated biofilm formation in 65 DFU patients, using in situ hybridization and scanning electron microscopy, and found that all 65 had biofilm formation present [53].

25.5.2 The Infection Challenge

Despite recent advances in the treatment of infection, the role that bacteria play in the wound environment, and how this can lead to complications, is still relatively unknown. There are still questions around whether bacterial bioburden alone is

responsible for poor outcomes in healing. There are some suggestions that the interaction between different bacteria is more important than the physical bioburden, whilst others cite the presence of anaerobic bacteria as one of the main causes of poor healing outcomes. This last point has been difficult to prove as there has been difficulty in identifying all the bacteria present in a wound. The recent gold standard had been based on isolating and growing up the bacteria in culture. Unfortunately, this has only been able to identify bacteria that grow under specific culture conditions, which are usually high nutrient agar and aerobic conditions. Interestingly, these techniques miss those bacteria that thrive under anaerobic conditions, which are thought to be problematic for wounds and delay healing times [54]. Recent advances in gene technologies have allowed the sequencing of the 16S ribosomal RNA region of bacteria which is a much more powerful technique for the identification of specific strains of bacteria, when compared to growth in culture. This technique also allows the analysis of microbial load, microbial diversity and the presence of pathogens, which provides a much fuller picture of wound infection. However, the technique is limited as it does not differentiate between live and dead bacteria and it does not distinguish how the pathogens are interacting [54].

25.6 Conclusion

Whilst our understanding of the cellular process of wound healing has improved in recent times there is much still to be learnt, in particular with respect to chronic wounds. There remains an unmet need for the treatment of these non-healing wounds, which in the worst cases can lead to amputation and death. They present a significant burden to Health Services in terms of cost and treatment times so much so that the Association for the Advancement of Wound Care has referred to chronic wounds as “the most important health problem you’ve never heard of”. A greater understanding of the wound healing process is therefore still a necessity for adequate treatments to be produced.

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Chapter 26
Pathophysiology and Principles of Management of the Diabetic Foot

Guilherme Pena, David G. Armstrong, Joseph L. Mills, and Robert Fitridge

Key Learning Points

- Diabetic foot complications are the most common cause of "non-traumatic" lower limb amputation. Eighty five percent of these amputations are preceded by foot ulceration.
- Diabetic neuropathy is the most prevalent chronic complication of diabetes, affecting at least half of all diabetic patients during their lifetime. The pathogenesis of diabetic neuropathy is complex, multifactorial and not fully understood. Metabolic abnormalities that are implicated in the pathogenesis of diabetic neuropathy including non-enzymatic glycosylation of neural structures, malfunction of polyol metabolism, activation of the hexosamine pathway and protein kinase C (PKC) isoforms.
- Approximately 50% of patients with a diabetic foot ulcer have coexisting PAD. PAD in diabetes tends to occur more distally than smoking-related PAD and is particularly common below the knee. These atherosclerotic lesions tend to be multilevel with a high prevalence of long occlusions.
- Conventional methods of assessing tissue perfusion in the peripheral circulation are frequently unreliable in patients with diabetes, and therefore it is challenging to determine the perfusion deficit in patients with diabetic foot ulceration.

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- WIfI classification should be used for limb staging in the diabetic foot and is based on grading Wound, Ischaemia and foot Infection on a scale from 0 to 3. WIfI can be used to assess the risk of major limb amputation at 1 year and also the need for revascularisation.
- The principles of management of diabetic patients with foot ulcers include offloading, wound management, management of infection, assessment of perfusion and revascularisation if required.
- Negative pressure wound therapy should be considered in patients with diabetes and a post-operative (surgical) wound on the foot.
- Infection severity guides the choice of the empiric antibiotic regimen and its route of administration. The presence of osteomyelitis has important diagnostic, therapeutic and prognostic implications. The current IWGDF guidelines recommend treatment of diabetic foot osteomyelitis with antibiotic therapy for no longer than 6 weeks.
- Assessment of perfusion in the diabetic foot can be challenging. The level of perfusion required to heal a foot ulcer depends on multiple factors such as ulcer size and location, presence/extent of gangrene and infection. The Global Vascular Guidelines on the management of chronic limb-threatening ischaemia recommends an evidence-based approach to diabetic patients with foot ulceration.
- Charcot neuroarthropathy is a serious but frequently missed condition in people with diabetic neuropathy. The hallmark of this condition is a warm, swollen and erythematous foot which can easily be misinterpreted as acute infection, gout or osteomyelitis. Early treatment of Charcot requires immobilization and non-weight-bearing in a cast until the acute inflammatory process subsides.

26.1 Introduction

Diabetes is a major public health challenge worldwide, which is associated with a variety of complications including cardiovascular, kidney, eye and foot disease. It is an important cause of mortality, morbidity, cost (to health systems and the patient) and disability worldwide. The number of adults living with diabetes worldwide has quadrupled over the last 35 years and will continue to rise [1]. In 2013, approximately 382 million people had diabetes and this number is expected to rise to 592 million by 2035 [2].

Diabetic foot may be defined as infection, ulceration or destruction of tissues of the foot associated with neuropathy and/or peripheral artery disease in the lower extremity of people with diabetes [3]. It is estimated that patients with diabetes have a 34% lifetime risk of developing a foot ulcer with more than 50% of these ulcers becoming infected and many of those requiring hospitalisation [4, 5]. The cost of care for diabetic patients with a lower extremity ulcer is a major economic burden for both society and individual patients [6–10]. These costs are significantly increased when ulcers became infected or when patients needed prolonged inpatient treatment or amputation [11].

Diabetic foot complications are the most common cause of non-traumatic lower limb amputation internationally [5, 12]. A history of foot ulcer is significantly associated with negative outcomes. The risk of death at 5 years for a patient with a diabetic foot ulcer is 2.5 times as high as the risk for a patient with diabetes who does not have a foot ulcer [13]. Of all amputations in diabetic patients, 85% are preceded by foot ulceration which subsequently deteriorates to gangrene or infection [14, 15]. Mortality after diabetes-related amputation is notoriously high; 70% at 5 years for all patients with diabetes and 74% at 2 years for those undergoing dialysis [5, 16].

More than three quarters of patients with diabetic foot ulcers can achieve primary healing within 1 year [5, 17, 18]. Unfortunately, patients with a DFU history have a high risk of re-ulceration [17]. Approximately 40% of patients have a recurrence within 1 year of the ulcer healing, almost 60% within 3 years, and 65% within 5 years [5]. Thus, it may be more useful to think of patients who have achieved wound closure as being in remission rather than being healed [5].

Foot ulcers in people with DM have a serious impact on health-related quality of life, particularly with respect to physical functioning and role limitations due to physical and emotional issues [19, 20]. They also represent a major use of health resources, incurring costs not only for dressings, but also staff costs, tests and investigations, antibiotics and specialist footwear.

26.2 Pathophysiology of the Diabetic Foot

The pathogenesis of foot ulceration is complex and requires an appreciation of the role of several contributory factors, including peripheral neuropathy, peripheral arterial disease (PAD), biomechanical problems including limited joint mobility and susceptibility to infection.

26.2.1 Neuropathy and Biomechanical Abnormalities

Diabetic neuropathy is one of the most prevalent chronic complications of diabetes, affecting at least half of all diabetic patients during their lifetime [21]. It creates a substantial burden on both the affected patients and the healthcare system [22, 23].

The pathogenesis of diabetic neuropathy is complex, multifactorial and not fully understood. There are several metabolic abnormalities that are implicated in the pathogenesis of diabetic neuropathy including non-enzymatic glycosylation of neural structures, malfunction of polyol metabolism and activation of the hexosamine pathway and protein kinase C (PKC) isoforms. Collectively these metabolic abnormalities cause an imbalance in the mitochondrial redox state and lead to excess formation of mitochondrial and cytosolic reactive oxygen species (ROS) promoting neuronal damage [21, 24–26]. The polyol pathway is probably the most studied of these metabolic abnormalities. Excess glucose is converted to sorbitol by aldose

reductase and results in osmotic imbalance in the cell. This activates a compensatory efflux of myoinositol and taurine. Myoinositol is an essential component of sodium/potassium (Na/K) ATPase and its loss impairs normal nerve physiology. The increase in aldose reductase activity, which also depletes cellular stores of NADPH, is needed for nitric oxide generation and regeneration of the essential antioxidant glutathione. This results in the generation of cytoplasmic ROS and consequent cellular dysfunction [21].

Another factor thought to contribute to the pathogenesis of diabetic neuropathy is related to impaired insulin signalling. Although insulin is not involved in glucose uptake into neurons, it has been demonstrated that it has important neurotrophic effects promoting neuronal growth and survival. Reduction of this neurotrophic signalling due to insulin deficiency in Type 1 diabetes promotes cellular injury.

The state of relative insulin deficiency (due to peripheral insulin resistance), could in part also be contributing to the aetiology of DN in Type 2 diabetes [22, 27]. Recently there has been interest in understanding the bioenergetic profile of the peripheral nervous system, especially the interaction between axons and Schwann cells (SC) and their association with neuropathy. There is growing evidence the SCs are critical sensors of axon activity and provide energy for axon activity [28, 29]. It is speculated that in diabetes SCs not only lose their ability to provide energy to myelinated and unmyelinated axons but also transfer toxic lipid species to the axons they contact [22].

The relative importance of the multiple pathways implicated in the pathogenesis of diabetic neuropathy varies with cell type, disease profile and time. As a likely consequence of differences in the underlying mechanisms, tight glucose control can reduce neuropathy in type 1 diabetic patients but appears not to be as efficacious in type 2 patients [30, 31].

Diabetic neuropathy affects the proximal and distal, somatic and autonomic nerves. Most relevant to the pathophysiology of diabetic foot ulcers is sensory neuropathy with loss of protective sensation (LOPS), motor neuropathy resulting in foot deformity and autonomic neuropathy associated with sudomotor dysfunction contributing to dry skin which is more prone to cracking and wound development.

Peripheral neuropathy must be severe before leading to LOPS and when present, increases vulnerability to physical and thermal trauma [4]. With an inability to detect the pain signals that warn of impending tissue trauma and impaired ability to distribute forces that are applied to the plantar surface, the insensate foot is exposed to increased pressures that hasten tissue damage leading to ulceration [32].

Motor neuropathy is believed to lead to weakness preferentially affecting the intrinsic muscles of the foot, thus causing imbalance between flexors and extensors of the toes (intrinsic minus foot). Atrophy of the small muscles responsible for metatarsophalangeal plantar flexion is thought to lead to the development of hammer toes, claw toes, prominent metatarsal heads, and pes cavus. These structural deformities and restriction of joint mobility are commonly associated with areas of increased peak plantar pressures [32–34].

Assessment of gait and dynamic plantar pressures are valuable to help understand the biomechanical abnormalities that contribute to the formation and

persistence of diabetic foot ulceration. Recent studies have shown that diabetic patients with foot ulcers have distinguishing gait parameters including reduced range of movement of joints; higher vertical and horizontal ground reaction forces and slower walking speeds with smaller step lengths [35] and higher plantar pressures than diabetic controls with no ulceration [36]. This provides supportive evidence of the importance of pressure-offloading in the management of diabetic foot ulcers.

26.2.2 Peripheral Artery Disease (PAD)

PAD is common in patients with diabetes [37–39], and approximately 50% of patients with a diabetic foot ulcer have coexisting PAD [18, 40, 41]. PAD in diabetes occurs predominantly in the infra-inguinal vasculature and is dissimilar to PAD in patients without diabetes in its characteristics, treatment and outcomes. The atherosclerotic lesions tend to be multilevel and particularly severe in the below knee vessels (popliteal and tibial arteries), with a high prevalence of long occlusions [38, 42]. The predilection for multiple crural vessel involvement combined with extensive calf arterial calcification increases the technical challenges associated with revascularisation using either open bypass or endovascular techniques [41]. Furthermore, in patients with diabetes, a similar degree of anatomical arterial disease can result in a more severe perfusion deficit because of paucity of collateral vessels as well as the influence of physiological factors associated with diabetes, such as arteriolar shunting [43]. The presence of PAD amongst patients with foot ulceration is associated with adverse outcomes such as poor wound healing and higher rates of lower extremity amputation [17].

Assessing foot perfusion is particularly challenging in patients with diabetes. This population commonly lacks typical symptoms of vascular insufficiency such as claudication or rest pain [44]. However, assessment of perfusion is an essential step in the management of patients with diabetic foot ulceration, in order to estimate the risk of amputation, likelihood of wound healing without vascular intervention, and likely benefit of revascularisation.

Foot perfusion needs to be measured and then assessed in terms of global and regional perfusion deficits rather than as an absolute measurement. Quantification of blood flow required to heal a foot lesion depends on several factors including the presence of infection, extent of tissue loss, abnormal mechanical loading of the foot during walking, and co-morbidities such as renal failure [45–47]. Conventional methods of assessing tissue perfusion in the peripheral circulation are frequently unreliable in patients with diabetes and it may therefore be difficult to determine the perfusion deficit in patients with foot ulceration (see below).

In summary, the combination of foot deformity, loss of protective sensation, dry skin, inadequate off-loading, and repetitive minor trauma can lead to tissue damage and ulceration. Once an ulcer has formed, healing may be delayed or not occur, particularly if significant ischaemia is present.

26.3 Clinical Assessment of the Diabetic Foot

26.3.1 History and Physical Examination

A thorough history and physical examination of each patient presenting with diabetic foot pathology should include a history of duration of diabetes and adequacy of diabetic control, significant medical co-morbidities and a history of pedal wounds, prior amputations, and lower extremity vascular interventions [48]. A history of foot tingling, burning and/or numbness, can help to identify those patients with neuropathy. A history of claudication or other walking impairment, ischemic rest pain, and nonhealing wounds are highly suggestive of periphery artery disease.

Physical examination should follow a systematic approach and the patient should be examined including both feet. Foot deformities (e.g., claw toes, hammer toes), bony prominences and limited joint mobility should be noted as they contribute to a high risk of ulceration. It is important to examine between all of the toes. Footwear must be inspected for appropriateness. Any wounds must be carefully assessed, and accurate documentation made regarding wound location, size, depth, characteristic of the wound base and margins, exudate (amount and type) and the presence and severity of infection.

The possibility of peripheral neuropathy and vascular insufficiency must be assessed. Sensory examination using the 10 g (5.07 Semmes-Weinstein) monofilament and/or tuning fork (128 Hz) is important for the assessment of pressure perception, vibration perception and tactile sensation. Lower extremity vascular examination should include palpation of lower extremity pulses (i.e., femoral, popliteal, dorsalis pedis, and posterior tibial), auscultation for femoral bruits, and inspection of the legs and feet. Pulse palpation is necessary but not sufficient to assess perfusion.

26.3.2 Assessment of Foot Perfusion

26.3.2.1 Ankle-Brachial Index (ABI)

ABI is the ratio of systolic pressure at the ankle to that in the arm; if the arm pressures are disparate, the higher of the two should be used as the denominator. It is a quick, simple and non-invasive test used to document PAD [49]. In addition to reflecting the presence of PAD, the ABI also is an indicator of generalized atherosclerosis [50, 51]. Patients with ABI $\leq$0.90 are diagnosed with PAD. Diabetic patients with an ABI above 0.9 may possibly have PAD and should undergo further assessment if clinical suspicion is present. Values >1.40 are abnormal and indicate that the arteries are calcified and not able to be compressed, which is more common among individuals with diabetes mellitus and/or advanced chronic kidney disease [52, 53]. In the setting of definite or suspected incompressible ABI values, additional testing should be undertaken. Individuals with diabetes frequently have

calcium deposition in the arterial media; a condition known as medial arterial calcification (MAC), which most commonly affect the calf arteries [54]. This condition causes arterial wall stiffness, which results in vessels that are more difficult to occlude in the calf and ankle. The consequence is an artefactually high ankle pressure and ABI [55, 56]. This should also be suspected even with near normal pressures if the Doppler arterial waveforms are blunted.

26.3.2.2 Toe Pressure and Toe:Brachial Index

An alternative to ABI is to measure toe pressures (TPs) and the toe:brachial pressure index (TBI). These may be more useful measures of perfusion in the diabetic patient because MAC frequently spares the pedal arteries [57, 58]. Toe pressures are obtained by placing a cuff around the base of the toe, ideally the hallux, with a digital flow sensor beyond the cuff. Toe pressures may be measured by photoplethysmography (detecting pulsatile flow and producing a pulse wave curve) or laser Doppler (detecting changes in wavelength when the laser encounters red blood cells) [59]. TBI is the ratio between toe pressure and the highest of the two brachial pressures. A TBI $\geq$0.75 is generally considered within the normal range, whilst a TBI <0.25 is consistent with severe PAD [60, 61]. An absolute systolic toe pressure of 30 mm Hg or greater has been correlated with a significantly higher probability of foot ulcer healing in diabetic patients [45].

26.3.2.3 Doppler Waveform Assessment

Audio and visual analyses of Doppler waveforms are useful tools for assessment of the presence of PAD. A normal Doppler waveform in the lower extremities has a characteristic triphasic pattern, composed primarily of a systolic forward-flow phase, a late-systolic reverse flow phase, and a smaller, diastolic forward-flow phase [21]. Detection of a triphasic pedal Doppler arterial waveform with a hand-held Doppler provides strong evidence for the absence of PAD [62]. The presence of monophasic flow with an isolated forward systolic waveform with diminished amplitude is usually associated with significant PAD.

26.3.2.4 Transcutaneous Oxygen Pressure and Skin Perfusion Pressure

Transcutaneous oxygen tension ($TcPO_2$) measures the transfer of oxygen molecules to the skin surface, allowing objective quantification of the degree of limb perfusion [63]. $TcPO_2$ maps the actual oxygen supply available for the skin tissue cells and it also responds to microcirculatory events. The measured PO_2 in the dermis is displayed in millimetres of mercury, with a normal healthy value in the foot for an individual breathing normobaric air being >50 mmHg [63, 64]. Skin perfusion pressure (SPP) is the blood pressure that is required to restore flow to capillaries following controlled occlusion and subsequent flow return.

$TcPO_2$ and SPP values can be used to predict the presence of vascular disease and the likely success of healing an ulcer and major/minor amputations with or without revascularization. $TcPO_2$ measurements with an oxygen challenge are also utilized as an indicator of whether or not hyperbaric therapy will be likely to be beneficial in wound healing [60]. $TcPO_2$ levels of less than 25 mmHg are indicative of severely reduced blood flow to the area of evaluation and strongly suggest that revascularization will be required to achieve healing. Patients with a $TcPO_2 \geq 25$ mmHg and SPP ≥ 40 mmHg have a higher likelihood of wound healing compared to wounds with evidence of a more severe perfusion deficit [45, 65, 66].

26.3.3 Diagnosis of Osteomyelitis

The accurate diagnosis of foot sepsis and in particular osteomyelitis (OM) in the diabetic foot is important for planning adequate treatment and affects prognosis. The prevalence of bone involvement is variable, depending on the context. It is found in approximately 60% of patients hospitalized for a DFI and 10–20% of apparently less severe infections presenting in an outpatient setting [67, 68]. The differentiation of bone infection from soft tissue infection may be challenging, as can be the differentiation of bone infection from non-infectious bone disorders such as acute gout and acute Charcot neuroarthropathy. To further complicate foot assessment, these conditions may co-exist with OM or foot sepsis, particularly when an adjacent ulcer is present.

A definitive diagnosis of OM ideally requires both the presence of histological findings consistent with bone infection (acute or chronic inflammatory cells, necrosis) and the isolation of bacteria from an aseptically obtained bone sample [69]. However, bone biopsy is frequently not able to be performed, most commonly due to commencement of antibiotics prior to review of the foot. The clinician may need to rely on clinical, laboratory and imaging findings for diagnosis. The presence of exposed bone, bone palpable with a probe ("probe to bone test"); erythematous and indurated ("sausage") toe, especially with an ulcer, an ulcer that is deep, failure to heal despite offloading or wound location over a bony prominence and the presence of a soft tissue sinus are highly suggestive of OM.

The probe to bone test (PBT) is a useful clinical diagnostic tool for diagnosing osteomyelitis. PBT is performed by using a sterile probe to gently explore the wound. If the probe encounters a hard or gritty substance that is presumed to be bone or joint space, the test is considered positive and this greatly increases the likelihood of osteomyelitis in a high-risk population where there is a high pre-test probability to diagnose OM [67]. The test also useful to rule out OM, as a negative probe-to-bone test in a patient at low risk strongly argues against the diagnosis of osteomyelitis [67, 68]. Occasionally, a viscous exudate (joint fluid) may be found discharging from a sinus, supporting a diagnosis of joint infection.

Imaging diagnosis of osteomyelitis usually begins with plain radiographs which provide an inexpensive, widely available tool for initial evaluation and are often adequate for imaging the foot in patients with suspected diabetic foot osteomyelitis

(DFO). Radiographic signs of osteomyelitis include decreased bone density, lytic changes and cortical erosion, trabecular destruction, bone necrosis, Brodie abscess, sclerosis, and periosteal reaction. X-ray has low sensitivity especially in the early stages of osteomyelitis as radiological changes may be delayed for up to 4 weeks following infection. Comparison with previous films or repeat radiographs at 2–6 weeks may be useful. If these studies are negative and clinical suspicion remains high, the patient will need additional imaging. MRI is the preferred advanced imaging modality for diagnosing osteomyelitis due to good sensitivity and specificity and good spatial resolution for assessment of both soft tissues and bone. In situations where MRI is contraindicated or unavailable, a nuclear medicine scan such as leukocyte scan preferably combined with a bone scan is the best alternative. Other imaging modalities may also be helpful in the diagnosis of OM [69, 70] (Table 26.1).

Table 26.1 Imaging modalities for diagnosis of osteomyelitis [66]

Imaging modality	Advantages	Disadvantages
Plain radiography	Readily available and inexpensive	Low sensitivity in early stages of osteomyelitis
	May be used to monitor response to antibiotic treatment	Specificity is limited by difficulty differentiating infection from Charcot's arthropathy and other pathologies (e.g. gout)
	Can reveal presence of radio-opaque foreign bodies, gas in soft tissues, calcified arteries, fractures or bony abnormalities	
MRI	Preferred advanced imaging modality for diagnosing osteomyelitis	Reduced performance with severe ischaemia
	Does not use radiation	Not all patients are suitable for MRI (e.g. pacemaker)
	Excellent spatial resolution and is very useful for evaluation of bone marrow as well as of soft tissue structures. Good for detection of sinus tracts, deep tissue necrosis, abscesses and other inflammatory changes	
Nuclear medicine scan	More sensitive than radiographs for detecting osteomyelitis during early stages of the disease	Poor specificity and low resolution of images
	Labelled leucocyte scintigraphy with either indium-111 (^{111}In) or technetium-99 (^{99}mTc), improves specificity	Includes a 24 h waiting period before imaging can begin as well as low resolution of the images
	White blood cell-labelled single-photon emission computed tomography can be combined with computed tomography (^{99}mTc WBC labelled-SPECT/CT) imaging to provide good spatial resolution	Limited availability
PET (PET/CT)	Excellent spatial resolution	Limited availability
	Does not require blood processing	High costs

26.4 Risk Classification/Staging of the Diabetic Foot

Based on a thorough history, physical examination and ABI/toe pressures, each patient should be carefully assessed and assigned to a specific foot risk stage. Limb staging is important to provide risk stratification of patients with respect to disease natural history (risk of amputation, likelihood of wound healing, and likely benefit from revascularisation). Staging also allows meaningful comparison of different treatment strategies. Various classification systems are used to stratify diabetic patients with foot complications in an attempt to predict the outcomes of likelihood of ulcer healing and risk of lower limb amputation and thus help plan treatment strategies.

The Meggit-Wagner wound classification system was previously widely used, and it is based on assessment of ulcer depth and the presence of osteomyelitis or gangrene [71]. The drawback of the Wagner classification system is that it does not specifically address two critically important parameters in diabetic foot: ischaemia and infection [72].

The University of Texas classification grades ulcers based on depth. Each grade is then staged according to the presence of infection, ischaemia or both. However, this classification lacks adequate assessment of infection and ischaemia as they are included only as dichotomised variables [73, 74]. The SINBAD system grades ulcer site, area and depth, the presence of sepsis, arterial disease and neuropathy as dichotomised variables [75]. The IWGDF recommends the use of SINBAD as a primary triage and audit tool for the diabetic foot [66]. SINBAD unfortunately lacks mandatory perfusion assessment.

26.4.1 WIfI Classification

In 2014 the Society for Vascular Surgery proposed a Lower Extremity Threatened Limb Classification System which represents a synthesis of multiple previously published classification schemes that focussed on diabetic foot ulcers and pure ischaemia models. This classification is referred as WIfI and is based on grading each of the three major factors (**W**ound, **I**schaemia and **f**oot **I**nfection) on a scale from 0 to 3, where 0 represents none, 1 mild, 2 moderate, and 3 severe [77].

Wounds are classified from grade 0 through grade 3 based on size, depth, severity, location and anticipated difficulty achieving wound healing (Table 26.2). Advanced gangrene with an unsalvageable foot is classified as WIfI clinical stage 5. Classification of ischaemia is based on ABI, Toe pressure (TP) or transcutaneous oxygen saturation ($TcPO_2$) (Table 26.3), with preference given to toe pressures, especially in patients with diabetes. Diabetic patients may have falsely elevated ABIs due to MAC and in this situation TP or $TcPO_2$ measurements are preferred for assessment of perfusion. Patients with TP < 30 mmHg have severe ischaemia and are likely to require revascularization to achieve wound healing and limb salvage. WIfI incorporates the classification used by the Infectious Diseases Society of America (the "infection" part of the PEDIS classification) to assess severity of infection (Table 26.4).

Table 26.2 WIfI wound grading [77]

Grade	Ulcer	Gangrene
0	No ulcer	No gangrene
	Ischaemic rest pain (requires typical symptoms + ischaemia grade 3); no wound	
1	Small, shallow ulcer(s) on distal leg or foot; no exposed bone, unless limited to distal phalanx	No gangrene
	Minor tissue loss. Salvageable with simple digital amputation (1 or 2 digits) or skin coverage	
2	Deeper ulcer with exposed bone, joint or tendon: generally not involving the heel; shallow heel ulcer, without calcaneal involvement	Gangrenous changes limited to digits
	Major tissue loss salvageable with multiple (≥3) digital amputations or standard transmetatarsal amputation ± skin coverage	
3	Extensive deep ulcer involving forefoot and/or midfoot; deep, full thickness heel ulcer ± calcaneal involvement	Extensive gangrene involving forefoot and/or midfoot; full thickness heel necrosis ± calcaneal involvement
	Extensive tissue loss salvageable only with complex foot reconstruction or non-traditional TMA (Chopart or Lisfranc); flap coverage or complex management needed for large soft tissue defect	

Table 26.3 WIfI Ischaemia grading [76]

Grade	ABI	Ankle systolic pressure (mmHg)	TP, $TcPO_2$ (mmHg)
0	≥0.80	>100	≥60
1	0.6–0.79	70–100	40–59
2	0.4–0.59	50–70	30–39
3	≤0.39	<50	<30

TP Toe pressure, *$TcPO_2$* Transcutaneous oxygen pressure
If TP and ABI measurements result in different grades, TP will be the primary determinant of ischaemia grade

Once the patient has been scored under the three categories, the appropriate spectrum score is then derived to give an overall amputation risk. Spectrum scores deemed low risk, moderate risk and high risk for limb amputation at 1 year are categorized as clinical stage 2, stage 3 and stage 4 disease, respectively, although the definitions of 'low', 'moderate' and 'high' risk are not given.

The three categories (wound, ischemia, and foot infection) with four grades of severity produces a grid with 64 theoretically possible clinical combinations (WIfI classes). A Delphi consensus of the members of the Society of Vascular Surgery Lower Extremity Guidelines Committee assigned a risk category to each of the combinations in regards to risk of limb amputation at 1 year (very low risk, low risk, moderate risk or high risk) and benefit of revascularisation (very low benefit, low benefit, moderate benefit or high benefit) for each of the possible combinations (Table 26.5). Several reported series have been published with analysis of outcomes of patients with threatened limb, including diabetic foot patients, based on WIfI clinical stage validating this model [47, 77–83].

Table 26.4 WIfI infection grading [77]

Grade	Clinical manifestation of infection
0	No symptoms or signs of infection
1	Infection present, as defined by the presence of at least two of the following items: (a) Local swelling or induration (b) Erythema >0.5–≤2 cm around the ulcer (c) Local tenderness or pain (d) Local warmth (e) Purulent discharge (thick, opaque to white, or sanguineous secretion). Local infection involving only skin and the subcutaneous tissue (without involvement of deeper tissues and without systemic signs as described below). Exclude other causes of inflammatory response of the skin (e.g., trauma, gout, acute Charcot neuro-osteoarthropathy, fracture, thrombosis, venous stasis)
2	Local infection (as described above) with erythema >2 cm, or involving structures deeper than skin and subcutaneous tissues (e.g., abscess, osteomyelitis, septic arthritis, fasciitis), and no signs or symptoms of systemic inflammatory response syndrome (SIRS)
3	Local infection (as described above) with the signs of SIRS, as manifested by two or more of the following: (a) Temperature >38 °C or <36 °C (b) Heart rate > 90 beats/min (c) Respiratory rate >20 breaths/min or $PaCO_2$ < 33 mmHg (d) White cell count >12,000 or <4000/mm^3 or 10% immature (band) forms

Table 26.5 WIfI clinical stages [76]

(a) Estimated risk of amputation at 1 year

	Ischaemia 0				Ischaemia 1				Ischaemia 2				Ischaemia 3			
Wound 0	VL	VL	L	M	VL	L	M	H	L	L	M	H	L	M	M	H
Wound 1	VL	VL	L	M	VL	L	M	H	L	M	H	H	M	M	H	H
Wound 2	L	L	M	H	M	M	H	H	M	H	H	H	H	H	H	H
Wound 3	M	M	H	H	H	H	H	H	H	H	H	H	H	H	H	H
	fI 0	fI1	fI2	fI3	fI 0	fI1	fI2	fI3	fI 0	fI1	fI2	fI3	fI 0	fI1	fI2	fI3

VL (Very Low) = Clinical Stage 1

L (Low) = Clinical Stage 2

M (Moderate) = Clinical Stage 3

H (High) = Clinical Stage 4

Unsalvageable limb = Clinical Stage 5

Table 26.5 (continued)

(b). Estimated likelihood of benefit of revascularization (assuming infection can be controlled first)

	Ischaemia 0				Ischaemia 1				Ischaemia 2				Ischaemia 3			
Wound 0	VL	VL	VL	VL	VL	L	L	M	L	L	M	M	M	H	H	H
Wound 1	VL	VL	VL	VL	L	M	M	M	M	H	H	H	H	H	H	H
Wound 2	VL	VL	VL	VL	M	M	H	H	H	H	H	H	H	H	H	H
Wound 3	VL	VL	VL	VL	M	M	M	H	H	H	H	H	H	H	H	H
	fI 0	fI1	fI2	fI3	fI 0	fI1	fI2	fI3	fI 0	fI1	fI2	fI3	fI 0	fI1	fI2	fI3

fI, foot Infection

VL = Very low = Clinical Stage 1

L = Low = Clinical stage 2

M = Moderate = Clinical stage 3

H = High = Clinical stage 4

26.5 Principles of Management of the Diabetic Foot

The goals of treatment of the patient with a diabetic foot ulcer are to achieve wound healing, avoid amputations (particularly major), improve quality of life and prevent ulcer recurrence. In order to achieve these goals, establishment of a multidisciplinary team to manage diabetic foot pathology is considered to be the best practice strategy [14, 84, 85]. This integrated approach acknowledges that no one specialist possesses all the expertise and knowledge to optimally manage the patient. In particular, the management of patients with chronic and complex wounds requires input from a number of healthcare professionals. The multidisciplinary team may include, but is not limited to, podiatrists, vascular surgeons, orthopaedic surgeons, vascular interventionalists, endocrinologists, infectious disease specialists, diabetes educators, wound care nurses, orthotists, radiologists and dieticians.

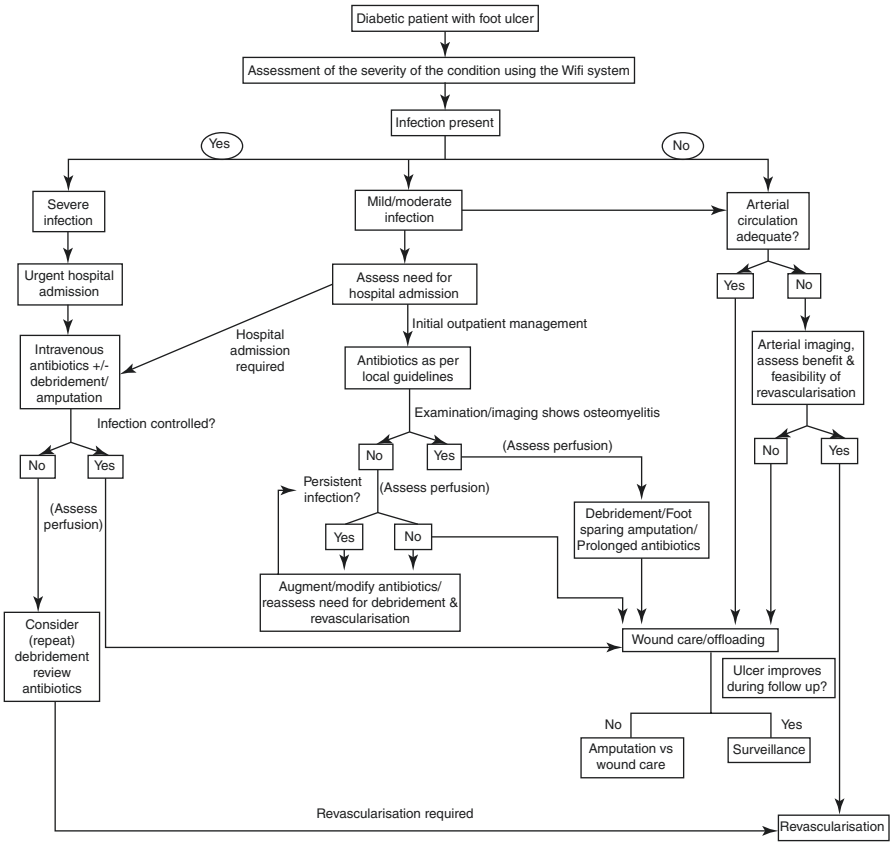

Fig. 26.1 A systematic approach to the assessment and management of the diabetic foot

At initial clinical assessment of a patient with a diabetic foot, a decision needs to be made whether the patient is suitable for outpatient management or if admission to hospital is required for intravenous antibiotics, surgical debridement and/or revascularisation. The principles of management of diabetic patients with foot ulcers include offloading, wound management, management of infection and revascularisation if required. Figure 26.1 summarizes an approach to patients with diabetic foot ulcers.

26.5.1 Offloading

People with diabetes should wear appropriate footwear that fits, protects and accommodates the shape of their feet in order to prevent ulceration. For patients with a plantar diabetic foot ulcer, prescription of appropriate offloading devices to heal the

ulcer is recommended. There are numerous products available to assist in redistributing pressure over a larger weight bearing area thus providing offloading. The gold standard for treatment of a heel or neuropathic plantar forefoot ulcer without ischemia or uncontrolled infection is a non-removable knee-high device. This could be a total contact cast (TCC) or removable cast walker made irremovable [86, 87]. There is strong evidence that pressure-relief devices that cannot be removed are associated with faster healing of ulcers than are removable devices. However, in situations where frequent wound care or wound review is required (such as ischaemic or heavily exudative ulcers) or if active infection is present, non-removable offloading devices are not suitable and a removable walker needs to be considered [66, 86, 88].

When knee-high devices are contraindicated or not tolerated by people with diabetic foot ulcers, other offloading devices such as forefoot offloading shoe, cast shoe, or custom-made temporary shoe should be considered. When removable offloading devices are prescribed, potential issues with patient adherence must be anticipated and strategies put in place to improve patient compliance [5].

Elective foot surgery such as Achilles tendon lengthening, digital flexor tenotomy and joint arthroplasty may be considered for recalcitrant forefoot plantar ulcers and prevention of ulcer recurrence in appropriate high risk patients [86]. Once a plantar ulcer is healed, the use of footwear that has a demonstrated plantar pressure-relieving effect during walking is indicated to reduce the risk of re-ulceration. Offloading for non-plantar ulcer depends on the type and location of the wound and various modalities can be considered, including shoe modifications, temporary footwear, toe spacers and orthoses [86].

26.5.2 Wound Management

The basic principles of wound management include regular cleaning with sterile water or saline, debridement if necessary to remove debris, slough, necrotic and infected matter from the wound surface, and dressing with a sterile, inert dressing with the aim of controlling exudate and maintaining a warm, moist environment to promote healing [66, 89, 90]. Debridement may be undertaken using physical (e.g. surgical, sharp or hydro-debridement), biological (larvae), autolytic (hydrogels) or biochemical (enzymes) methods.

Depending upon the severity of the foot wound, urgent surgical debridement may be required to drain necrotic tissue and pus. This also permits adequate assessment of the extent of infection and enables deep specimen(s) to be obtained for culture to determine the true causative organisms and their antibiotic sensitivities. As a general rule all necrotic and infected tissue should be removed, ensuring that no bone is left exposed, while leaving part of the wound open to allow drainage.

Bone resection and minor amputation is often necessary when there is osteomyelitis, extensive soft tissue necrosis and/or deep abscess present. Minor amputation may consist of simple removal of a toe, ray amputation (toe and metatarsal), or

transmetatarsal amputation. Once infection is under control and the necessary surgical drainage/debridement has been performed, attention to the long-term function of the foot is a key issue. Patients who have undergone previous surgeries or amputations may have biomechanical consequences that can potentially result in an unstable foot or lead to a foot prone to re-ulceration [70].

Wound care is an essential aspect in the management of diabetic foot ulcers and post-operative wounds. There are several types of dressings available from basic wound contact dressings to more advanced gels, films, and antimicrobial dressings. Dressings aim to control exudate, maintain a warm and moist environment to promote healing, control the growth of microorganisms and protect the wound [91]. In general, selection of dressings should principally be made on the basis of exudate control, comfort and cost [90, 92–94]. However, in non-infected neuro-ischaemic diabetic foot ulcers that are difficult to heal despite best standard care, sucrose-octasulfate-impregnated dressings should be considered [95].

Negative pressure wound therapy (NPWT) assists in wound management by physical and biological responses that influence wound healing [96, 97]. An RCT demonstrated benefit of NPWT compared to standard care in both the time to healing and the proportion of wounds healed for complex post-operative diabetic foot wounds [98, 99].

Hyperbaric oxygen therapy has theoretical benefit as an adjunct in wound healing in the diabetic foot. Treatment involves placing the patient in a compression chamber, increasing the environmental pressure within the chamber, and administering 100% oxygen for respiration. While HBOT might be of benefit in nonhealing diabetic ulcers there is insufficient evidence of a benefit in long term follow up and it does not appear to reduce minor amputation rate in people with foot ulcers due to diabetes [100, 101]. The International Working Group in Diabetic Foot recommend that HBOT might be considered as adjunct therapy, however stated that further research is necessary to determine which patient group might benefit most from this treatment and also to establish cost-effectiveness [66, 90].

26.5.3 Management of Infection

While most DFIs are relatively superficial at presentation, microorganisms can spread contiguously to subcutaneous tissues, including fascia, tendons, muscle, joints and bone, and infection can become limb- or life-threatening [70]. The system proposed by the Infectious Diseases Society of America (the “infection” part of the PEDIS classification), which is also incorporated into the WIfI classification, categorises the severity of infection in the diabetic foot. Infection severity guides the choice of the empiric antibiotic regimen and its route of administration and helps the clinician to determine the need for hospitalisation, the requirement and timing for surgery and influence the likelihood of amputation. Table 26.6 describes features

Table 26.6 Characteristics suggesting a more serious diabetic foot infection and potential need for hospital admission (adapted from IWGDF—Guidelines (2019) [66]

Wound specific	
Wound	Penetrates to subcutaneous tissues (e.g., fascia, tendon, muscle, joint or bone)
Cellulitis	Extensive, >2 cm distant from ulceration or rapidly progressive
Local signs	Severe inflammation or induration, crepitus, bullae, discoloration, necrosis, or gangrene, ecchymoses or petechiae, new anaesthesia.
General	
Presentation	Acute onset/worsening or rapidly progressive
Systemic signs	Leukocytosis, very high C-reactive protein or erythrocyte sedimentation rate, severe/worsening hyperglycaemia, acidosis, deterioration of renal function, electrolyte abnormalities
Complicating features	Presence of a foreign body, puncture wound, deep abscess, arterial or venous insufficiency, lymphoedema, immunosuppressive illness or treatment, lack of home support, and unable to comply with the required outpatient treatment regimen
Current treatment	Progression while on apparently appropriate antibiotic and appropriate supportive therapy

associated with more serious foot infection and potential need for admission to hospital. Mild infections are usually treated with oral antibiotics while limb and life-threatening infections requires intravenous antibiotic therapy and may need surgical debridement.

The empirical antibiotic regimen should be based on the anticipated spectrum of infecting organisms and local protocol. S. aureus and beta-haemolytic streptococci are widely recognized as pathogens in acute DFIs. In chronic wounds, especially in the setting of prior antimicrobial therapy, infections are more frequently polymicrobial and the causative pathogens are more diverse, often including aerobic gram-negative bacilli and obligate anaerobic bacteria [102]. A major problem in treating DFIs has been the increased rate of isolation of antibiotic resistant pathogens, particularly methicillin-resistant S. aureus (MRSA). Therefore, depending upon patient risk and local prevalence of MRSA an antimicrobial agent active against these bacteria should be added to the empirical regimen.

It is of paramount importance to collect and process specimens for culture appropriately. Superficial wound swabs are easy to obtain, however they frequently grow contaminants and are less likely to yield the true pathogens. Specimens should be obtained only after cleansing and debriding the wound and ideally should include tissue obtained by curettage or biopsy [102].

In some chronic infections, such as osteomyelitis, if deemed safe, it is advisable to discontinue antibiotic therapy for at least a few days before obtaining deep cultures or bone biopsies because prior antibiotic therapy can cause false-negative results.

As stated previously, surgery remains a cornerstone of treatment for many deep infections. Bone resection and minor amputations are often required when there is osteomyelitis and/or extensive soft tissue infection present. A specimen

Table 26.7 Suggested route, setting, and duration of antibiotic therapy, by clinical presentation

Site of infection, by severity or extent	Route of administration	Setting	Duration of therapy
Soft tissue only			
Mild	Oral	Outpatient	1–2 weeks; may extend up to 4 weeks if slow to resolve
Moderate	Oral (or initial parenteral)	Outpatient / inpatient	1–3 weeks
Severe	Initial parenteral, switch to oral when possible	Inpatient, then outpatient	3–4 weeks[a]
Bone or joint			
No residual infected tissue (e.g., postamputation)	Parenteral or oral	Inpatient, then outpatient	2–5 days
Residual infected soft tissue (but not bone)	Parenteral or oral	Inpatient, then outpatient	1–3 weeks
Residual infected (but viable) bone	Initial parenteral, then consider oral switch	Inpatient, then outpatient	Up to 6 weeks
No surgery, or residual dead bone postoperatively	Initial parenteral, then consider oral switch	Inpatient, then outpatient	Up to 6 weeks

From 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections [114] and the 2019 International Working Group Guidelines for the Diabetic Foot [66]

[a]Re-evaluate the need for further tests, alternate antibiotics or alternate management strategy if not settling within 3–4 weeks

of proximal bone should be obtained at the time of surgery for analysis by culture and histopathology. The wound should be washed, and a clean instrument used when collecting these specimens to reduce the risk of contamination. These results have clinical implications as patients with residual bone infection require a longer duration of postoperative antibiotics and may carry an increased risk for re-amputation [103].

Table 26.7 summarises the Infectious Diseases Society of America and IWGDF recommendations [66] for the duration of antibiotic treatment according to the clinical presentation. Management of osteomyelitis has been an area of controversy particularly in relation to selection of patients for non-operative management and duration of antibiotic treatment if surgery is not performed.

Osteomyelitis has traditionally been treated with prolonged (≥3 months) course of antibiotics. However more recent trials have demonstrated that shorter period of treatment (6 weeks) may be as effective [104–106] and associated with significantly fewer adverse effects related to antibiotics such as diarrhoea [105]. As a result, the most recent IWGDF guidelines in 2019 recommend 6 weeks of antibiotic therapy for patients who do not undergo resection of infected bone [66]. A key issue is to appropriately select patients in whom non-operative management is safe and likely

to be successful. Patients with infection confined to a small, forefoot lesion; without severe or necrotizing soft-tissue infections or significant peripheral arterial disease are more likely to respond well to non-surgical treatment. Other factors such as fitness for surgery, likely foot function if surgery is undertaken and patient preference needs to be considered when deciding treatment [107].

26.5.4 Lower Limb Arterial Revascularisation

All patients with diabetic foot ulceration should be evaluated for the presence of peripheral artery disease at the time of presentation by clinical assessment and basic non-invasive testing (most frequently, ABI and toe waveforms and pressures). All diabetic patients with a foot ulcer and PAD should be considered for vascular imaging and possibly revascularisation. Colour duplex ultrasound, CT-angiography, MR-angiography or intra-arterial digital subtraction angiography can be used to obtain anatomical information of the arterial system which is essential when planning revascularisation [45].

The aim of revascularisation in the patient with DFU is to treat the perfusion deficit by restoring direct flow to at least one of the foot arteries, preferably the artery that supplies the anatomical region of the wound. The level of perfusion required to heal a foot ulcer depends on multiple factors such as ulcer size and location, presence/extent of gangrene and infection [108]. A patient with a shallow, uninfected toe ulcer is likely to need less perfusion to heal the foot compared to a patient with extensive tissue necrosis who is likely to require much better perfusion to achieve wound healing [108]. As a general rule, a toe pressure ≥30 mmHg; or, a TcPO2 ≥25 mmHg should be achieved post revascularisation [45].

The decision regarding the revascularisation technique is complex and the clinician must take into consideration the morphology and length of the arterial lesion, availability of autogenous venous conduit, patient comorbidities and available expertise. Revascularization is increasingly attempted by endovascular means in the first instance. However open bypass remains an effective approach particularly for patients with severe ischaemia, long segment disease and major tissue loss who have available vein conduit and acceptable operative risk [108]. Patients with more advanced tissue loss (higher WIfI stage) need significantly more reinterventions after endovascular therapy to heal the foot [109]. In addition to revascularization, aggressive medical therapy and cardiovascular risk management including support for cessation of smoking, treatment of hypertension and prescription of a statin and antiplatelets should be ensured in diabetic patients with PAD. A detailed evidence-based assessment and treatment algorithm has recently been published as part of the Global Vascular Guidelines for chronic limb threatening ischaemia document in 2019 [110].

26.6 Charcot Neuroarthropathy

Charcot arthropathy occurs in 1–2% patients with diabetes and peripheral neuropathy. It is characterised by pathological fractures, joint dislocation, deformity and severe destruction of the foot [111]. It has serious implications for the patient as it may result in significant foot deformity, ulceration, and subsequent limb loss. The pathogenesis of Charcot arthropathy appears to be multifactorial with a genetic predisposition, altered levels of neuropeptides (calcitonin gene-related peptide [CGRP] and nitric oxide) in the foot, increased inflammatory cytokines and disordered bone turnover contributing to the condition [112].

Acute Charcot arthropathy presents with a warm, swollen and erythematous foot which can easily be misinterpreted as acute infection, gout or osteomyelitis. The absence of ulceration, lack of other signs of infection and a WCC within normal range favours the diagnosis of Charcot foot over an infective process. Plain radiography is the initial imaging modality for assessment of Charcot foot. If such imaging is normal and the clinical suspicion is high, MRI or nuclear imaging can be useful as they are more sensitive for assessment of bone pathology.

Typically the affected individual has preserved blood flow in the foot with good pedal pulses. Repetitive cumulative injuries to an insensate foot may progress into the destructive stage of Charcot arthropathy and lead to gross foot deformity. The hallmark deformity of this condition is midfoot collapse, described as a "rocker-bottom" foot. The process leading to gross deformities of the foot and/or ankle is relatively painless given the neuropathy.

The most important strategies for management of active Charcot foot are early diagnosis, offloading and immobilisation. The use of total contact cast (TCC) is considered the treatment of choice. The cast needs to be changed every 1–2 weeks to accommodate the decreasing oedema. Patients presenting with a very swollen foot may be immobilised in a backslab until the initial swelling subsides (with bed rest and immobilisation). Opinion varies in relation to whether patients should be weightbearing or not and regarding the length of time that the cast should be applied. Casting should be continued until resolution of the erythema, swelling, warmth and improvement in radiological signs which may take several months. Antiresorptive therapy (bisphosphonates) and calcitonin have been used in the acute phase however there is lack of conclusive evidence for the benefit of these adjunct therapies. After the TCC has been removed, different offloading modalities can be used, including the Charcot restraint orthotic walker (CROW). Surgical procedures may be performed to correct bone alignment, excise exostoses and relieve areas of high pressure. The goal for treatment for patient with chronic Charcot changes is to maintain a stable foot free from ulceration and infection, which frequently requires significant orthopaedic, podiatry and orthotics input [113].

26.7 Prevention

Diabetic foot disease is potentially preventable, and every effort should be made to ensure that high-risk patients are identified and receive early treatment of foot complications. The International Working Group for the Diabetic Foot emphasizes the importance of prevention of foot problems [66]. Successful efforts to prevent and treat diabetic foot complications depend upon a well-organised multidisciplinary approach. Ideally a foot care programme should provide the following:

1. Education for people with diabetes, their caregivers and for healthcare staff.
2. A system to detect all high-risk patients.
3. Measures to reduce risk of foot ulceration, such as podiatric maintenance care and appropriate footwear.
4. Prompt and effective treatment of any foot complication.
5. Focussed care of patients in diabetic foot remission to maximize ulcer-free, hospital-free and activity-rich days.
6. Auditing all aspects of the diabetic foot to identify problems and ensure that local practice meets accepted standards of care.

Diabetic foot ulcers should be seen as a chronic potentially limb-threatening condition and strategies should be designed to meet the needs of patients requiring chronic care, rather than simply responding to acute problems when they occur. Aggressive preventive strategies should aim to provide an efficient and cost-effective solution to a challenging and costly disease process.

26.8 Conclusion

Diabetic foot complications are a major public health challenge worldwide and one of the ten major causes of disability worldwide. Unfortunately, the number of people affected by diabetic foot pathology is likely to continue to rise due to the population ageing and the globally increasing incidence of diabetes.

The prevention of diabetic foot ulcers is essential to reduce the risks to the patient and the resultant economic burden to society. Once an ulcer has developed the management is complex, and requires a multidisciplinary team approach to optimise outcomes. Treatment should be evidence-based and may include offloading, wound management, management of infection and revascularisation.

Significant and exciting advances in the management of diabetic foot have occurred in the past decades. There has been the development and implementation of international treatment guidelines for the diabetic foot, such as the IWGDF guidelines, and growing implementation of diabetic foot programs across the globe. There is now a better understanding of the pathophysiology of the diabetic foot which allows development of therapeutic interventions.

There are innovative technologies, such as negative pressure wound therapy and endovascular interventions, that have been adopted and have changed the management of diabetic foot. This is an evolving field and there are several new technologies which have the potential to improve outcomes of patients with foot complications.

Despite all the recent advances, much remains to be done. Continuous investment in prevention, management and research of the diabetic foot syndrome is of paramount importance.

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Chapter 27
Lymphoedema

Matt Waltham and Kristiana Gordon

Key Learning Points

- The lymphatic system has several functions, including (1) maintenance of fluid balance by returning lymphatic fluid from interstitial spaces to the blood circulation, (2) maintaining immune function with the help of lymphoid organs, and (3) nutritional, as fat is absorbed via the intestinal lymphatics. The lymphatic system also plays a role in disease, such as inflammatory disorders and cancer metastasis.
- The lymphatic system contributes to immune function by mediating the transport of soluble antigens, macrophages, lymphocytes and dendritic cells to draining lymph nodes. It also assists with the trafficking of leucocytes to and from lymph nodes.
- The differentiation of lymphatic endothelial cells (LECs) is thought to be governed by the action of three transcription factors: Prox1, Sox18 and COUP-TFII. Prox1 is required for the differentiation of LECs during embryogenesis and for the maintenance of LEC fate during adult life. Prox1-expressing LECs upregulate Vascular Endothelial Growth Factor Receptor 3 (VEGFR3), LYVE1 and other lymphatic endothelial-specific molecules. Sox18 is expressed in the cardinal vein and is responsible for regulating Prox1 expression as it binds to Prox1 promoter. COUP-TFII has been shown to establish venous endothelial cell identity and LEC specification by suppressing Notch signalling.
- Lymphoedema occurs when the rate of microvascular filtration overwhelms lymphatic drainage capacity. This can occur because either the microvascular filtration rate is increased (e.g. in the case of venous insufficiency), or the rate of lymphatic flow has decreased, or a combination of the two. Failure of the

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lymphatic system leads to the accumulation of protein-rich lymph fluid within interstitial spaces, manifesting as lymphoedema.

- Primary lymphoedema arises as a result of a genetic predisposition. The lymphatic system fails to develop normally or to be maintained adequately, causing impaired drainage of lymph and swelling of the affected region. Primary lymphoedema can be divided into five different categories
  - Genetic Syndromes associated with lymphoedema
  - Lymphoedema with systemic (internal) lymphatic abnormalities
  - Lymphoedema in association with overgrowth of tissues
  - Congenital lymphoedema
  - Late-onset primary lymphoedema (primary lymphoedema that develops after the first year of life)
- A diagnostic pathway has been developed which describes the specific primary lymphoedema phenotypes and guides clinicians on relevant gene testing that may be available.
- Secondary lymphoedema develops from an acquired disruption to lymph drainage routes, e.g. damage to the lymphatic vessels and/or lymph nodes. Causes of secondary lymphoedema include infection (e.g. recurrent cellulitis, or Filariasis), malignancy, surgery, inflammatory conditions, trauma, venous disease and immobility.
- The management of lymphoedema is aimed at reducing swelling through physical treatments designed to stimulate flow through existing or collateral drainage routes. This physical therapy management approach is the same for patients with primary and secondary lymphoedema.
- Patients with primary or secondary lymphoedema are susceptible to a number of complications include infections with bacteria and fungi (almost certainly due to reduced immune surveillance secondary to lymphatic dysfunction). Recurrent cellulitis is a particular problem. The use of prophylactic penicillin has been shown to reduce the rate of infection. Lymphoedema may also be complicated by the development of cutaneous malignancy.

27.1 Introduction

The lymphatic system forms part of the human circulatory and immune systems. It has several functions, including (1) maintenance of fluid balance by returning lymphatic fluid from interstitial spaces to the blood circulation, (2) maintaining immune function with the help of lymphoid organs, and (3) nutritional, as fat is absorbed via the intestinal lymphatics. The lymphatic system also plays a role in disease e.g. inflammatory disorders and cancer metastasis.

Lymphoedema occurs when the rate of microvascular filtration overwhelms lymphatic drainage capacity. This can occur because either the microvascular filtration rate is increased (e.g. in the case of venous insufficiency), or the rate of lymphatic

flow has decreased, or a combination of the two. Failure of the lymphatic system leads to the accumulation of protein-rich lymph fluid within interstitial spaces, manifesting as lymphoedema, a debilitating swelling with associated changes of the skin.

Primary lymphoedema arises as a result of a genetic predisposition; the lymphatic system fails to develop normally, or to be maintained adequately, causing impaired drainage of lymph resulting in swelling of the affected region. Primary lymphoedema may occur as a non-syndromic Mendelian condition, or less commonly as part of a complex syndromic disorder. A diagnostic algorithm has been developed in order to facilitate diagnosis [1].

Secondary lymphoedema develops from an acquired disruption to lymph drainage routes, e.g. damage to the lymphatic vessels and/or lymph nodes. Causes of secondary lymphoedema include infection (e.g. recurrent cellulitis, or Filariasis), malignancy, surgery, inflammatory conditions, trauma, venous disease and immobility [2–4].

Patients with primary or secondary lymphoedema are susceptible to a number of complications. These include infections, due to reduced immune surveillance secondary to lymphatic dysfunction. Recurrent cellulitis is a particular problem. Constitutional symptoms such as fever, vomiting or headaches may develop suddenly without heralding skin signs of cellulitis. Repeated episodes of infection will exacerbate lymphoedema and predispose to further infections, thus creating a vicious cycle of events. The use of prophylactic penicillin has been shown to reduce the rate of infection [5].

Unfortunately there is no proven curative treatment for lymphoedema. Management is aimed at reducing the swelling through physical treatments designed to stimulate flow through existing or collateral drainage routes. This physical therapy management approach is the same for those patients with primary and secondary lymphoedema. Several surgical techniques have been implemented in an attempt to improve lymphatic drainage, or achieve limb volume reduction via liposuction. However, in the absence of robust data to support most surgical techniques, we must rely on so-called “conservative” physical therapies for the majority of patients until new and improved therapeutic options are available.

27.2 Lymphangiogenesis

Lymphangiogenesis is the term used to describe the growth of new lymphatic vessels. “Developmental lymphangiogenesis” occurs in the foetus as the lymphatic system is developing. However, lymphangiogenesis may also occur post-natally in association with inflammatory disease and malignancy. Research has identified lymphatic endothelial cell-specific molecular markers and genes involved in lymphatic development and lymphangiogenesis. There is much heterogeneity in expression of these endothelial markers in both lymphatic and blood vessels, which may explain why there is a high rate of coexistent venous abnormalities in patients with primary lymphoedema.

27.2.1 Origin and Differentiation of Lymphatic Endothelial Cells

The most widely accepted model of the origin of lymphatic endothelium was proposed by Florence Sabin in 1902 [6]. Other models have been proposed but are less widely accepted and not supported by mammalian animal models. The current literature reports that lymph sacs develop in the human embryo at 6–7 weeks gestation, nearly 1 month after the blood vasculature has started to develop [7]. Research in animal models has demonstrated that a subpopulation of blood endothelial cells, arising from cardinal and peripheral veins, differentiate into lymphatic endothelial cells (LECs) [8]. The LECs then bud off as strings of cells to form primitive lymphatic structures called "lymph sacs" [9]. Differentiation of LECs is thought to be governed by the action of three transcription factors: Prox1, Sox 18 and COUP-TFII [10]. Prox1-expressing LECs up-regulate Vascular Endothelial Growth Factor Receptor 3 (VEGFR3), LYVE1 and other lymphatic endothelial-specific molecules [11]. Sox18 is expressed in the cardinal vein and is responsible for regulating Prox1 expression [12]. COUP-TFII has been shown to establish venous endothelial cell identity and LEC specification by suppressing Notch signalling [13].

27.2.2 Sprouting of Lymphatic Endothelial Cells

In embryogenesis, individual lymphatic endothelial cells connect to other LECs by adherens junctions and start "sprouting" into primitive lymphatic structures (lymph sacs) [14]. A direct connection is created between the primordial thoracic duct and the cardinal vein. Valves develop at the connection site in order to prevent retrograde flow of blood into the newly formed lymphatic system [15]. The LECs produce Podoplanin (a mucin-type protein) that interacts with Clec-2 in platelets to induce platelet aggregation and induce a "sealing" process and subsequent separation of the two vascular systems [16]. Animal studies suggest that the vegfc/vegfr3 and ccbe1 (collagen and calcium binding EGF domain-1) signalling pathways regulate sprouting. Venous sprouting is inhibited by the absence of vegfc or ccbe1, resulting in the complete absence of lymphatic vessels in animal models [15].

The ligands VEGFC and VEGFD bind and activate the tyrosine kinase receptors VEGFR2 and VEGFR3 on LECs. VEGFR3 is considered the main VEGFC receptor for lymphangiogenesis [17]. Both VEGFC and VEGFD promote migration and proliferation of LECs *in vitro*, and lymphatic vessel hyperplasia *in vivo*. However, it appears that only VEGFC is required for embryonic lymphatic development [18, 19]. CCBE1 does not appear to have lymphangiogenic activity on its own but enhances the lymphangiogenic effects of VEGFC *in vivo* [20]. CCBE1 up-regulates levels of mature VEGFC *in vitro*. CCBE1 also promotes proteolytic cleavage of the otherwise poorly active 29/31-kDa form of VEGFC by the A disintegrin and metalloprotease with thrombospondin motifs-3 protease (ADAMTS3), resulting in the mature a form of VEGFC, which subsequently induces increased VEGFC receptor signalling [21].

Whilst we do not yet fully understand all mechanisms involved in lymphangiogenesis, the identification of mutations in *SOX18*, *VEGFR3, VEGFC, CCBE1* and *ADAMTS3* in humans with lymphatic insufficiency supports the critical role these genes play.

27.2.3 Remodelling of a Lymphatic Vascular System

Further development of the lymphatic system occurs by lymphatic vessel sprouting from the primitive lymph sacs mentioned above. The primitive lymphatic vessels must now mature into a functional network with vessel-type features that serve their critical functions. Remodelling processes lead to the formation of flap valves in lymphatic capillaries, the establishment of pre-collecting and collecting vessels via smooth muscle cell (SMC) recruitment, and luminal valve development.

Flap-like openings called "primary valves" form between the LECs of lymphatic capillaries to create button-like intercellular junctions. These facilitate the entry of interstitial fluid and leucocytes into the initial lymphatic vessels [22]. Prox1 and FOXC2 transcription factors are expressed by clusters of cells within developing collecting vessels to form intraluminal valves [23]. FOXC2 is a significant regulator of lymphatic valve formation. FOXC2 deficiency in humans results in lymphatic vessel valve aplasia [24].

The lymphatic pre-collectors and collecting lymphatic vessels are covered by smooth muscle cells (SMCs), except in the luminal valve areas, to assist with proximal propulsion of lymph [25]. SMCs play a significant role in lymphatic remodelling and function. Unlike the larger collecting vessels, initial lymphatic capillaries have a discontinuous basement membrane. The gaps permit entry of interstitial fluid, macromolecules and leucocytes [26]. Initial lymphatic capillary LECs remain attached to the interstitial matrix by anchoring filaments [27].

It is clear how some primary lymphoedema subtypes are caused by abnormal gene function (e.g. *VEGFR3* mutations and Milroy disease; *CCBE1* and Hennekam syndrome; *FOXC2* and Lymphoedema distichiasis syndrome), as the causal genes play a significant role in the lymphangiogenic pathway. However, genes implicated in other forms of primary lymphoedema are not known to play a role in lymphangiogenesis. There is clearly more to learn about lymphangiogenesis and the genes that regulate it.

27.3 Structure and Function of the Lymphatic System

The lymphatic system is comprised of a network of lymphatic vessels and lymph nodes. The primary function is to drain materials from tissue spaces that cannot directly return to the bloodstream. These include capillary filtrate, proteins, macrophages, lymphocytes and malignant cells [28]. The lymphatic system ensures that capillary ultrafiltrate and plasma proteins are returned to the blood circulation via the thoracic duct.

The lymphatic system is not a circular system, unlike the blood vascular system. Lymphatic vessels are essentially of three types: (1) non-contractile initial lymphatic vessels that absorb capillary filtrate, leucocytes and macrophages, (2) pre-collectors that are sparsely covered by smooth muscle cells, and (3) the larger collecting lymphatic vessels that actively pump lymph to the regional lymph nodes.

27.3.1 Maintenance of Fluid Balance

Lymphoedema occurs when microvascular (capillary and venular) filtration overwhelms the lymph drainage system. This can occur because (1) the microvascular filtration rate is increased, or (2) the rate of lymphatic flow has decreased, or a combination of the two. The rate of capillary filtration is governed by the Starling principle of fluid exchange. Essentially, microvascular filtration of fluid from the capillary into the interstitium is driven by the hydraulic pressure gradient across the blood vessel wall and is opposed by the osmotic pressure gradient, which is the suction force retaining fluid within the vessel [29]. Accumulation of capillary filtrate (i.e. lymphoedema) in tissue spaces is prevented by lymph drainage and not, as was previously thought, through venous reabsorption.

If lymphatic drainage fails to cope with excessive microvascular filtration caused by an increase in capillary pressure (e.g. heart failure), or reduction in plasma colloid osmotic pressure (e.g. malnutrition, liver disease), or increased endothelial conductance (e.g. in inflammatory conditions) then oedema will develop. If the lymphatic system fails to adequately drain the capillary filtrate due to (1) damage to a previously healthy lymphatic system (as in a secondary lymphoedema scenario), or (2) an abnormal development of the lymphatic system (as in primary lymphoedema), then the clinical signs of lymphoedema will develop.

27.3.2 Immunity and Infection

The lymphatic system plays a vital role in immune function by mediating the transport of soluble antigens, macrophages, lymphocytes and dendritic cells to draining lymph nodes via afferent lymphatic vessels. It also assists with the trafficking of leucocytes to and from lymph nodes [30]. This transport system allows for an adaptive immune response to be generated to pathogens.

Local infections (e.g. warts, cellulitis or athlete's foot) are a frequent concern for patients with lymphoedema of any aetiology. Lymphoedema has been reported to convey a relative risk of 71 times the normal for development of cellulitis [31]. The underlying mechanism is likely to be a disturbance in immune cell trafficking that compromises local tissue immune surveillance.

27.3.3 Lymphatics, Fat and Inflammation

The intestinal lymphatics (lacteals) are responsible for the majority of fat absorption. They absorb dietary fats in the villi of the small intestine in the form of a milky substance called chyle. The lacteals merge to form larger lymphatic vessels and the chyle is transported to the thoracic duct and later to the subclavian vein [32].

The relationship between fat and lymphatics may extend beyond the gut. Clinicians are now realising that fat deposition may be a defining clinical characteristic of lymphoedema. Management of lymphoedema by liposuction has confirmed the swelling is not just fluid, but includes large volumes of fat [33]. Evidence is emerging that the lymphatic system is involved in the regulation of lipids, adipocytes and inflammatory cells. Lymphatic impairment (i.e. lymphoedema) leads to disorders of inflammation and fat homeostasis and deposition, and would explain the increased adipose deposition seen in chronic lymphoedema [34].

27.4 Lymphoedema

27.4.1 Introduction

Failure of the lymphatic system leads to the accumulation of protein-rich lymph fluid within interstitial spaces, manifesting as lymphoedema. This may present as debilitating swelling, usually of a limb or limbs, with associated brawny changes of the skin. Primary lymphoedema arises due to an intrinsic abnormality involving a genetically determined aplasia, hypoplasia, malformation or dysfunction of lymphatic vessels. Secondary lymphoedema results from lymphatic damage due to extrinsic factors such as surgical lymphadenectomy, radiotherapy or chronic venous disease. Recurrent lower limb cellulitis can also lead to lymphoedema. Interestingly, a significant number of the latter group will have an intrinsic lymphatic abnormality predisposing them to cellulitis indicating that this can be both a cause of lymphoedema and occur secondary to a primary lymphoedema [35].

27.4.2 Epidemiology

Estimates of prevalence have been difficult to calculate as it is presumed that many affected individuals do not come to medical attention. However, it is acknowledged that lymphoedema/chronic oedema is an underestimated health problem that remains widely unrecognised despite affecting between 200,000 and 420,000 men, women and children in the UK [36, 37].

27.4.3 Clinical Features

The clinical signs of lymphoedema can range from mild swelling to that of massive enlargement in chronic cases that have not received adequate treatment. Protein-rich materials, lipids and debris accumulate in addition to water. This results in "solid" and "fluid" components to the swelling, giving rise to the brawny nature of chronic oedema that resists pitting [38]. Skin changes may be present, including brawny fibrotic skin and the presence of the Kaposi-Stemmer sign (the failure to pinch/pick up a fold of skin at the base of the second toe as a result of its thickness) that is pathognomonic of lymphoedema [39]. Papillomatosis (small flesh-coloured papules) occurs as a result of dilatation within the upper dermal lymphatics and subsequent fibrosis of the dermis. Lymphangiectasia appear as small blisters on the skin surface as a result of engorgement of lymphatic vessels. Lymph fluid frequently leaks as a result of minimal trauma and is termed lymphorrhoea (Fig. 27.1). The severity of lymphoedema may be classified according to the clinical features (Table 27.1), and this may be useful in monitoring response to therapy over a prolonged period.

27.4.4 Complications of Lymphoedema

Patients with primary or secondary lymphoedema are susceptible to a number of complications. These include infections with bacteria and fungi, almost certainly due to reduced immune surveillance secondary to lymphatic dysfunction.

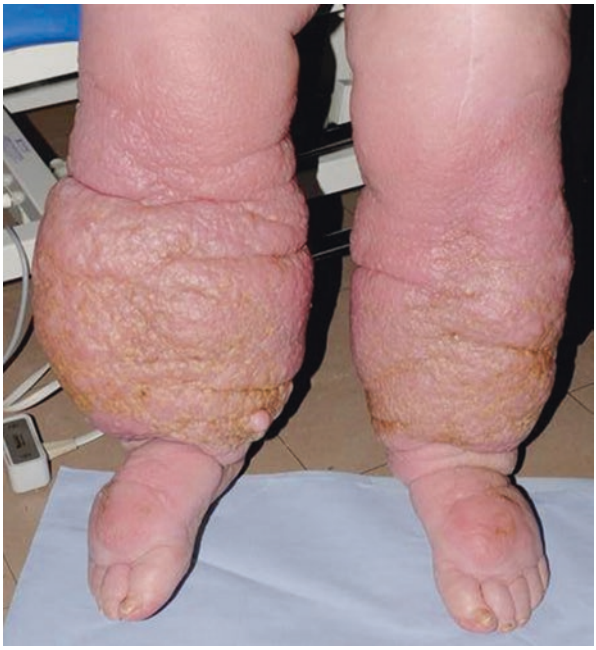

Fig. 27.1 Bilateral lower limb lymphoedema due to lymphovenous disease and immobility (note the left knee replacement) with signs of lipodermatosclerosis, papillomatosis and lymphorrhoea (the yellow encrusted areas)

Table 27.1 Stages of lymphoedema, adapted from the ISL Consensus Documents [40, 41]

Stage	Clinical signs
Stage 0	A latent/subclinical state where swelling is not evident despite impaired lymphatic transport. This stage may exist for many months or years before oedema becomes evident
Stage I	Early onset of lymphoedema where there is accumulation of tissue fluid that reduces with limb elevation; pitting may be present
Stage II	Accumulation of fluid that does not reduce on elevation; at later stages may be non-pitting
Stage III	Fibrotic tissue and absent pitting; elephantiasis skin changes develop e.g. acanthosis, fatty and fibrous tissue changes, and warty overgrowths

Recurrent cellulitis is a particular problem. Constitutional symptoms such as fever, vomiting or headaches may develop suddenly without heralding skin signs of cellulitis. Repeated episodes of infection will exacerbate lymphoedema and predispose to further infections, thus creating a vicious cycle of events. The use of prophylactic penicillin has been shown to reduce the rate of infection [5].

Rarely, lymphoedema may be complicated by the development of cutaneous malignancy. The most reported malignancy is lymphangiosarcoma [42]. Stewart-Treves syndrome refers to the development of lymphangiosarcoma in patients with chronic post-mastectomy lymphoedema. However, it is now accepted that lymphangiosarcoma may occur in longstanding lymphoedema of any primary or secondary cause [43, 44]. Patients with longstanding lymphoedema are also at increased risk of local skin tumours e.g. Kaposi's sarcoma, carcinoma, squamous cell carcinoma and malignant melanoma, presumably as a result of impaired immune surveillance within the lymphoedematous region [45, 46].

27.5 Secondary Lymphoedema

Secondary lymphoedema develops from any acquired disruption of lymph drainage routes, e.g. damage to the lymphatic vessels and/or lymph nodes. Lymphatic filariasis is the most common cause of lymphoedema worldwide, affecting an estimated 40 million people [47]. Mosquitoes are the vectors that transmit larvae to the lymphatics where they hatch into adult worms, causing progressive disruption to lymphatic function. Filarial infection is concentrated in the tropics and does not account for the majority of lymphoedema seen in temperate zones. The World Health Organisation (WHO) is currently undertaking a Global Programme to Eliminate Lymphatic Filariasis (GPELF) with large-scale preventative chemotherapy treatment.

Other causes of secondary lymphoedema include infection (e.g. recurrent cellulitis), malignancy, surgery, inflammatory conditions, trauma, venous disease and immobility [2–4] (Table 27.2).

Table 27.2 Causes of the swollen limb

A: Congenital				
Vascular	**Lymphatic**	**Other**		
Vascular malformation	Lymphoedema	Overgrowth Spectrum		
Klippel-Trenaunay syndrome	Lymphatic malformation			
Parkes weber syndrome	Lymphangiomatosis			
Maffucci syndrome				
B: Acquired				
Vascular	**Lymphatic**	**Inflammatory**	**Musculoskeletal**	**Tumours**
Venous; DVT Post-thrombotic syndrome Dependency syndrome Venous injury (e.g.: IV drug use)	Secondary Lymphoedema	Cellulitis Pre-tibial myxoedema	Rheumatoid arthritis Ruptured Baker's cyst Pathological fracture	Lymphoma sarcoma Metastases
Arterial; Acute arterial ischaemia Ischaemia-reperfusion injury				
Other; Drugs (e.g. calcium channel blockers) Idiopathic/cyclical oedema in women				

Perhaps the most recognised form of secondary lymphoedema in the developed world is that of breast cancer related lymphoedema (BCRL). Despite recent advances in breast cancer treatment, including the introduction of minimal surgery guided by sentinel lymph node biopsy, the prevalence of BCRL remains stubbornly high at 20% [48]. The reasons why some patients develop BCRL and others do not, despite similar treatment plans, remain a mystery. Other unanswered questions include 'why is there a delay of years before lymphoedema manifests in some patients', and 'why can the distribution of swelling within the limb vary' e.g. the arm may be swollen but the hand spared. The detection of Connexin47/*GJC2* gene mutations in a number of women with BCRL, suggests that underlying gene mutations lead to an increased susceptibility for developing lymphoedema following breast cancer treatment [49].

Venous lymphoedema results from venous valve failure and subsequent venous hypertension creating excessive microvascular filtration into the interstitial spaces. Varicose veins or post-thrombotic syndrome are the commonest causes. If the lymph drainage is compensating then the patient will not develop lymphoedema. Therefore, oedema in the presence of venous disease indicates lymphatic failure and treatment

should address improvements in lymph drainage as well as control of the venous disease. Surgical treatment of varicose veins will often not resolve 'venous' oedema because lymph drainage is compromised and surgery will not improve it. Therefore compression is the treatment of choice for venous oedema because compression garments (hosiery) have the advantage of reducing microvascular filtration (from the venous hypertension) and at the same time improved lymphatic drainage. Swelling of just one leg suggests a local cause such as venous obstruction from a deep vein thrombosis.

27.6 Primary Lymphoedema

Primary lymphoedema occurs as a result of a genetic predisposition causing the lymphatic system to fail to develop normally, or to be maintained adequately, causing abnormal drainage of lymph which results in swelling of the affected region. Primary lymphoedema may occur as an inherited condition, or less commonly as part of a complex syndromic disorder [50]. A patient with primary lymphoedema may only have problems with swelling, but some forms of primary lymphoedema occur in association with other health problems e.g. congenital heart disease, systemic/internal lymphatic abnormalities (pleural and/or pericardial effusions, ascites), or very rarely haematological malignancy (leukaemia). The various subtypes of primary lymphoedema are discussed below, together with a classification pathway that has been developed in order to facilitate diagnosis for clinicians involved in the care of patients with primary lymphoedema [1].

Specific phenotyping facilitates the identification of subgroups of patients with the same type of primary lymphoedema. This leads to a better understanding of the natural history and management of the specific conditions and more accurate recurrence risks for future offspring/generations.

Primary lymphoedema is not one disease, but the presenting feature of several distinct clinical entities. Historically, primary lymphoedema was categorised into three groups depending on the patient's age at onset of swelling: congenita (presenting at birth, Fig. 27.2), praecox (pubertal onset) or tarda (onset after 35 years of age). Mutations in several genes are known to cause primary lymphoedema. Some, but not all, of these genes have been shown to play a role in lymphangiogenesis (the process of developing and maintaining a healthy lymphatic system). The discovery of these gene mutations has changed our diagnostic approach in the clinic, which is now based on clinical phenotyping (the process of associating a patient's lymphoedema with other health problems) and genotyping (DNA tests looking for the underlying causal gene mutation) in addition to age of onset of swelling. Our experience and research has led us to realise that primary lymphoedema can be broadly divided into five different categories (see below, Fig. 27.3). Causal gene mutations have been identified for a number of disease subtypes within the five categories. A colour-coded diagnostic pathway has been developed that describes specific primary lymphoedema phenotypes and guides the clinician on gene tests that may be available for their patient (annotated in red within the pathway boxes).

Fig. 27.2 Primary lymphoedema of one limb in a baby

27.6.1 Genetic Syndromes Associated with Lymphoedema (Blue Section of the Pathway)

The swelling is not the predominant feature of the child's health problems, but simply part of their overall condition. Examples include Turner syndrome where girls are born with swollen feet ± hands because they are missing one X chromosome. Some children with Noonan syndrome also develop lymphoedema of their legs ± genitalia in childhood.

27.6.2 Lymphoedema with Systemic (Internal) Lymphatic Abnormalities (Pink Section of the Pathway)

This rare form of primary lymphoedema does not affect many children. These children may be swollen at birth (usually affecting several limbs/body parts) and have internal lymphatic problems. These internal problems may include fluid around the

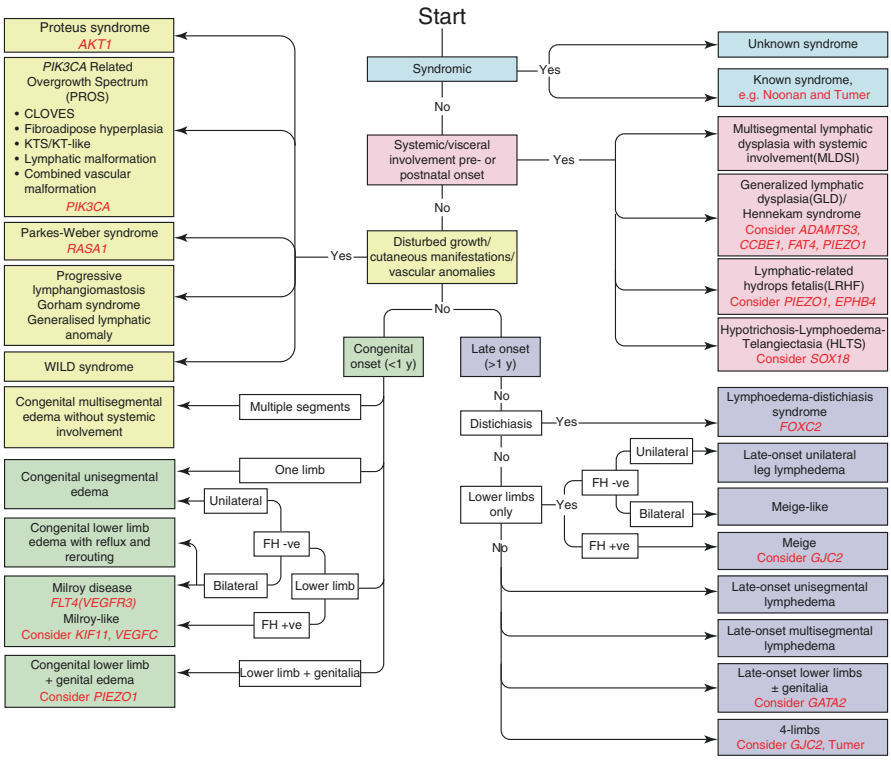

Fig. 27.3 Diagnostic algorithm of primary lymphoedema. (Updated from Connell et al. [1] Clin Genet)

heart and/or lungs. They may also have abnormal lymphatic drainage of the small intestine which means they cannot absorb fats properly, causing them to have diarrhoea when they eat fatty foods. One example of this rare problem is Hennekam syndrome due to a mutation in the *CCBE1 or FAT4* genes.

27.6.3 Lymphoedema in Association with Overgrowth of Tissues (Yellow Section)

Lymphoedema may develop in patients that have overgrowth of tissues within the swollen limb—e.g. the muscle, bone or fatty tissues are increased in size. There are several conditions within this group, including Klippel-Trenaunay syndrome where the child may have lymphoedema, varicose veins, red birthmarks of the skin and a longer limb. We are realising that many of these conditions develop because of a *PIK3CA/AKT1* genetic pathway mutation just within the swollen limb (i.e. a mosaic/somatic mutation), and the gene mutation will not be found in their blood.

27.6.4 Congenital Lymphoedema (Green Section)

Congenital lymphoedema describes children with lymphoedema that is present at birth (or develops within the first few months of life). There are several different causes but the most recognised is Milroy disease. This condition causes swelling of the feet and ankles at birth, varicose veins after puberty, and one third of boys will develop a build-up of lymphatic fluid in the scrotum (hydrocoeles). Milroy disease is due to a mutation in the *VEGFR3* gene. Children of affected individuals have a 50% risk of inheriting the problem.

27.6.5 Late-Onset Primary Lymphoedema (Purple Section)

The term "late-onset lymphoedema" is used to describe a primary lymphoedema that develops after the first year of the patient's life (i.e. non-congenital lymphoedema). Lymphoedema distichiasis syndrome is one example. It presents with pubertal onset of bilateral lower limb lymphoedema. Distichiasis (extra eyelashes) is present from early childhood and may cause eye irritation (Fig. 27.4). Other problems may include varicose veins, cleft palate and rarely congenital heart disease (heart valve abnormalities). Lymphoedema distichiasis syndrome occurs as a result of mutations in the *FOXC2* gene, and children of affected individuals have a 50% risk of inheriting the problem. Other examples of late-onset primary lymphoedema include Emberger syndrome (a rare type of primary lymphoedema associated with a risk of developing leukaemia), and Meige disease (the commonest form of primary lymphoedema and is not associated with other health problems).

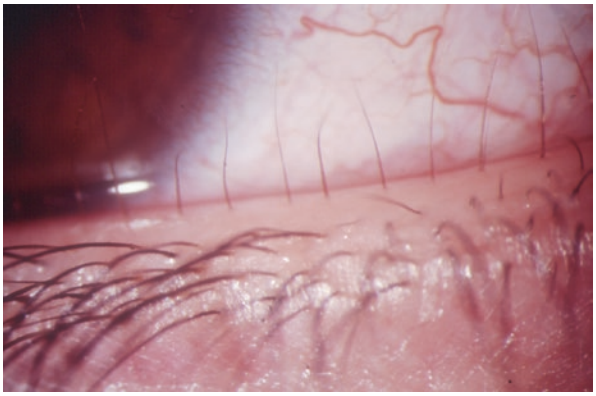

Fig. 27.4 Distichiasis with accessory eyelashes along the posterior border of the lid margin in the position of the Meibomian glands

The diagnostic pathway helps the clinician to offer appropriate genetic testing (assuming the underlying gene mutation is known), and screen and treat for associated health problems. Patients and families benefit hugely from receiving a formal genetic diagnosis of their primary lymphoedema as it allows the clinician to confidently predict the clinical prognosis and offer screening for family members.

Unfortunately there is no curative treatment for primary lymphoedema. Hopefully gene therapy will be available to treat some forms of primary lymphoedema within the next 5–10 years. Until then, management is aimed at controlling swelling through physical treatments designed to stimulate flow through existing or collateral drainage routes. This physical therapy management approach is the same for those patients with secondary lymphoedema. Several reconstructive surgical techniques have been implemented in recent years in a bid to improve lymphatic drainage in patients with secondary lymphoedema. These are unlikely to benefit patients with primary lymphoedema as the fault with lymphatic drainage is not an "obstruction", rather a molecular/genetic abnormality that cannot simply be bypassed.

27.7 Investigating Lymphoedema

Most patients with lymphoedema are diagnosed from the history and clinical findings. The use and value of imaging techniques is highly dependent on availability and expertise. Techniques used to investigate the lymphatic system are discussed below, but some patients will benefit from additional imaging in order to fully understand the cause of their swelling. For example, magnetic resonance imaging (MRI) and computerised tomography (CT) scanning may be indicated to assess for the presence of intra-abdominal or pelvic pathology/masses obstructing lymphatic drainage. MRI may also be requested to assess for tissue hypertrophy or signs of overgrowth (e.g. in Klippel-Trenaunay syndrome). Venous duplex imaging may be requested to investigate the possibility that a patient's lymphoedema is associated with venous incompetence. Table 27.3 compares the different techniques available for imaging the lymphatic system.

27.7.1 Lymphography and Lymphoscintigraphy

Imaging with X-ray contrast lymphography (lymphangiography) was previously the best investigation tool for demonstrating lymphatic collectors and lymph nodes. However, it is now rarely used as the technique requires the invasive procedure of direct cannulation of the lymphatics (Fig. 27.5).

Lymphoscintigraphy (isotope lymphography) is the current gold standard investigation for determining whether chronic oedema may be due to lymphatic failure. It involves a simple intra-dermal/subcutaneous injection of a radio-labelled tracer protein, exclusively cleared by lymphatics. Measurement of tracer uptake and transit through the lymphatics permits the qualitative and quantitative analyses of

Table 27.3 A comparison of the different techniques available for imaging the lymphatic system

Imaging technique	Contrast agent	Depth of imaging	Invasiveness	Availability
Lymphography/ lymphangiography	Intra-lymphatic injection of a radio-opaque oil (e.g. Lipiodol)	Anatomical imaging of lymphatic collectors and lymph nodes	Very invasive: Requires the identification of a lymphatic collecting vessel by exploratory surgery	Rarely performed anymore
Lymphoscintigraphy	Simple intra-dermal/ subcutaneous injection of a radio-labelled tracer protein (e.g. ^{99}Technetium-labelled dextran)	Lymphatic collectors and lymph nodes Poor resolution compared to lymphography technique Limited functional data acquired Not anatomical	Minimally	Readily available in hospitals
Fluorescence microlymphangiography	Simple intra-dermal injection of fluorescent contrast agent (FITC-dextran)	Superficial dermal lymphatic vessels	Minimally	Research tool
Near infra-red lymphangiography	Simple intra-dermal/ subcutaneous injection of indocyanine green	Superficial dermal lymphatic vessels and superficial lymph nodes. Peristalsis can be visualised but not quantified	Minimally	Increasing availability in departments offering lymphatic microsurgery
MR lymphangiography	Simple intra-dermal/ subcutaneous injection of a gadolinium-based contrast agent	Lymphatic collectors and lymph nodes. Subcutaneous fat and oedema also visualised	Minimally	Research tool in the UK. Offered in a few centres in USA & Europe.

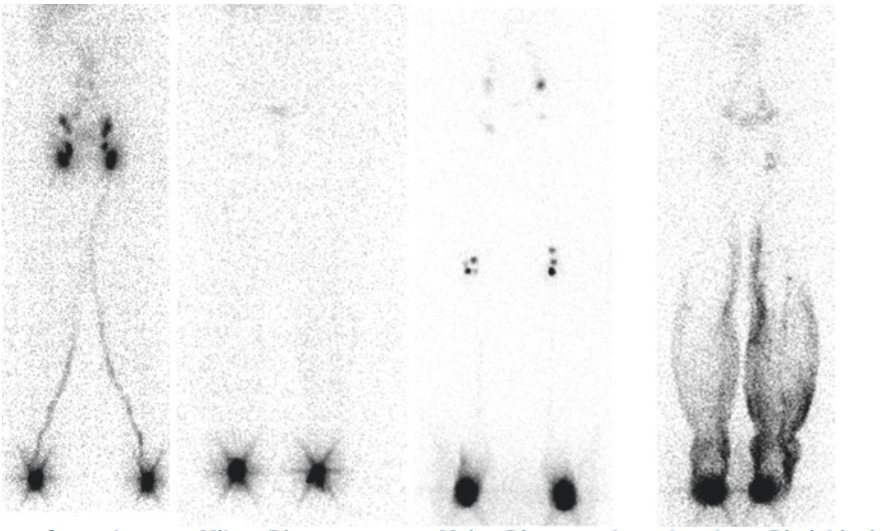

Fig. 27.5 Comparison of lymphoscintigraphy results in primary lymphoedema. (1) A healthy control demonstrating uptake of tracer from the injection sites in the feet to the inguinal lymph nodes via the main lymphatic tracts. These images are taken 2 h after the tracer was injected into the subjects' feet. (2) A patient with Milroy disease is unable to transport tracer from the injection sites in the feet because the initial lymphatic vessels are unable to function adequately. After 2 h there is no uptake of tracer within the lymphatic vessels or lymph nodes due to a "functional aplasia" of the lymphatic system. (3) A patient with Meige disease is able to transport tracer from the injection site in the feet up to the inguinal lymph nodes. However, tracer is re-routed via the deep lymphatic system, as demonstrated by uptake of tracer within the popliteal lymph nodes. (4) Patients with lymphoedema distichiasis syndrome have abnormal lymphatic function due to valve defects within the vessels. This is demonstrated on lymphoscintigraphy imaging: the tracer is absorbed by the initial lymphatic vessels and begins to be transported up the limb, but then refluxes back down, creating an outline of the limb in the process

lymph drainage and may discriminate lymphoedema from lipoedema [51, 52]. Lymphoscintigraphy has the potential to distinguish between different types of primary lymphoedema and their mechanisms of lymphatic failure i.e. initial lymphatic dysfunction in Milroy disease, versus lymph collector reflux in lymphoedema-distichiasis syndrome (Fig. 27.5). However, lymphoscintigraphy suffers from poor spatial resolution and cannot provide functional measurements of flow [53].

27.7.2 Near Infra-Red Lymphangiography

Near infra-red lymphangiography (NIR) using indocyanine green (ICG) is a recently introduced and potentially useful technique used to demonstrate superficial lymphatic vessels [54]. NIR imaging techniques use tracers which fluoresce under the excitation of near infra-red waves and external fluorescent detectors. ICG is a dye

with properties of NIR absorption and fluorescence emission. ICG-solution is injected intra-dermally or subcutaneously and enables real-time detection of the lymphatic vessels.

NIR has been used to assess lymphatic vessel structure and function [55]. NIR is now widely used to assess lymphatic vessel patency prior to lymphatico-venous or lymphatico-lymphatic anastomosis surgery [56].

27.7.3 Magnetic Resonance Lymphangiography (MRL)

The use of contrast-enhanced magnetic resonance lymphangiography has been used in the investigation of patients with lymphoedema [57]. MRL has been used to demonstrate the presence of abnormally enlarged and tortuous lymphatic vessels in patients with unspecified limb lymphoedema [58] (Fig. 27.6). However, it has yet to prove helpful in refining the subtype of primary lymphoedema as no clear correlation exists between the phenotype of primary lymphoedema and the MRL findings [59]. A developing area is the use of MRL to visualise central lymphatic abnormalities and assist with interventional treatment e.g. in patients with chylous effusion of the heart/lungs/ascites [60].

27.8 Management of Lymphoedema

Unfortunately there is no proven curative treatment for lymphoedema. Management is aimed at controlling swelling through physical treatments designed to stimulate flow through existing or collateral drainage routes. Several surgical techniques

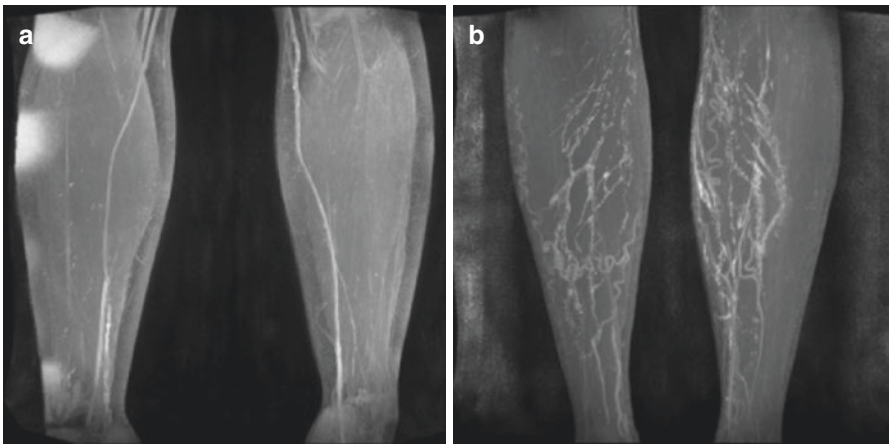

Fig. 27.6 (**a**) Post-contrast MRL of a healthy volunteer demonstrating multiple lymphatic vessels within the lower legs. The vessels appear to have small valves within them (on close inspection). (**b**) A patient with Lymphoedema distichiasis syndrome' MRL images (post-contrast) demonstrating multiple dilated, tortuous lymphatic vessels are seen in the below-knee regions

have been implemented in recent years in a bid to improve lymphatic drainage, or achieve limb volume reduction via liposuction. However, in the absence of robust data to support most surgical techniques, we must rely on so-called "conservative" physical therapies for the majority of patients. Hopefully gene therapy will be available as an additional treatment for some forms of primary lymphoedema in the future.

27.8.1 Physical Therapies

Compression is the most important component of lymphoedema treatment and cannot be replaced by any other modality. The International Society of Lymphology has developed guidelines regarding the management of lymphoedema [40]. The physical treatment of lymphoedema, often called "Decongestive Lymphatic Therapy (DLT), should occur in two distinct stages. The goal of the intensive first stage (Reduction Phase) is to "disorganise" the lymphoedema, reduce the volume and discomfort. In order to do so, a combination of techniques should be used; manual lymph drainage, multi-layered bandages, exercise and skin care. The use of intermittent pneumatic compression has also been suggested. The goal of the second stage of treatment (Maintenance Phase) is to maintain the results achieved in the reduction phase. The techniques used in this phase are: compression with low-stretch elastic hosiery garments, specific exercises and skin care.

27.8.1.1 Reduction Phase Treatment

The intensive reduction phase of lymphoedema treatment is usually offered to patients with significant swelling (of any body site) in order to achieve considerable volume reduction (with inelastic bandages or Velcro strapping systems) before fitting them with compression hosiery with a view to maintaining the smaller (limb) volumes. The majority of patients with mild lymphoedema, typically of a limb, do not require intensive bandaging therapy and can be directly measured for appropriate compression hosiery.

The duration of reduction phase therapy varies across the globe but typically involves daily MLD (massage), bandaging and exercises once daily for an approximate duration of 2–4 weeks. Treatment duration will be determined by the assessing physician or lymphoedema therapist, but should be continued until pitting oedema has resolved and long-term compression hosiery commenced. A small number of patients require a prolonged course of treatment if they have significant limb distortion or severe, neglected chronic lymphoedema.

27.8.1.2 Maintenance Phase Treatment

Compression hosiery limits the amount of fluid accumulation within the tissues affected by lymphoedema. It acts as a counterforce to muscle contractions to improve lymphatic drainage. Compression garments have a graduated compression profile (more strength at the foot/hand than at the top of the garment), ensuring lymphatic fluid is directed towards the root of the limb (groin/axilla). Effects are enhanced when a patient wears their compression hosiery during exercise. Compression hosiery is available in many different styles (below-knee or full-length stockings, half or full tights and sleeves) and degrees of asserted pressure. Complex limb lymphoedema may require the use of high compression and/or double layers of garments. Most garments last no more than 6 months. A minimum of two sets of garments should be provided (one to wear whilst the other is washed). The patient's technique for the application, removal and care of garments is crucial for a successful outcome. Compression garments are beneficial in the management of lymphoedema and it is possible to achieve volume reduction with compression hosiery and exercise alone.

Compression can also be achieved with the use of Velcro strapping systems applied to swollen limbs (Fig. 27.7). These adjustable garments are utilised in the 'maintenance' phase of lymphoedema treatment, often for patients with significant comorbidities who are unable to comply with standard compression hosiery. These Velcro garments can be applied by the patient and/or carers after minimal training. One advantage over bandaging is the option to adjust them (i.e. apply more pressure

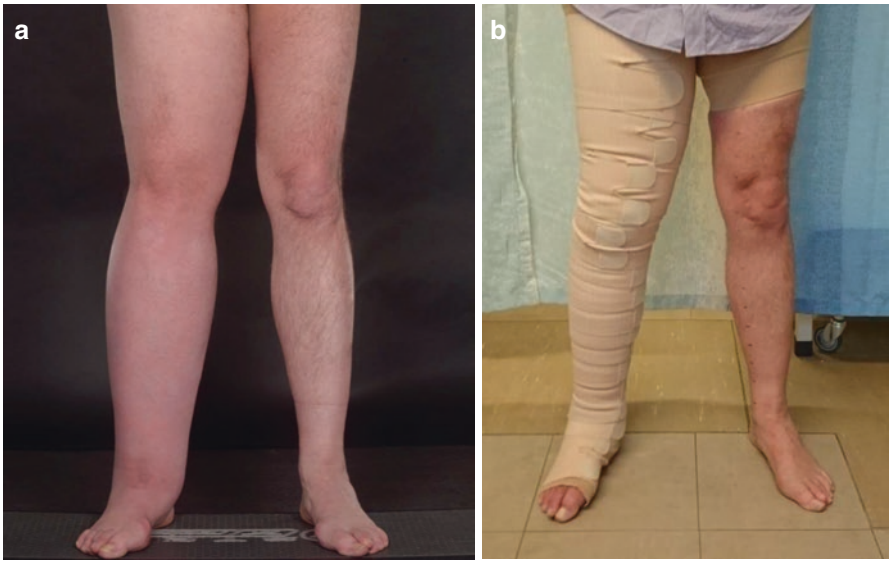

Fig. 27.7 (**a**) Patient with right lower limb secondary lymphoedema. (**b**) Same patient as above (**a**) receiving compression treatment with Velcro-strapping system

to the limb) during the day to overcome issues with 'slippage' during periods of limb volume reduction. There is a recent realisation that these Velcro strapping systems can also be utilised in the intensive reduction phase of treatment. A recent randomised controlled trial demonstrated significantly more leg volume reduction from the use of the Velcro system when compared to conventional inelastic bandages [61].

27.8.1.3 Manual Lymphatic Drainage (MLD)

Manual lymphatic drainage is a massage technique performed by lymphoedema therapists. The aim is to re-route the accumulation of lymph from the swollen region via collateral lymphatic pathways to lymphatic basins that are able to drain normally. The initial step in MLD is to decongest the central /proximal areas before massaging the oedematous region. This facilitates the drainage of lymph via lymphatic vessels and pathways that have been stimulated by the massage technique. Tissue movement must be gentle if it is to stimulate lymph flow without increasing blood flow [62]. There is little evidence in the literature to support its use [63–65]. However, MLD is widely practised and many patients, therapists and physicians advocate the benefits.

The recent development of lymphofluoroscopy (Indocyanine Green Lymphography) as a tool to map patient's superficial lymphatic drainage pathways is proving of interest to patients and lymphoedema therapists alike. Lymphatic drainage pathways differ between patients as a result of differences in normal anatomy and damage from external factors (e.g. cancer treatment or infection). Lymphofluoroscopy mapping involves the intradermal injection of indocyanine green tracer (ICG) that is taken up by the initial lymphatic vessels and the superficial lymphatic pathways can be mapped out on the skin, and documented for future MLD treatments. This imaging technique could facilitate the development of a bespoke treatment protocol, maximizing the benefits of MLD for each patient.

27.8.1.4 Intermittent Pneumatic Compression

Pneumatic compression therapy (intermittent sequential pneumatic compression) should not be used in preference to compression garments and exercise but can be a useful adjunct in the treatment of some cases of lymphoedema [66]. An inflatable boot or sleeve is connected to a motor-driven pump and lymph is displaced proximally towards the root of the limb. If hosiery is not fitted immediately following the use of pneumatic compression, the lymphoedema will rapidly recur. Few studies support the beneficial use of pneumatic compression in lymphoedema [67]. Pneumatic compression may soften the tissues and reduce limb volume during treatment, but it is doubtful that any long-term benefit is gained over the use of compression hosiery and exercise.

27.8.2 Pharmacological Therapies

There is little use for drug therapy in the management of lymphoedema. Diuretics alone demonstrate minimal improvement in lymphoedema, as their mode of action is to reduce capillary filtration by a reduction in circulating blood volume. Paroven (an oxerutin) and Coumarin (a benzopyrone) have been trialled in lymphoedema and may create a small reduction in limb volume by reducing vascular permeability and thus the amount of fluid forming in the subcutaneous tissues. However, this has been shown to be of little clinical benefit to the patient [68].

The link between lymphatic function and inflammation has recently been explored from a therapeutic perspective. Research in animal models has demonstrated the efficacy of specific topical immunomodulatory therapies in treating secondary lymphoedema [69]. This exciting development has led to human trials with specific anti-inflammatory agents, with the results eagerly awaited by patients and clinicians alike [70].

27.8.3 Surgical Options

27.8.3.1 Excisional Methods

Excisional surgical procedures for the management of lymphoedema, regardless of the underlying cause, have been employed for more than a century. They are now rarely employed as the post-operative complications can be disastrous (e.g. with exacerbation of swelling and/or recurrent infections). An example of a recognised lymphatic surgical technique includes the "Charles procedure" where the patient undergoes complete resection of the skin and subcutaneous tissue of the lymphoedematous region, and receives a full thickness skin graft to restore the tissue defect [71]. It has largely been abandoned because of problems with massive skin transplant necrosis, poor cosmetic results, and worsening lymphoedema distal to the surgical site. None of the excisional procedures have curative potential as all chances of restoring effective lymphatic transport have been surgically removed. Volume reduction may be achieved through tissue reduction, but not through lymphatic drainage improvement. The indications for this type of surgery must be restricted to rare cases lacking alternative conservative or surgical treatment options.

27.8.3.2 Lymphatico-Venous Anastomosis (LVA) Surgery

Recently there has been much interest in a surgical procedure called lymphaticovenous anastomosis surgery (LVA). LVA is a type of lymphovenous bypass, utilising a supermicrosurgical technique to anastomose distal subdermal lymphatic

vessels with adjacent venules less than 0.8 mm in diameter in an attempt to improve regional lymph drainage and potentially remove the need for using hosiery (a scenario that many patients are desperate to achieve). It is popular amongst surgeons as it is performed under local anaesthetic and the surgical incisions are small. Post-operative complications include lymphatic vessel occlusion possibly due to thrombus formation within the lumen [72]. Prior to LVA surgery, the patients undergo indocyanine green fluorescent imaging to locate functional patent lymphatic vessels within the affected limb.

A review of the literature suggests that the efficacy of LVA surgery is questionable. Campisi et al. published significant volume reduction in 83% of their 1800 patients, with a mean volume reduction of 67% [73]. Other groups report different outcomes. For example, Damstra et al. performed LVA surgery on the upper limbs of 11 patients and reported a mean volume reduction of only 4% after 3 months and no significant volume reduction seen after 1 year. Lymphoscintigraphy failed to demonstrate patency of any bypass procedures, with the authors suggesting the evidence for LVA success is limited [74].

In summary, there is a lack of long-term data as the technique is relatively new. However, some surgeons favour its use because of the low risk of complications. There is currently no method of determining its effect on lymphatic function. The development of imaging techniques could provide a tool to answer the question of its place in lymphatic treatment strategies.

27.8.3.3 Lymph Node Transfer Surgery

Autologous transplantation of normal lymphatic tissue within a local or free flap to a region that is deficient of lymph nodes and vessels has been performed by surgeons. The aim is to transplant normal lymph nodes to encourage and improve lymphatic drainage in a previously oedematous region. Becker et al. reported a series of 24 patients receiving Deep/Superficial Inferior Epigastric Perforator flap (DIEP/SIEP) transposition to the axillary region with post-operative MLD for 6 months. Ten patients were apparently cured of their lymphoedema (41.6%), an additional 6 patients experienced more than 50% permanent volume reduction, 6 patients achieved less than 50% reduction and 2 patients had no improvement [75]. Similarly, Lin et al. transferred a vascularised groin lymph node flap to the wrist with anastomosis of superficial circumflex iliac vessels to the radial artery in 13 patients and reported a mean reduction in arm circumference reduction of 50.5% [76]. Fewer surgeons seem to be performing this type of surgery but the reported results appear encouraging. However, this surgery relies on the transfer of normal lymph nodes in order to improve lymphatic drainage. This may be possible in a patient with secondary lymphoedema, but concerns exist over its use in patients with primary lymphoedema who may not have “normal” lymph nodes, or “normal” lymphatic vasculature, even at unaffected sites.

27.8.3.4 Liposuction

Chronic lymphoedema may be associated with excess fatty tissue deposition within the swollen region in some patients. The mechanism is thought to be due to abnormal adipogenesis secondary to inflammatory changes within lymphoedematous tissue [77].

Excess adipose tissue will not respond to decongestive lymphoedema treatments, anastomosis surgery or lymph node transfer procedures. Liposuction creates significant volume reduction in therapy-resistant lymphoedema of the extremities, when combined with lifelong compression therapy [78]. This adjunctive therapy is only offered to compliant patients willing to wear lifelong compression garments. Warren reports that if patients disregard regular use of their garments, a relapse or worsening of lymphoedema is observed as liposuction may cause injury to remaining subcutaneous lymphatics within lymphoedematous extremities [78]. Brorson reports complete reduction of excess volume in compliant patients that were followed for up to 17 years [33]. Liposuction techniques, although not curative, offer an effective symptomatic treatment [79].

27.9 Summary

This chapter aims to provide the clinician with a greater understanding of the aetiology of lymphoedema and increased knowledge for managing this condition. Lymphoedema is a chronic debilitating condition that may lead to significant physical and psychological morbidity. Swelling results in discomfort, reduced mobility and impaired function. Recurrent infections further complicate the management of this challenging condition. Many patients currently receive inadequate care as lymphoedema fails to receive appropriate recognition by the medical community. Surgical management will only benefit select individuals, but daily compression therapy is proven to be effective in the majority. Emerging evidence of the relationship between the lymphatic system and chronic inflammation should lead to the development of adjunctive oral therapies. The future of lymphoedema care will undoubtedly improve thanks to research, but clinicians must still endeavor to diagnose and treat lymphoedema in a timely manner in order to avoid complications.

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Chapter 28
Graft Materials: Present and Future

Mital Desai and George Hamilton

Key Learning Points

- Synthetic materials such as polyethylene terephthalate (Dacron) and expanded polytetrafluoroethylene (ePTFE) have been successfully used in large diameter vascular grafts but their results are consistently poor as diameter decreases below 6 mm.
- Thrombosis, lack of endothelial coverage, compliance mismatch and haemodynamic imbalance are the main factors responsible for early and late graft failure.
- Both Dacron and ePTFE have undergone several modifications to address their limitations, including heparin bonding, electrospinning, gelatin/polymer coatings and modification with sulfonated silk fibroin, without any significant success.
- Polyurethane grafts have been investigated with studies showing superior thrombo-resistance, rapid ingrowth of living tissue and reduced anastomotic hyperplasia. However, the major problems have been relative thrombogenicity and aneurysmal degeneration.
- Biografts, vascular grafts made from biological sources such as bacterial cellulose, allogeneic grafts and biotubes have been used but not yet gained widespread clinical acceptance.
- Over the last decade, we have seen significant progress in cardiovascular tissue engineering. The three essential components of tissue engineering consist of (1) the cells that are seeded onto the graft, (2) the scaffold onto which the extracellular matrix (ECM) forms, and (3) the humoral and mechanical signals that regulate neotissue formation and maintenance.

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- The current climate of vascular tissue engineering is very promising. Significant progress has been made in the development of recent technologies in scaffold engineering and cell engineering, including gene delivery and improvement of endothelial progenitor cells.
- With on-going development to match as closely as possible both the mechanical characteristics and biological functions of normal human arteries there is real potential for new graft development within the next decade.

28.1 Introduction

Polyethylene terephthalate (PET or Dacron) and expanded polytetrafluoroethylene (ePTFE) grafts have been very successful in replacing medium and large diameter vessels. However, as diameters decrease below 6 mm, their performance has been consistently poor. Autologous vein remains the conduit of choice for infrageniculate bypasses. However almost a third of patients do not have a suitable available vein [1] due to the patient having varicose veins, previous removal, bypass or phlebitis, fibrosis or trauma, or if the vein is too small. In these patients, prosthetic grafts are required. The ideal properties of vascular grafts are summarised in Table 28.1. Prosthetic materials have a tendency to fail due to thrombosis and insufficient healing with incomplete endothelial cell coverage. Compliance mismatch and haemodynamic imbalance are further factors leading to intimal hyperplasia (IH), one of the fundamental causes of graft occlusion.

As a result, much effort has been directed to developing better small-calibre vascular grafts, surgical techniques have been modified, novel biomaterials have been investigated and cell and tissue culture technologies have been adopted. Partly or fully tissue-engineered vascular grafts have been produced and experimentally and clinically evaluated with some promising results. This chapter will review

Table 28.1 Properties of an ideal vascular conduit [2]

Mechanical features
• Strong
• Compliant
• Kink resistant
• Good suture retention
Biocompatibility
• Nontoxic
• Nonimmunogenic
• Low thrombogenicity
Availability
• Readily available in a variety of lengths and diameters
• Economic
• Low manufacturing cost

vascular graft materials in current use and in development with special attention to graft modifications and tissue engineering.

28.2 Pathophysiology of Graft Healing

The tissue response to implantation of a prosthetic graft is complex with many variable factors involved such as the material used, its construction, its porosity, and its length. Further important factors relate to the interaction between the graft and the host artery at the anastomotic areas. A major stimulus for study of this area is the still unresolved puzzle of man's inability to endothelialise a prosthetic graft beyond the immediate 2 cm or so from the anastomosis.

28.2.1 The Peri-Anastomotic Area

Prosthetic grafting involves injury to the artery from the direct trauma of implantation, and subsequent exposure of the anastomotic areas to haemodynamic stress (compliance mismatch, turbulent flow and altered shear stress). Following implantation, in arteries or veins and especially in the peri-anastomotic area, there is fibroblast and smooth muscle cell (SMC) accumulation in the intima with extracellular matrix (ECM) deposition. The end result of this excessive cellular deposition is expansion of the intimal layer, termed as "neointima" which is associated with loss of peri-anastomotic luminal area.

The three phases of intimal hyperplasia develop quite rapidly with the first being proliferation of medial smooth muscle cells as soon as 24 h after injury and lasting up to 4 weeks. The second phase of SMC migration into the intima starts as early as 4 days after injury and continues for about a month. The final phase is of intimal expansion due to the dual action of SMC migration and intimal proliferation by deposition of matrix proteins such as collagen, elastin and proteoglycans. This phase is complex and is mostly self-limiting but may continue unabated if certain factors are present (Fig. 28.1a–c).

Low or changing shear stress direction (turbulent flow) promotes endothelial proliferation and apoptosis (programmed cell death), shape change, and reduced secretion of nitric oxide (NO). Conversely flow change towards high shear stress and endothelial regrowth in the injured area alters the balance between stimulatory and inhibitory factors leading to a decreased drive towards intimal hyperplasia. If this balance is not achieved because of ongoing factors such as lack of endothelial cover, major haemodynamic disturbance such as severe compliance mismatch or turbulent flow with areas of stagnation and low shear stress, then the drive towards intimal hyperplasia will continue unabated leading to severe narrowing at the anastomosis and graft failure.

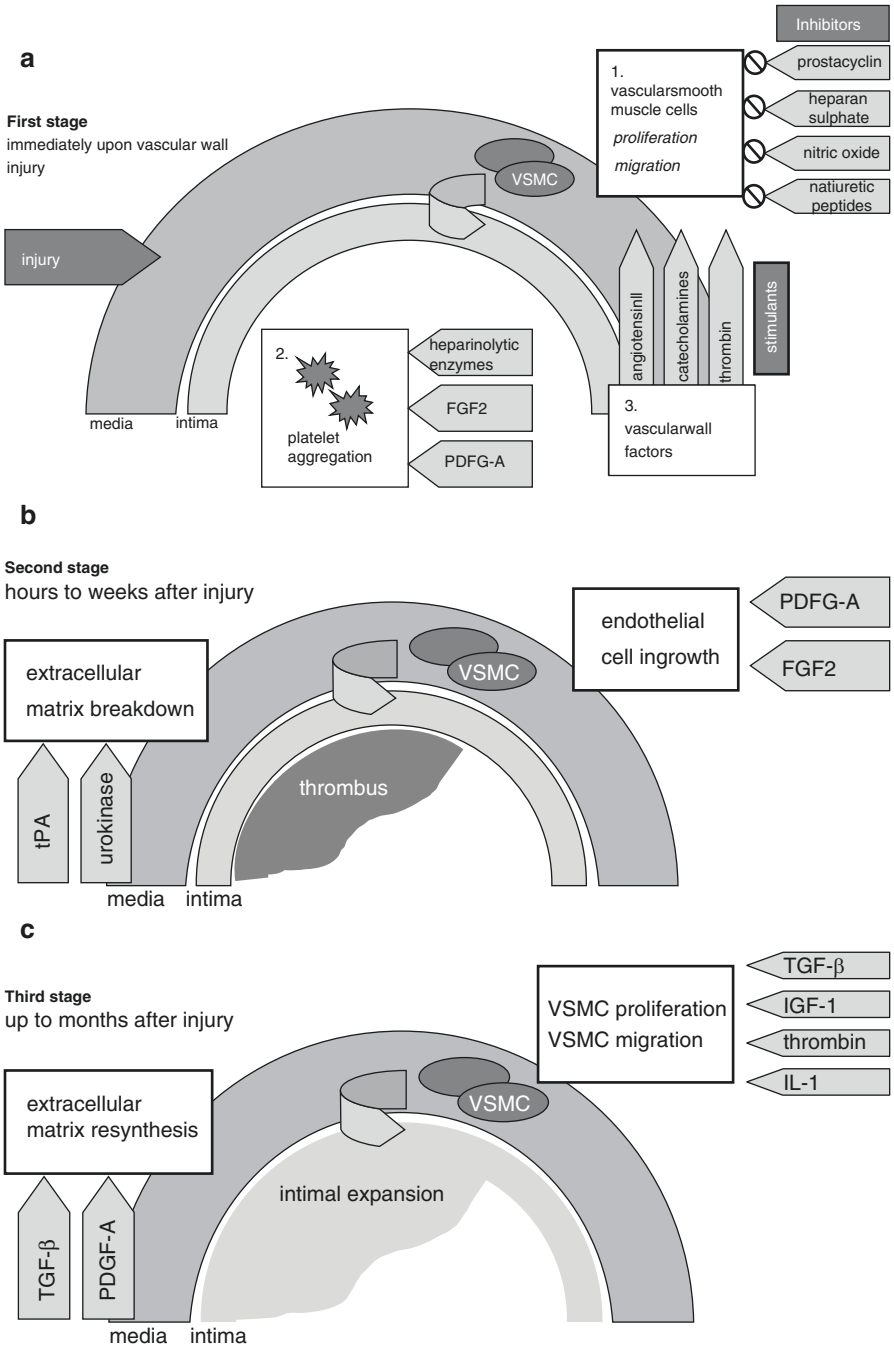

Fig. 28.1 (**a–c**: first stage to third stage) Stages of intimal hyperplasia formation (From Scharn et al [3] *reproduced by permission*)

28.2.2 Healing of Prosthetic Grafts

Healing of prosthetic grafts takes place by two main mechanisms, capillary ingrowth through the graft wall, and growth of endothelial cells along the luminal surface of the graft from each anastomosis [4]. Almost all studies of prosthetic graft healing in animal models have used short lengths of graft, typically 10 cm or less, which readily developed a full lining of endothelial cells. Virtually all animals used in these studies were young. In man however, endothelialisation is restricted to the first centimetre or two of the anastomotic regions with no evidence of healing having taken place beyond this area.

None of the clinically used prostheses spontaneously develop a neo-intima, except for sporadically observed small islands of endothelium. "Fall-out healing" characterised by isolated endothelial islands with no connection to either the anastomotic or adventitial areas, occurs more often on PET than ePTFE grafts. However, these scanty, late-occurring islands do not result in significant healing or endothelialisation in contemporary vascular prostheses [5].

28.2.3 Graft Porosity and Permeability

Porosity and permeability are two distinct terms that describe different characteristics of vascular prostheses. Porosity is a measure of the void fraction within the prosthesis wall and gives a rough prediction of the capacity of the graft to anchor newly formed surrounding tissue after implantation. Permeability indicates the rate at which fluid can flow through the prosthesis wall when measured under physiological pressure conditions. Zilla suggests that to facilitate transmural healing and endothelialisation, graft spaces should be wide enough to allow ingrowth of a capillary tuft with accompanying fibroblasts or pericytes requiring minimum pore diameters of 60–80 μm [6]. Currently available grafts, even those described as having high porosity fail in this regard (Table 28.2).

Soon after graft implantation, the interstices become filled with fibrin and matrix similar to any early wound. Macrophages form part of a normal inflammatory response releasing cytokines to stimulate migration and proliferation of fibroblasts and endothelial cells. In the later stages however, macrophages persisting in large number may have an adverse effect on healing and ingrowth. Consistently the outer portion of the graft has high concentrations of macrophages and foreign body giant cells while the deeper layers lose these cells, probably due to the dense impermeable and impenetrable nature of the fibrinous pannus. PET seems to be more inflammatory than PTFE where less giant cells are found.

Table 28.2 The effects of graft porosity [7]

Low porosity ePTFE grafts: <45 μm of internodal distance
• Low porosity ePTFE grafts (<30 μm): no difference in healing between animal and human
• Within 2 weeks surface is covered with fibrin and platelet thrombus 15 μm thick, which over following months increases to between 80 and 300 μm
• Pannus persists for years and is actively thrombogenic
• Ingrowth of connective tissue is limited to the outer graft wall
High porosity ePTFE grafts: >45 μm internodal distance
• First layering similar to that of low porosity ePTFE grafts
• In older animals very little ingrowth—luminal thrombus without any cellular component
• Early and spontaneous endothelialisation is found in young animals
• These changes happen as early as 1–2 weeks
– Patches of endothelial cells and capillary orifices found approximately 100–500 μm apart which proceed to confluence
– These endothelial cells lie over a layer of arterial smooth muscle cells probably derived from pericytes
– Stable neo-intima evenly distributed along the surface, as compared to the limited peri-anastomotic coverage in low porosity grafts
– This extensive endothelialisation arises from cells reaching the luminal surface by transmural ingrowth
– In older primate models and also in the dog these developments take longer but only with sprouting capillaries reaching the outer third to one half of the graft wall
Low porosity PET grafts (woven)
• Immediately after implantation thin pannus of fibrin and platelets deposited on the surface.
• Thrombus compacts over time and in man stabilises after 1 year
• Endothelialisation does not happen either in animals or in humans
– Small islands of endothelial cells found after many years in explants in man
• Narrow graft interstices filled with fibrin
• Foreign body giant cell reaction present
• Variable spread of some capillaries and fibroblasts into interstitial spaces never breaks through the compacted fibrin of the inner lining
High porosity PET grafts (knitted)
• Initial pannus same as woven PET but develops to a thickness of 100–120 μm increasing to 500 μm by 6 months
• In dogs and other animals this inner lining replaced with a confluent layer of smooth muscle cells resting directly on the graft surface, covered by endothelium
• These come from anastomotic ingrowth but in longer grafts endothelialisation in the mid-graft region fails to occur despite partial ingrowth of capillary fibroblasts from the adventitia

28.3 Vascular Graft Failure

The processes involved in vein graft failure are illustrated in Fig. 28.2. The main causes of prosthetic graft failure can be linked to the time of occurrence.

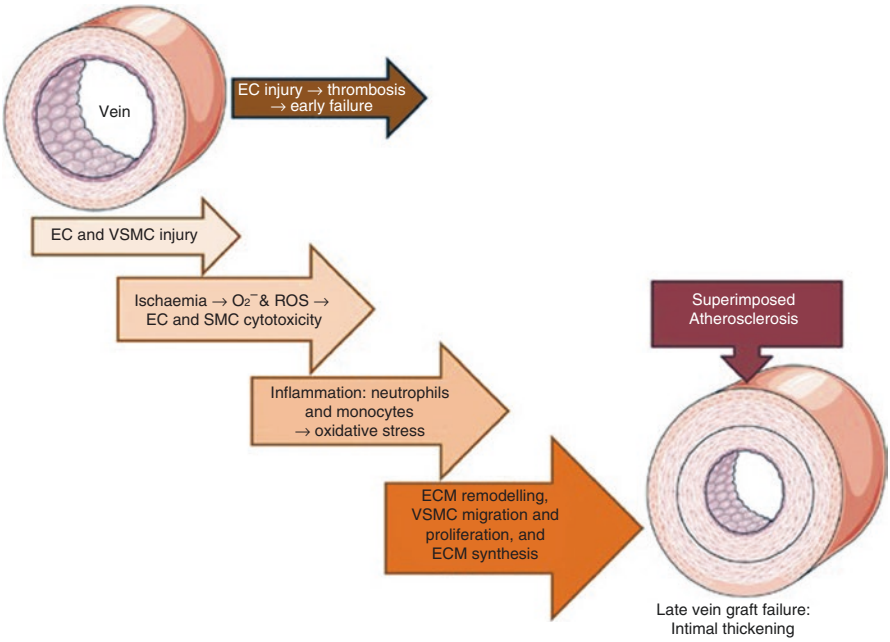

Fig. 28.2 Processes involved in vein graft failure (Reproduced from Wan et al [8] *by permission*)

28.3.1 Early Failure

Thrombosis is the main cause of early graft failure. It depends primarily on the surface properties of the graft due to the activation of a cascade of events at the blood-graft interface; this ultimately leads to formation of thrombin that facilitates cleavage of fibrinogen to fibrin.

28.3.2 Late Failure

Development of IH following prosthetic graft implantation is the main cause of late failure and occurs through two major mechanisms: (1) immune system related and (2) compliance mismatch related.

In the immune system related mechanism, the foreign body response activates macrophages and platelets, which release growth factors such as TGF-β and PDGF, and promote chemotactic migration and proliferation of vascular SMCs to the intima [9].

In the compliance hypothesis of graft failure, arterial wall pulsatility depends on a combination of elastic and viscous components inherent in the structure of the artery, which can therefore be described as being viscoelastic. Most commonly this

property is measured as compliance, defined as the ratio of change in diameter over change in blood pressure (% mmHg × 10^{-2}). Arterial compliance is complex, having both longitudinal and circumferential components but only this latter measurement is commonly quoted when the elasticity of different materials is compared. Compliance mismatch has been implicated as an important factor in the performance of vascular grafts since 1976 [10].

The compliance-related mechanism may be explained as a wall stretch and flow-related cascade of events. Over proliferation of SMCs is the main mechanism involved in intimal hyperplasia development [11] and this suggests a strong link between the reduction in cyclic stretch due to low compliance of the grafts and the development of IH. On the other hand, several studies have shown the influence of compliance mismatch on haemodynamic factors. Stewart et al. [12] numerically showed that low compliance of grafts disturbs the transport and distribution of growth factors and lowers the wall shear stress. Low shear stress and flow separation in the anastomotic region cause platelet adhesion and activation, which release growth factors [9].

Although the immune system-related intimal hyperplasia development may be treated by pharmaceutical interventions and surface modification [13], the compliance mismatch-related intimal hyperplasia is dependent on the physical characteristics of the graft material and thus seems to be the Achilles' heel of most available prosthetic grafts.

Compliance mismatch results from two major components, tubular and anastomotic.

28.3.3 Tubular Compliance Mismatch

Mismatch of tubular compliance is present when there is a significant difference in elasticity between the prosthetic graft and native artery. At the interface between a compliant artery and a non-compliant graft, changes in impedance (defined as the resistance to pulsatile flow) will diminish pulsatile energy by as much as 60% [14]. Furthermore, optimal organ perfusion depends on pulsatile blood flow with a change from pulsatile to static perfusion shown to increase peripheral resistance by 10% [15]. Finally, at the graft to artery interface, there is wave reflection of pulsatile energy, which can lead to increased velocity gradients and turbulence. As a result of these increased vibratory movements and mechanical stresses, endothelial damage and IH occur.

28.3.4 Anastomotic Compliance Mismatch

A sutured anastomosis usually generates a decrease in diameter and drop in compliance determined primarily by the lack of elasticity of the suture material. Interrupted sutures give a more compliant anastomosis, while a continuous technique results in

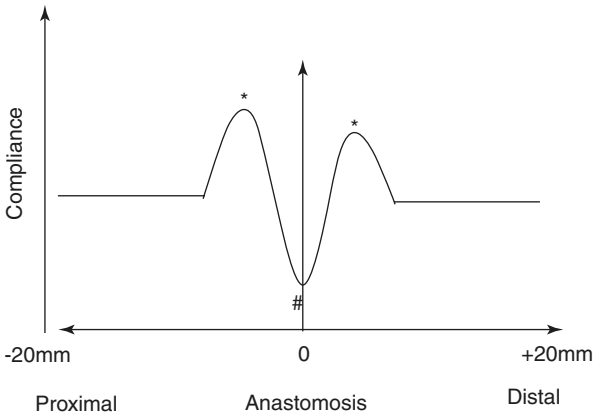

Fig. 28.3 The peri-anastomotic hypercompliant zones (PHZ); compliance at the anastomosis is lower due to the suture (#) while compliance is increased compared to the vessel wall several mms from the anastomosis (*). This effect further aggravates compliance mismatch in bypass grafting. (Source—Adelaide University Press. Permission granted)

a ring of non-compliant suture material—both prolene and PTFE sutures are profoundly non-elastic. Within a few millimetres on either side of the suture line, there is a paradoxical increase of compliance, which is known as the para-anastomotic hyper-compliant zone (PHZ) [16] (Fig. 28.3). Intimal hyperplasia develops typically in these areas of hyper-compliance.

Compliance mismatch will lead to a region of excessive mechanical stress which can give rise to subtle arterial wall injuries and initiate the first phase of intimal hyperplasia. Cyclical stretching is known to have a positive influence on proliferation of vascular smooth muscle cells and production of extracellular matrix. This increased cyclical stretch at the zones of PHZ, will cause proliferation of the smooth muscle cells.

Finally changes in compliance are known to affect flow and shear stress. Where there is turbulent flow, there will be areas of low shear stress and this is known to promote endothelial proliferation, apoptosis and reduce production of nitric oxide (NO). The evidence for the compliance hypothesis is not conclusive but analysis of the clinical performance of grafts of differing compliance reveals a positive correlation between compliance and patency rates. The most commonly used prosthetic grafts, namely PTFE and PET are profoundly rigid over the physiological pressure range. A physiologically important feature of the visco-elastic nature of human artery is compliance which diminishes with increasing pressure but increases exponentially as mean pressure falls below 80 mmHg, thus maximally conserving pulsatile energy in shock. The ideal prosthetic graft should share this property.

28.4 Prosthetic Grafts

The history of prosthetic grafts began in 1952 with successful placement of Vinyon–N tubes into the abdominal aorta of dogs, and subsequent human implantation in 1954 in 18 patients [17]. An explosion of interest followed, with prosthetic grafts being made from various textiles but their major problem was loss of tensile

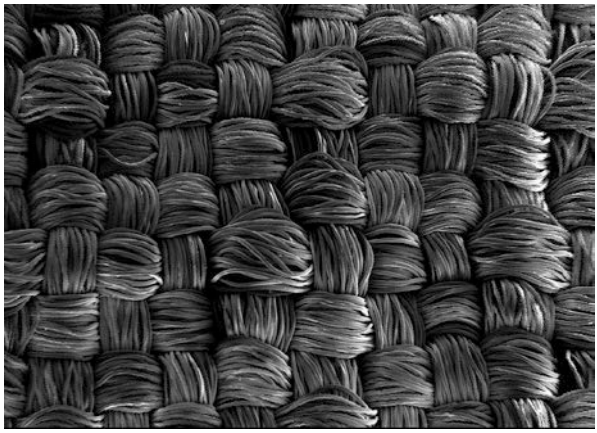

Fig. 28.4 Scanning electron micrograph of woven PET graft; note the tight interlacing of the yarn and minimal potential for porosity

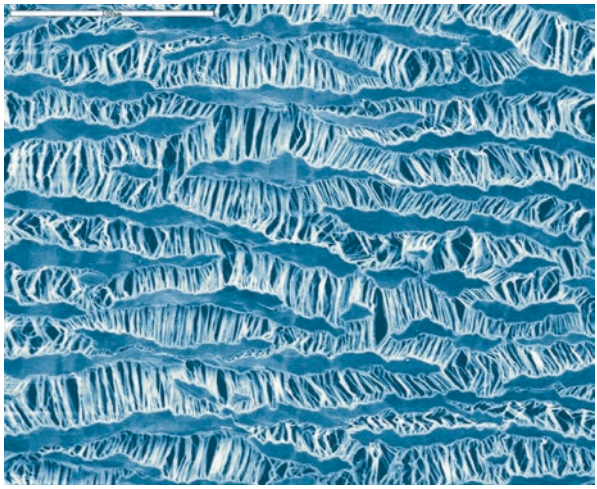

Fig. 28.5 Scanning electron micrograph of inner surface of ePTFE graft; note the characteristic node-fibril structure

strength. Two materials proved to be resistant namely PET (Fig. 28.4) and PTFE (Fig. 28.5), and because of their bio-durability have dominated graft development to this day.

28.4.1 Modifications of PET Grafts

28.4.1.1 Heparin Bonding

Heparin coating has been utilised for improving biocompatibility of PET. Besides enhancing the function of heparin-binding proteins, immobilised heparin also potentially reduces PET hydrophobicity. This change in surface chemistry might alter the proteins present at the interface, thereby influencing biocompatibility

independent of the biological action of heparin. It has been shown that this is associated with exposure of the fibrinogen P2 epitope as well as the adhesion of monocytes [18]. Independent of the inflammatory response, the hydrophilic nature of the heparin coating may affect tissue interaction (reduction in cell adhesion, growth and mobility). Overall, compared to human umbilical vein (HUV) or PTFE, heparin-bonded PET shows significantly better primary patency up to 2 years but not at 5 years of follow-up [19, 20].

28.4.1.2 Electrospinning

Electrospinning (see Glossary) is capable of producing fibrous interconnected network scaffolds at the micro and nano scales mimicking the morphology of the native extracellular matrix (ECM) with the potential to stimulate surface endothelialisation [21]. Electrospun PET is a promising material for small calibre vascular graft applications owing to its tuneable mechanical properties, biocompatibility, and nanofibrous structure that mimic the morphology of natural extracellular matrix [22]. However, the inherent inertness of PET impairs the luminal adhesion and proliferation of endothelial cells on electrospun tubular grafts, hindering the formation of a functional endothelium.

28.4.1.3 Gelatin Coatings

Gelatin coatings, owing to their ability to promote endothelialisation, are a valuable approach to overcome some of these limitations. The proposed coating technique by Pezzioli et al. [22] successfully combined the advantages of the nanofibrous structure of electrospun PET with incorporation of cross-linked gelatin, thus providing both biological cues and mechanical reinforcement. This technique is promising for the development of biocompatible small calibre vascular grafts with superior biomimetic and mechanical properties.

Giol and colleagues [23] have described a proof-of-concept surface modification procedure specifically engineered to simultaneously promote surface endothelialisation and antithrombotic properties. This is via covalent bonding of gelatin through photoactivated azide derivatives. Superior bio- and haemo-compatibility was confirmed for the gelatin-covalently modified PET surfaces compared to the conventional surface-modification procedures.

28.4.2 Modifications of ePTFE Grafts

ePTFE grafts in the below-knee (BK) position have shown poor 1-year cumulative patency rate of 65% and at 2-years of 29% [24]. Prosthetic materials in the above-knee position (AK) have also shown lower patency rates as compared to autologous

vein. The ePTFE graft has been modified in various ways. Thin wall ePTFE grafts have improved handling characteristics but still have an outer wrap to provide strength. Stretch ePTFE grafts have improved longitudinal rather than circumferential elasticity with better handling characteristics but no clinical benefit has been demonstrated in clinical studies. External support, either rings or spirals, is thought to improve patency in extra-anatomic (axillo-femoral or femoro-femoral) or below knee grafts.

28.4.2.1 Heparin-Bonded ePTFE Grafts

New ePTFE grafts with long-term covalent linkage bonding of heparin reduce platelet aggregation and inhibit IH on the prosthetic surface [25]. Some of the ideal functional characteristics of a heparinised graft include uniform heparinisation, retention of heparin on the graft surface, and maintenance of heparin bioactivity. The GORE PROPATEN™ vascular graft (W.L. Gore & Associates, Inc., Flagstaff, AZ) a heparin-bonded ePTFE graft, is commercially available in Europe and has been approved for use in the United States since 2006. The first randomised comparison of the Propaten™ graft with standard ePTFE confirmed significantly decreased relative risk of losing primary and secondary patency by 36% and 40%, respectively [26]. The benefit was most marked in critical ischaemia and in femoropopliteal bypasses, in which the risk of losing primary patency was halved. The 5-year reported outcomes suggest no significant difference in primary patency as compared to standard ePTFE grafts. However in patients with critical limb ischaemia, the use of heparin coating reduced the 5-year risk of loss of primary patency by 37% [27]. This is at the expense of higher cost, although overall heparin coated grafts may be cost-effective due to longer patency in patients with critical limb ischaemia [28]. Whether the results of the Propaten™ graft can be generalised to other heparin-bonded PTFE grafts may be questionable, as the manufacturers claim Propaten™ to be more bioactive due to end-bonding of heparin. Other available techniques result in side-bonding, which could impair the bioactive component of heparin, but published data confirming this possible adverse effect is not available [26].

Another heparin-bonded PTFE graft, the Jotec™ graft, has been tested in a large multi-centre randomised trial in Germany, but the study failed to show overall clinical difference, with the exception of significantly better patencies concerning femoro-popliteal bypass above the knee [29].

Overall these trials suggest that heparin bonding does improve the patency of PTFE grafts. These advantages are related to reduced platelet aggregation, graft thrombogenicity, and inhibition of intimal hyperplasia as compared to untreated PTFE, without causing any measurable systemic anticoagulation effect [30]. In an era of endovascular enthusiasm, with conflicting results for the treatment of long or complex lesions of the superficial femoral artery, above knee bypass with the use of heparin-bonded ePTFE graft remains an effective option, with low rate of perioperative complications and satisfactory long-term results [31].

28.4.2.2 Sulfonated Silk Fibroin Modified ePTFE Grafts

Silks are commonly defined as protein polymers, which are present in the glands of arthropods such as silkworms, spiders, scorpions, mites, and bees, and then spun into fibres during their metamorphosis. Silk is composed of two major proteins: silk fibroin (SF) and sericin. SF offers excellent biocompatibility along with acceptable degradation rates and minimally toxic degradation products, and importantly, elicits few adverse immune responses in host systems [32].

SF-modified experimental grafts may have good long-term patency due to the anticoagulant function of the SF film potentially encouraging endothelialisation. However, further in vivo studies with a longer follow-up of at least 2 years and toxicology analyses are needed before the graft can be applied clinically.

28.4.2.3 Polymer Coated ePTFE Grafts

Bastijanic et al. [33] evaluated the potential for biomimetic self-assembling fluorosurfactant polymer (FSP) coatings incorporating heptamaltose (M7-FSP) to block nonspecific protein adsorption, the cell adhesive RGD peptide (RGD-FSP), or the endothelial cell-selective CRRETAWAC peptide (cRRE-FSP) to improve patency and endothelialisation in small-diameter ePTFE vascular graft implants. Their results in pig carotid artery interposition model indicate that self-assembling polymer coatings can promote selective endothelial cell attachment while reducing platelet adhesion. This technology has potential, pending future studies.

28.5 Polyurethane Grafts

Polyurethanes are segmented polymers initially formulated in the early 1960s to provide elasticity in garment materials (Lycra). These are a very large polymer family of which the most important component is the urethane group present in repeating sequences on the main chain of the polymer. This forms the hard segment providing strength with the soft segment being the other main component (macromonomers ranging from hundreds to over a thousand Daltons). These hard and soft components have a degree of incompatibility, which allows microphase separation and inter-component movement delivering superior visco-elastic and compliant properties. Polyurethane has gained substantial interest because of its tunable biological, biochemical and biomechanical properties. It has high wear resistance, no toxicity, excellent physical/mechanical properties and relative haemo-compatibility with human blood and cells. Polyurethanes also possess excellent blood and tissue compatibility and are in extensive use in access catheters and linings of various prosthetic devices. Clinical experience of conventional polyurethane grafts confirmed their superior thrombo-resistance, rapid ingrowth of living tissue and reduced

anastomotic hyperplasia [34]. The major problems with polyurethane vascular grafts have been relative thrombogenicity and aneurysmal degeneration.

28.5.1 Modifications of Polyurethane Grafts

28.5.1.1 Polycarbonate Polyurethane

Conventional polyurethanes are biodegradable at the soft segment of the polymer particularly at the ester and ether groups found in poly (ester) urethane and poly (ether) urethane. Recent interest has focused on replacing these susceptible groups with other moieties in particular polycarbonate, which is more hydrolytically and oxidatively resistant. A polycarbonate polyurethane is currently available for clinical use, Corvita (Corvita Inc.) and also a renal access graft composed of polyether polyurethane, the Vectra graft (Bard Inc.).

Polycarbonate urethane can be electrospun to produce nanofibrous grafts with addition of functional amide groups by plasma treatment to help in heparin conjugation using end-point immobilisation. These grafts have shown superior endothelialisation in a rodent model; however, the results will need to be confirmed in different animal models as rodents re-endothelialise synthetic grafts much more readily than humans do [35].

28.5.1.2 Composite Polyurethane Grafts

Soldani et al. have developed a new compliant small diameter graft with a poly (ether) urethane–polydimethylsiloxane semi-interpenetrating polymeric network and featuring two different porous wall layers; this showed superior compliance and patency rates in comparison with standard ePTFE, with remodeling in vivo leading to gradual replacement by natural tissue with no sign of calcification [36]. Small-diameter poly (epsiloncaprolactone) polyurethane grafts are a second promising alternative polyurethane with better healing characteristics in vivo compared with ePTFE, with faster endothelialisation and extracellular matrix formation, accompanied by resistance to structural deterioration during remodeling [37].

Using polyester filament yarns knitted into tubular fabrics, a composite reinforced polyurethane vascular graft has been demonstrated to be 5–10 times stronger than pure polyurethane grafts [38]. A bioengineered microporous polycarbonate siloxane polyurethane graft has been developed for coronary artery bypass grafting. Biological agents including heparin and sirolimus can be impregnated into its absorbable collagen and hyaluronan microstructural component, giving a unique drug-eluting graft with endothelialisation without excessive intimal hyperplasia [39]. Biodegradable polymer systems provide the opportunity for release of various growth factors to promote vascular wall regeneration.

28.5.1.3 Endothelial Progenitor Cell (EPC) Homing

Studies have also evaluated the role of circulating EPC homing as a potential means to achieve *in situ* endothelialisation. These cells move toward inner artificial vascular surfaces during early transplantation stages, after which they can differentiate and proliferate, leading to in situ vascular endothelialisation. Many biochemical factors influence EPC mobilisation, homing and differentiation. Amongst these, is stromal cell-derived factor (SDF)-1a/vascular endothelial growth factor (VEGF)—which incorporated into polyurethane (PU) conduits, prepared via electrospinning, showed superior patency at 6 months in a canine femoral artery model [40]. However, while many factors remain unknown, the potential role of EPCs in spontaneous endothelialisation continues to be debated.

28.6 Other Graft Modifications

28.6.1 Modifications to Reduce Thrombogenicity

The presence of a functional and intact endothelium has been shown to improve the patency of prosthetic small diameter grafts. However, endothelialised prosthetic grafts by pre-implantation culture have not received widespread adoption, mostly due to difficulties with the invasive autologous sourcing and need for culture facilities of endothelial cells (ECs). Recently there has been great interest in EC-like cells with high proliferation potential which can be isolated non-invasively from peripheral blood. Endothelial progenitor cells (EPCs) have been used to coat vascular grafts to produce an antithrombotic surface and prevent thrombosis [41]. In vitro studies have shown that EPCs, even when harvested from patients with cardiovascular disease, support a similar antithrombotic phenotype to differentiated ECs when exposed to laminar shear stress [42]. They can be expanded to higher densities with minimal contamination by other cell types, can maintain firm adhesion to the underlying substrate, and can reduce clot formation by releasing anti-thrombotic factors.

In addition to Heparin bonding, Hirudin, one of the most potent thrombin inhibitors, has also been used for graft coating. Grafts coated with recombinant Hirudin displayed minimal thrombus deposition and thinner layers of platelet deposition and protein absorption in animal models [43]. In addition to thrombin inhibitors, attachment to graft surfaces of platelet inhibitors, nitric oxide, tissue factor inhibitors, factor Xa inhibitors, thrombomodulin, activated protein C and fibrinolytics have all been attempted with some positive results in animal models; these have to be interpreted with caution with appreciation of the variable haematology of particular animal species.

28.6.2 Modifications to Reduce Intimal Hyperplasia

IH affects all forms of vascular grafts, including both venous and prosthetic conduits used in coronary and peripheral arterial bypass, and arteriovenous fistulae created for haemodialysis access. An estimated 30–60% of vascular grafts are complicated by clinically detectable IH, with the incidence and clinical consequences varying depending on the type of graft. The predisposing factors include surgical trauma, mechanical forces at the anastomotic site and, in the case of prosthetic conduits, biocompatibility.

28.6.3 Mechanical Prevention

Multiple different strategies have been used to prevent the formation of IH in bypass grafts, including novel techniques aimed at reducing flow variation at the anastomosis and pharmacologic interventions targeting the molecular pathways involved in IH [44]. Various boots, patches and cuffs have been introduced to limit compliance mismatch between prosthetic grafts and native arterial vessel. In a Cochrane review, although improved graft patency rate is reported, the authors have concluded that using pre-cuffed PTFE, PTFE with a vein cuff or spliced vein bypass graft, are not likely to have any effect on the most important clinical outcome, limb salvage [45]. Other strategies adopted include using nitinol mesh to constrict vein diameter with an aim to limit size mismatch between vein and the vessel and creation of a distal arteriovenous anastomosis to increase outflow and minimise areas of sheer stress but none of these have been adopted in clinical practice.

28.6.4 Pharmacological Prevention

As discussed earlier, heparin-coated PTFE grafts have shown promise with excellent short-term patency rates. Recently, interest in NO as a regulator of vein graft adaptation has led to attempts to increase NO production to limit IH. Work in animal models has demonstrated that increased NO production can significantly limit vein graft thickening [46]. There have been clinical trials using NO to limit IH, including the PATENT trial, which uses vein grafts bathed in a solution of nona-L-arginine to provide a sustained reservoir of L-arginine, the substrate for production of NO, thereby increasing NO production [47]. In other investigated methodologies, research into venous remodelling from the Dardik group has suggested that regulation of Eph-B4 pathway, found during embryonic development and also expressed in the adult venous tissue, can be a novel therapeutic target to inhibit intimal hyperplasia and prevent vein graft failure [48].

28.6.5 Other Potential Therapies

In vitro coating of PTFE grafts with autologous endothelial cells has been performed; however, the techniques involved for this treatment have proven difficult and time consuming, with a complex cell culture process and months of advance planning preventing routine clinical use. Another newer strategy has been to apply endothelial cells to the adventitial surface of grafts, capitalising on the ability of endothelial cells to secrete multiple growth regulatory proteins, such as fibroblast growth factor-2 (FGF-2) and heparan sulfate, to prevent abnormal smooth muscle cell (SMC) proliferation. Endothelial cell-loaded gel foam wraps for grafts have been trialed and early clinical trials are underway with potentially positive results [49].

28.6.6 Modifications to Improve Haemocompatibility

The conventional polymeric biomaterials PET and PTFE generally have hydrophobic (low surface energy), chemically inert, and nonpolar surfaces which must cope with many challenges such as undesirable absorption and cell adhesion. Therefore, surface modification has become an effective tool to improve surface function in the design of new and improved biomaterials. Plasma surface modification has been extensively studied over several years. This technology can not only chemically and physically alter the surface composition and microstructure via processes such as etching, chemical reactivity, sterilisation, and ion radiation, but can also manufacture biomimetic surface structures such as thin film coatings, grafting of biomacromolecules, and immobilisation of physiologically relevant proteins [50].

Various bioengineering research strategies aspire to induce endothelialisation of graft surfaces either prior to implantation or by accelerating in situ graft endothelialisation. The longstanding focus for such strategies is a confluent endothelium which can address the inherent thrombogenicity of prosthetic graft surfaces and improve long-term patency of vascular grafts. Adversely, the immobilisation of natural materials on the surface of cardiovascular implants (biomimetic modification) may prove to be thrombogenic by acceleration of platelet aggregation before a fully functional endothelial layer can develop. Hence, surface modification with anticoagulants before endothelialisation is an essential first step [50]. A desirable surface for cardiovascular bypass grafts is one that would be thromboresistant at implantation while attracting circulating endothelial cells (ECs) to attach or adhere upon exposure to eventually result in a fully functional endothelium. Our group has adopted a multidisciplinary approach and demonstrated the potential of plasma treatment and topographical structures on luminal graft surface to enhance self endothelialisation potential of nanocomposite polymer grafts. Although, still in infancy, this methodology merits greater research focus in surface modulation of graft materials [51].

28.7 Biological Vascular Grafts

Biografts, vascular grafts made from biological sources, have been used over many years. Allografts (sourced from the same species) in current use are primarily umbilical and saphenous vein. Other types of grafts, such as decellularised bovine internal jugular xenografts and human allograft vessels from cadavers are prone to aneurysm formation, calcification, and thrombosis and therefore have not gained widespread clinical acceptance [52].

28.7.1 Bacterial Cellulose

Bacterial cellulose (BC) is a novel vascular material with the potential to reduce surface thrombogenicity. BC is a polysaccharide produced by Acetobacter Xylinum bacteria, which exhibits high-mechanical strength, high-water content, high crystallinity, and an ultrafine highly pure nanofibril network similar to that of collagen [53]. The similarity of the cellulose fibre structure in BC with the collagen network in arteries suggests that elastinogenesis in the remodeling process in BC grafts could create a similar structure to arteries showing higher compliance, as compared to saphenous vein, ePTFE and PET [54]. One promising concept is the use of the hydrogel bacterial nanocellulose (BNC) designed in tubular shape. The biodegradability of BC in a physiological environment, however, remains to be investigated by in vivo experiments in order to better understand the long-term evolution of its mechanical properties following implantation.

28.7.2 Allogeneic Grafts

The in-vivo evaluation of cryopreserved human umbilical arteries treated with poly (styrene sulfonate)/poly (allylamine hydrochloride) has demonstrated a high graft patency after 3 months of implantation [55]. L'Heureux et al. have demonstrated the feasibility of assembling arterial bypass grafts exclusively from autologous cells in primate models [56]. No prosthetic or exogenous materials were used; instead, the vessels were created with the use of autologous fibroblasts and endothelial cells harvested from a small biopsy specimen of skin and superficial vein. In vivo results indicated that the grafts were antithrombogenic and mechanically stable for 8 months, with histology and microscopy displaying complete tissue integration, regeneration of a vascular media, as well as elastogenesis and a collagen fibre network.

28.7.3 Biotubes

In-body tissue architecture, a cell-free, in vivo tissue engineering technology that can produce autologous implantable tissues of the desired shape by subcutaneously embedding specially designed moulds, has been used to develop long tubular collagenous tissues called Biotubes. The 25-cm Biotubes functioned as arterial grafts with no need for luminal modification or mechanical support, and demonstrated vascular reconstruction within 3 months after implantation into dogs [57]. Tseng et al. have demonstrated the formation of functional endothelial cells aligned on the inner wall surface achieved by seeding with adipose stem cells. These small-sized adipose stem cell-seeded vascular Biotubes may decrease the rate of intimal hyperplasia during longer implantation times and have potential clinical applications in the future [58].

28.8 Vascular Tissue Engineering

Over the last decade, we have witnessed a dramatic paradigm shift in cardiovascular tissue engineering that has driven the field away from biomaterial-focused approaches and towards more biology-driven strategies. The three essential components of tissue engineering consist of (1) the cells that are seeded onto the graft, (2) the scaffold onto which the extra-cellular matrix (ECM) forms, and (3) the humoral and mechanical signals that regulate neotissue formation and maintenance [59].

Historically, the first tissue engineered blood vessel substitute was created by Weinberg and Bell in 1986 when they cultured bovine endothelial cells (ECs), SMCs, and fibroblasts embedded within a collagen matrix and formed into a tubular structure [60]. An EC lining is vital to maintain a nonthrombogenic luminal surface, and full functionality cannot be obtained without a SMC layer. Likewise, vessel architecture and robustness depend on a biologically active ECM. These requirements raise the inevitable question of where to obtain these cells and how exactly to arrange them into a structured tubular vessel [61]. The main approaches in the development of a tissue-engineered vascular graft (TEVG) are outlined below:

28.8.1 Self-Assembled TEVG

TEVGs formed by culturing autologous fibroblasts and ECs without a scaffold have shown promising results in early clinical trials [62]. This patient-specific graft requires a 6- to 9-month culture period in which autologous fibroblasts produce sheets of tissue. Because of long wait times in addition to high production cost, it is

unlikely that this approach will become standard clinical practice or will be useful for patients who require expeditious intervention. In an ideal setting, the next-generation TEVG would no longer require seeded cells, thus greatly eliminating some of the expense and time required to generate a TEVG and facilitating the manufacture of an 'off-the-shelf' graft.

Tissue engineering by self-assembly (TESA) can generate completely biological constructs by using the tissue that is naturally assembled by mesenchymal cells.

Sheet-based tissue engineering (SBTE) was the first platform developed from the TESA principle. In SBTE, self-assembled sheets are rolled into the many distinct layers that compose a natural blood vessel. This method was used to construct the first tissue-engineered human blood vessel that displayed physiological mechanical properties without the need for an exogenous scaffold [63]. The blood vessel was composed of three distinct biological layers: a functional endothelium seeded onto an 'internal membrane' (IM), a 'media' made of SMCs, and an 'adventitia' made of living human skin fibroblasts. This study has shown the feasibility of using cell-generated matrix and finally challenged the dogmatic view that an exogenous scaffold is a prerequisite to build a successful tissue-engineered graft [64].

28.8.2 Scaffold-Based TEVG

The scaffold-based approach represents the most diffuse strategy to build TEVGs. The popularity of this methodology is justified by the fact that the presence of physical support enables the cells to follow a structural pathway during their colonisation and proliferation [65].

Matsuda and co-workers developed a graft composed of a combination of purified collagen and cells. Despite reasonable outcomes at 6 months and positive tissue remodeling, the presence of permanent prosthetic scaffold suggested that potential benefits of this approach did not appear to outweigh its complexity [66]. As an alternative to collagen scaffolds, a number of groups have also researched the use of cell-impregnated fibrin gels to create vascular grafts. A possible advantage of using fibrin is that fibrinogen and thrombin, the precursors to fibrin gel formation, can be readily obtained from a patient's own blood. However, like collagen-based grafts, constructs created from fibrin-gels have a typically low mechanical strength.

Electrospinning microfabrication technique is often used to generate tubular structures composed of nanofibres from different polymers. Composite scaffolds made of Polycaprolactone (PCL)/collagen grafts containing stromal cell–derived factor-1α–derived peptide and SP (substance P) for in situ vascular regeneration have been developed. They have shown promising results with endothelialisation and the grafts also promoted smooth muscle cell regeneration, endogenous stem cell recruitment, and blood vessel formation [67].

Chitosan, a biocompatible natural polymer, can provide a novel biological scaffold for TEVG. Chitosan-based hydrogels displayed promising in vitro biocompatibility and haemo-compatibility properties as well as in vivo short-term performance.

Although the results of this study are promising and support further investigation, the main limitations are the short length of the implanted vascular graft, the short duration of implantation and the small number of animals [68].

28.8.3 Biodregadable Prosthetic Polymer-Based Constructs

As an alternative to permanent prosthetic scaffolds, many groups have explored the use of biodegradable prosthetics. Popularised by the laboratory of Robert Langer, polyglycolic acid (PGA)- and polylactic acid-based constructs have dominated the field of tissue engineering in the last 10 years. However, balancing scaffold degradation with tissue deposition to maintain appropriate mechanical strength can be a considerable challenge affected by polymer design, indication, and interpatient variability [64].

Dahl and colleagues reported their development of human vascular grafts by culturing smooth muscle cells from human cadavers (that is, allogeneic cells) on tubular scaffolds made from a biodegradable polymer PGA [69]. The smooth muscle cells produced collagen and other molecules that formed an ECM. When the scaffold degraded, fully formed vascular grafts were left behind. These human vascular grafts were 6 mm or greater in diameter and retained their strength, elasticity and patency even after storage in phosphate-buffered saline solution for a year. Interestingly, the authors suggest that with this approach of using allogeneic human cells to produce TEVGs, one human donor can provide grafts for dozens of patients. This approach differs significantly from the one-donor-to-one-recipient model, which pertains to autologous tissue engineering and to cadaveric human or animal blood vessels [69]. These data support a possible future for these decellularised human TEVGs, especially as they will be available without significant patient wait time.

Bioresorbable vascular scaffold (BVS) technology was introduced more than two decades ago, with the seductive idea of providing transient mechanical support and drug delivery, while avoiding the adverse events associated with permanent metallic stents such as late stent thrombosis, restenosis and neo-atherosclerosis. The hypothesis was that after complete resorption of the scaffold, there would be full restoration of cyclic pulsatility and physiological vasomotion, adaptive vascular remodelling capability and plaque regression [70]. The poly-L-lactic acid (PLLA) everolimus-eluting Absorb™ BVS (Abbott Vascular, Santa Clara, CA, USA) quickly became the leading technology, supported by pre-clinical and clinical data. In the earlier trials, Absorb™ BVS failed to meet its co-primary endpoint of superior vasomotor reactivity and non-inferior late luminal loss compared with the cobalt-chromium everolimus-eluting stent. In a recent randomised trial, with refinement of technology, the results were non-inferior [71]. There are exciting times ahead to see how this technology will evolve and how its development in the coronary population may get adopted in the peripheral vascular disease population. A group from Australia have already reported mid-term 3 year follow up with the use

of Absorb™ BVS in popliteal and tibial arteries showing excellent safety, patency and freedom from clinically driven target vessel revascularisation [72]. Another potential area for this technology is in the paediatric population with congenital heart disease, as a bioresorbable scaffold can degrade over time, and be replaced with autologous tissue that can repair, remodel and even grow with the patient [73].

28.8.4 Decellularised Tissue Grafts

The decellularisation of a tissue-engineered vessel is more efficient than engineering a vessel for an individual patient and eliminates the extended lead-time for autologous vessel culture that may limit the widespread use of completely autologous grafts [62]. Decellularised ECM has been proposed for the tissue engineering of blood vessels. ECM isolated from different tissues and organs possesses different properties in terms of collagen type, content and density, glycosaminoglycans composition and vulnerability to different decellularisation procedures [74]. Decellularisation of the native tissue was shown to be successful in arteries, but its effect on matrix composition, mechanics, cell matrix interaction, and remodelling needs to be further studied. In addition, procuring native allogeneic vessels for decellularisation is a significant practical hurdle, which has limited the extensive application of such an approach.

The Yale and Duke groups have reported an interesting integration of a tissue-engineered vessel, decellularisation, and EPC population. Because the engineered connective tissues (vessels) were produced from allogeneic cells, the months-long process required to culture a collagen-rich and mechanically robust tissue was moved “off-line”, not requiring cells from the actual recipient. This decellularised tissue worked well as an arterial graft and was gradually remodelled in vivo by host cells [75].

28.8.5 Hybrid TEVG

Natural and synthetic polymers can be used together to create a composite scaffold in order to improve their properties. A “hybrid” approach to in situ tissue engineering could be adopted, based on the use of semi-degradable scaffolds combining a natural, biodegradable material—typically showing superior bioactivity and biocompatibility—with a synthetic, non-degradable one, granting a permanent backbone with adjustable and stable mechanical properties. Silk fibroin and polyurethane are two possible candidates to pursue the “hybrid” goal. Van Uden et al. report the successful blending of regenerated silk fibroin with a medical-grade, non-degradable polyurethane using formic acid and dichloromethane, and the manufacturing of hybrid, semi-degradable electrospun tubular meshes with different ratios of the two materials showing potential [76]. Though the hybrid approach offers an opportunity

Table 28.3 TEVG applied in human studies [65]

Approaches	Applications	Scaffold materials and fabrication methods	Groups
Natural material-based TEVG	Graft as an extrahepatic portal vein bypass	Decellularised human iliac vein seeded with Autologous cells **No commercial product**	Sumitran-Holgersson and colleagues [77]
	Lower extremity bypass surgery	Decellularised bovine carotid artery graft **Commercial name:** Artegraft (North Brunswick, NJ)	Lin and colleagues [78]
Synthetic material-based TEVG	Arteriovenous (AV) shunt for haemodialysis	Decellularisation of PGA scaffolds seeded with cadaver SMCs **Commercial name:** Humacyte (Humacyte Incorporated, RTP, NC)	Niklason and colleagues [79]
Self-assembled TEVG	AV shunt for haemodialysis access	Cell-sheet of human fibroblast in a shape of conduit. ECs were seeded in the graft after devitalisation of the luminal side **Commercial name:** Cytograft (Cytograft Tissue Engineering, Inc.)	L'Heureux and colleagues [80]
	AV shunts for haemodialysis access	Cell-sheet of human fibroblast in a shape of conduit, without further endothelialisation Dehydrated and stored (−80 °C) before clinical application **Commercial name:** LifeLine™ (Cytograft Tissue Engineering, Inc.)	L'Heureux and colleagues [80]

of exploiting the qualities of natural and synthetic polymers, a typical drawback is a need for long conditioning and the requirement of high technological skills during the manufacturing process [65].

The TEVGs used in human studies are summarised in Table 28.3. The materials used for TEVGs are schematically illustrated in Fig. 28.6.

28.9 Arteriovenous Grafts

The creation of a functional and durable dialysis vascular access is crucial in the treatment of patients with end stage renal disease and is a challenging quest for vascular surgeons. Arteriovenous fistula is currently the preferred mode of vascular access for haemodialysis, because it has the highest primary patency rate combined with the lowest associated risk of morbidity and mortality; however, when they are unavailable or fail to mature, arteriovenous grafts (AVG) are needed to avoid the use

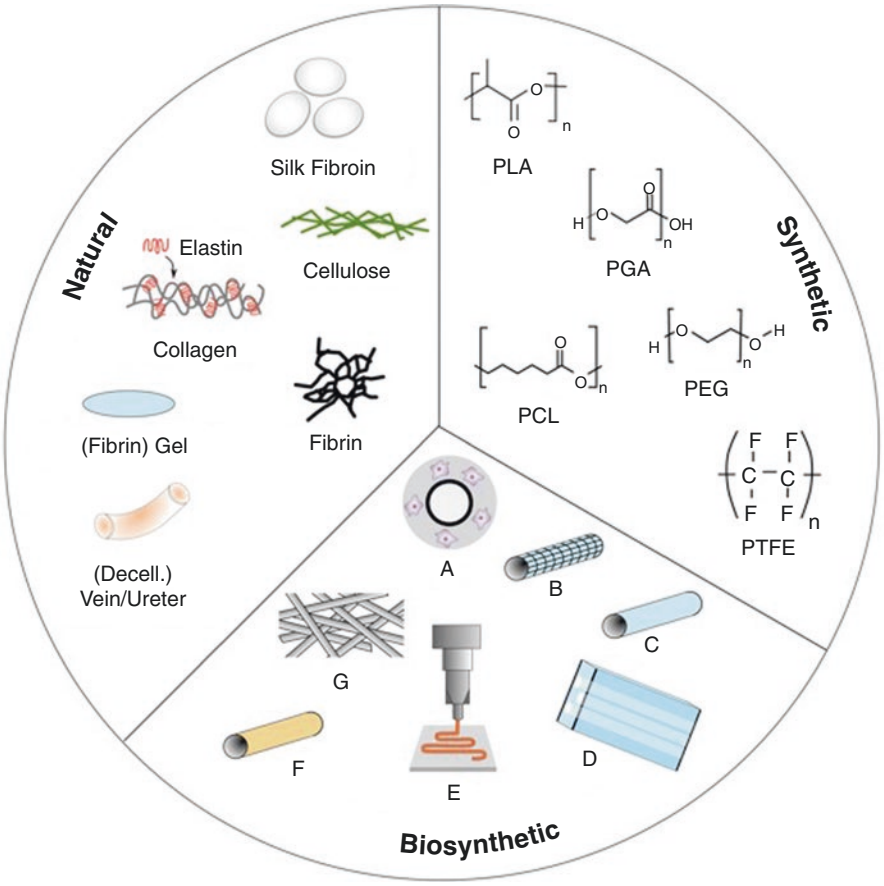

Fig. 28.6 Graft material used in vascular tissue engineering. Natural materials used for vascular grafts contain decellularised ECM such as collagen, silk fibroin or fibrin. Furthermore, decellularised vein allografts display satisfactory mechanical stability, minimal evidence of antigenicity and a recolonization with smooth muscle cells. Besides natural, synthetic materials are an attractive alternative due to their flexibility, mechanical strength and stiffness. Combining natural and synthetic materials (biosynthetic) opens more possibilities for tailoring the grafts properties to suit the needs of a particular application. Possible materials are collagen layers containing cells and an acellular support sleeve (A), fibrin-based scaffolds (B), nanostructured polyurethane blended with gelatin (C), microchannels in methacrylated gelatin (D), 3D printed scaffolds (E), hybrid-meshes (F) or heparin functionalised polymers (G) (With permission from Hielscher et al. [81])

of central venous catheters. Currently, ePTFE grafts are the most commonly used prosthetic AVGs. Tissue engineering (TE) has led to some promising alternatives. In the most recent clinical success pioneered TE—growing a tissue tube using donor cells is combined with regenerative medicine (RM) implanting an acellular scaffold conducive to recellularisation by host cells via decellularisation of the tissue tube. This new TE/RM approach has yielded promising results in a phase 2 clinical trial as an AVG [79]. Completely biological AVGs developed from neonatal human

dermal fibroblasts entrapped in bovine fibrin gel have also been evaluated and they have potential to become readily available "off-the-shelf" graft by showing reasonable patency rates and matrix remodelling with extensive recellularisation and endothelialisation in baboons [82]. However, response to trauma can be a potential concern limiting its clinical use. Polyurethane grafts have also been evaluated for arteriovenous access with several available for clinical use including Flixene (Atrium, Hudson, NH), Avflo (Nicast Ltd., Lod, Israel), Rapidax (Vascutek, a Terumo Company, Inchinnan, United Kingdom), and Accuseal (W. L. Gore, Flagstaff, Ariz) and Vectra (Thoratec Corporation, CR Bard, Inc., Pleasanton, Calif). Polyurethane grafts offer the advantage of early cannulation and have patency and complication rates similar to ePTFE grafts. There are concerns, however that early cannulation may increase the risk of infection [83].

28.10 Graft Materials for Aortic Stent-Grafts

Nitinol has a long-track record of successful uses in many endovascular applications. However, nitinol-based devices are relatively bulky when used with synthetic graft materials such as ePTFE or PET. Thin film nitinol has unique mechanical properties (e.g., superelasticity), excellent biocompatibility, and ultra-smooth surface, as well as shape memory behaviour. All these features along with its low-profile physical dimension (i.e., a few micrometres thick) make this material an ideal candidate in developing low-profile medical devices (e.g., endovascular devices). Both in vitro and in vivo test data demonstrated a superior haemocompatibility of the thin film nitinol when compared with commercially available endovascular graft materials such as ePTFE or PET [84]. Further research in material design for aortic stent-grafts is warranted. Whilst in small vessels, the main issue with compliance mismatch is intimal hyperplasia, in large diameter vessels such as aorta, poor compliance and stiffness of the graft materials, especially when treating long segments of diseased aorta or implanting the devices close to the aortic arch, can have potential impact on cardiopulmonary function by causing physiological strain [85].

28.11 Conclusions and Future Directions

To this day, we have neither prosthetic small-diameter vascular grafts nor autologous grafts, which can be considered ideal. The problem is that after 60 years of intensive study on development of novel biomaterials and modifications of current materials, we do not have a truly thromboresistant surface and we have been unable to abolish compliance mismatch. In most cases, it is impossible to find a single material that meets all of the necessary criteria of mechanical strength, viscoelasticity, and surface properties to match the promotion of EC adhesion, proliferation, and differentiation when coupled with antithrombogenic surface properties.

However, the current climate of vascular tissue engineering is very promising given recent clinical success and ongoing bench-top innovations; the field is poised to produce the next-generation TEVG that will be free of some current hindrances limiting their commercial availability. Significant progress has been made in the development of recent technologies in scaffold engineering and cell engineering, including gene delivery and improvement of endothelial progenitor cells. Over the years, the strategy to fabricate and modify the vascular tissue grafts has shifted towards biomimicry and in vivo endothelialisation. The development of these studies in clinical settings will determine the future of the vascular implants. There are still many scientific questions that need to be addressed and there is still enough space for creativity and innovation in this field of research. There are many improvements that have been made and that need to be achieved [86].

As we develop a better understanding of neotissue formation and elucidate the signalling pathways involved in graft remodeling, effective molecular targets will emerge to further improve neotissue growth and vessel patency [61]. Undeniably, economic feasibility is a key factor in clinical availability but, ultimately, a technology that outperforms the standard of care will evolve to become more affordable with time (e.g. MRI scanners) whereas an inexpensive technology that marginally works will be abandoned [64]. Identification of patient-specific factors that predict graft failure, probably based on individual genetic variation, may provide personalised therapeutic strategies in the future [44].

Over the next 5 years improved prosthetic grafts will become available with the introduction of biodurable and compliant materials. Lumen modulation by anticoagulant molecules, cell ligands and growth factors will further enhance performance thus adding thromboresistance to compliance. Attachment technology will allow PET and PTFE to be similarly modified although these can never be sufficiently compliant to abolish compliance mismatch. With on-going development to match as closely as possible both the mechanical characteristics and biological functions of normal human arteries there is real potential for new graft development within the next decade.

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Chapter 29
Vascular Graft Infections

Mauro Vicaretti

Key Learning Points

- Vascular graft infection is associated with significant mortality and morbidity (including limb loss).
- Bacteriology of graft infections varies with the affected site of the body.
- Early graft infections (within the first 4 months of graft implantation) are uncommon (approx. 1%) and are caused by virulent micro-organisms.
- Graft infections are more common following emergency surgery and surgery in the groin.
- Late graft infections tend to be caused by less virulent organisms such as Staphylococcus epidermidis.
- Late graft infections often present insidiously with sinus tracts, perigraft fluid collections, graft occlusion and development of pseudoaneurysms.
- Many bacteria can produce biofilms, which form a capsule of extracellular polymer substances.
- Biofilms protect bacteria from conventional antibiotics.
- Small colony variants often form biofilms and are difficult to isolate. They are likely to be a common cause of recurrent and relapsing infection.
- The management of vascular graft infection is complex and a multi-disciplinary approach is required to optimise outcomes. The "gold standard" approach is total graft excision and extra-anatomical revascularisation. More conservative approaches may be required in individual cases.
- The management of stent graft infection is challenging and best outcomes are obtained with explantation and revascularisation. Conservative management is associated with significantly inferior outcomes.

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29.1 Introduction

The introduction of prosthetic grafts has revolutionised the management of vascular disease but graft infection, although uncommon, remains a dreaded complication with significant morbidity and mortality. Mortality occurs in approximately one third of all graft infections [1], with mortality highest when an aortic prosthesis is involved [2, 3]. As many as 75% of survivors of an infected aortic prosthesis require amputation of a limb [3], with the incidence of amputation highest when the infection involves more distal prosthetic grafts [4]. The incidence of graft infections is difficult to quantify as infection may be manifested many years after implantation [1], with many reports being isolated or as part of case series. Nevertheless, the reported incidence is in the order of 5%, varying according to the site of operation (Table 29.1) [1], being higher when a groin incision is used, or if the procedure is an emergency or a redo procedure. However, to date there is a paucity of randomised controlled trial literature with respect to the optimal management of graft infection. This is understandable considering the complexity of such a medical and surgical problem. As such, treatment strategies are generally based on individual surgical experience with recommendations for treatment.

29.2 Natural History of Prosthetic Vascular Graft Infections

Early prosthetic vascular graft infections typically occurring in the first 4 months following placement are relatively uncommon (1%) and are usually caused by the more virulent micro-organisms, such as *S. aureus, E. Coli, Pseudomonas, Klebsiella, Proteus and Enterobacter* [1]. Late prosthetic vascular graft infections are the result of two possible mechanisms. Firstly, by haematogenous seeding from a septic focus elsewhere [11] or by the prosthetic graft becoming infected with enteric contents following a graft-enteric erosion. In both the haematogenous and graft-enteric erosion situations the usual causative organisms are those with high virulence and

Table 29.1 Incidence of prosthetic graft infection according to location

Author and year	Location of graft	Incidence (%)
Hoffert et al. (1965) [5]	Aortoiliac/femoral	2.2
	Femoropopliteal	6
Fry and Lindenauer (1967) [6]	Aortoiliac/aortofemoral	1.34
Szilagyi et al. (1972) [7]	Aortoiliac	2.2
	Aortofemoral	1.6
	Femoropopliteal	3.0
Criado (1982) [8]	Carotid-subclavian	1.5
Lorentzen et al. (1985) [9]	Aortofemoral	3.0
	Femoropopliteal	3.5
Rubin et al. (1985) [10]	Femoropopliteal	2.4

Modified from Back and Klein [1]

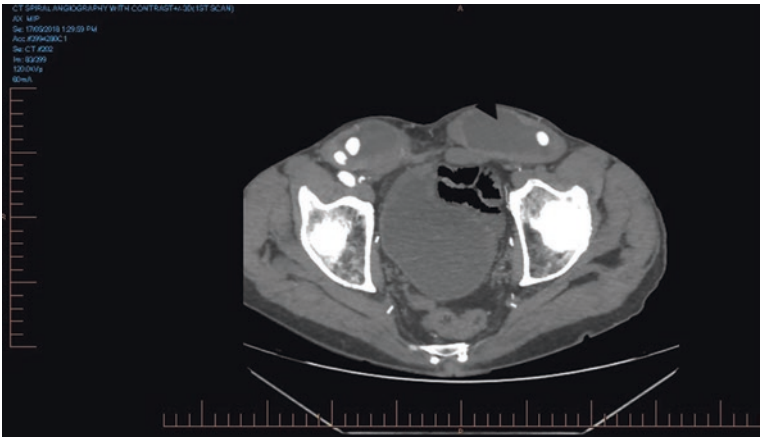

Fig. 29.1 Collections in both groins around prosthetic graft

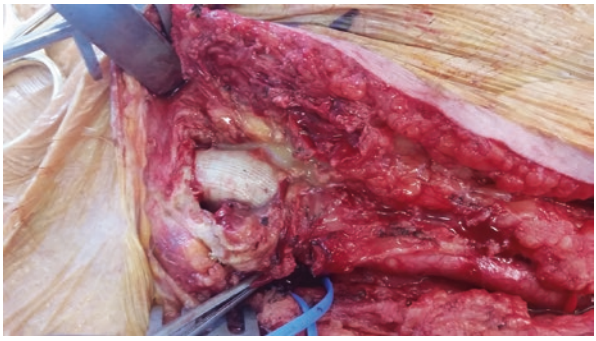

Fig. 29.2 Unincorporated Dacron graft with biofilm infection

clinical manifestations are signs and symptoms of sepsis. The second mode of presentation is insidious, caused by the less virulent coagulase negative staphylococci such as *S. epidermidis* with contamination likely occurring at the time of implantation [1]. Clinically the mode of presentation of these late appearing infections are as sinus tracts, perigraft fluid collection and anastomotic pseudoaneurysm formation [2]. This is illustrated in Fig. 29.1 which shows an extensive perigraft fluid collection around a prosthetic femoro-femoro cross over graft. Figure 29.2 demonstrates an unincorporated graft with perigraft fluid collection.

29.3 Mechanisms of Graft Contamination at Operation

There is general agreement that the most common mechanism by which grafts become infected is at the time of implantation either by contamination from the surgical team or by colonised microorganisms on the patient. It has been demonstrated that the majority of patients undergoing arterial revascularisation are

colonised with coagulase negative staphylococci [12] and colonisation of patients with nosocomial bacteria is enhanced when the preoperative hospitalisation is lengthy [13].

The incidence of graft infection following emergency aneurysmorrhapy has been reported to be as high as 7.5% [14]. The evidence of other potential mechanisms such as division of lymph nodes [15], infected transudated fluid during aortic surgery [16] and infected laminated thrombus [4, 16, 17] is conflicting. In addition, patients who develop superficial and/or deep surgical infections are 7.1 times more likely to develop graft infections and/or mycotic aneurysms [18].

29.4 Pathogenesis of Graft Infections

The exact aetiology of vascular graft infections is not completely understood but likely to be multifactorial. According to Bandyk and Esses [19], the risk of vascular graft infection as demonstrated by animal models can be predicted by the formula:

$$\text{Risk of biomaterial infection} = \text{Dose of bacterial contamination} \times \text{virulence} / \text{Host resistance}$$

The dose of bacterial contamination is dependent on the infecting microorganism. Experimentation in a canine aortic model has demonstrated that the infective threshold for bacteria to cause graft infection in over 50% of grafts was 10^7, 10^9, and 10^2 for *S. aureus, S. epidermidis and P. Aeruginosa* respectively [20]. Virulence of microorganisms is often associated with the production of secreted toxins and enzymes with a resultant decline in structural integrity of the artery wall [19, 20]. Many bacterial strains, including *S. epidermidis, S. aureus and P. aeruginosa, Klebsiella pneumoniae and Escherichia coli* are known to produce extracellular polymer substances (EPS) (slime), forming a capsule incorporating the bacteria. This is referred to as a biofilm and protects the micro-organism against host defences and antibiotic therapy and allows greater adherence of the microorganism to the biomaterial [21, 22]. Biofilms protect the invading bacteria by increasing their resistance against conventional antibiotics and in a sense the biofilm constitutes a protected mode of growth that allows survival in a hostile environment, allowing bacteria to resist biocides [23].

Essentially the steps involved in the development of biomaterial associated infection include the following: (a) bacterial adherence; (b). microcolony formation within a biofilm; (c) activation of host defences; and (d) the inflammatory response involving perigraft tissues and the graft/artery anastomoses (Fig. 29.3) [2].

Bacterial adherence is initiated when planktonic bacteria adhere to a surface and become irreversibly attached only after a few minutes [24]. Adherence is influenced by microorganism characteristics, graft properties including graft electronegativity, hydroporosity and smoothness of the graft [21]. Microcolony formation within the biofilm ensues as the microorganisms secrete a protective

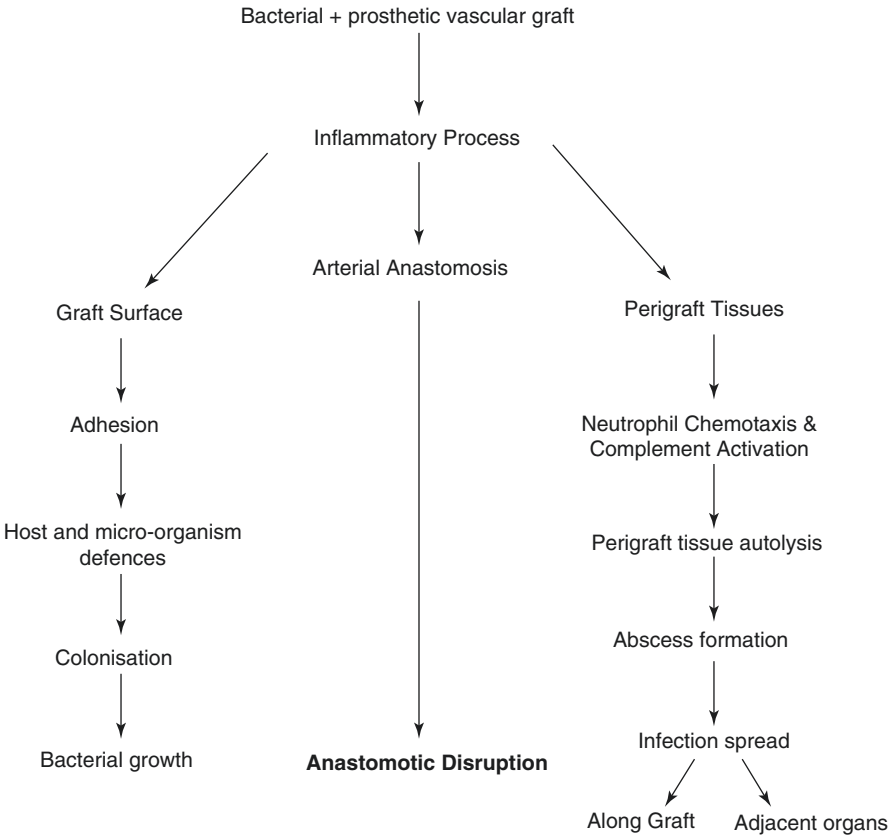

Fig. 29.3 Pathogenesis of vascular biomaterial-associated infections

layer of exopolysaccharides giving the biofilm the so-called slimy appearance. It is the interaction between the graft and the host that produces inflammatory stimuli activating the host's humoral and cellular immune system with the release of cytokines and polymorphonuclear granulocytes [2]. In addition, the acidic, ischaemic microenvironment produced by the immune foreign body reaction to the synthetic vascular grafts promotes bacterial biofilm formation and proliferation. The isolation of the graft and the microenvironment produced prevents antibiotics and immune complexes exerting their maximal effect, which contributes to the ultimate manifestations of graft infection, including tissue autolysis, arterial vessel wall or anastomotic disruption and haemorrhage [2]. The dynamic communities within the biofilm continually shed new planktonic cells [24].

It is the nature of the biofilm protecting the resident microorganisms which not infrequently mandates prosthetic graft removal, often followed by antibiotic treatment to prevent regrowth and to target bacteria released into the bloodstream or

surrounding tissues [25]. When this is not possible then aggressive alternate antimicrobial strategies are implemented [23].

Significant advances have been made in the understanding of the biofilm yet the dynamics of the microenvironment *in vivo* is different from that seen *in vitro* [23]. To act on the microorganisms, therapies have been targeted to disrupt the EPS matrix. Components of the EPS matrix could be vital targets in the disruption of biofilm structure. Equally, formation of biofilms could be prevented by inhibition of the attachment of cells to surfaces [23] and improved biofilm drug penetration [23, 26]. It is likely that therapies will be multiple, exploiting potential synergistic effects [26].

29.5 Bacteriology of Vascular Graft Infections

Gram positive, gram negative, anaerobic and fungal micro-organisms all have the potential to infect vascular prostheses but in general the majority of infections are the result of a small number of micro-organisms (Table 29.2) [27]. Staphylococci are the most prevalent organism associated with prosthetic graft infection [2, 28, 29]. Of the staphylococci, *S. aureus* is generally regarded as the most common causative bacteria [2, 28], particularly MRSA [29]. *S. epidermidis* is now being recognised as the leading cause of vascular graft infection, particularly chronic and late onset infections [17].

The gram negative organisms, *E. Coli, Pseudomonas, Klebsiella, Enterobacter* and *Proteus,* although relatively uncommon causative organisms for graft infections, are of particular interest and concern because of their high virulence and their tendency to destroy the vessel wall [19, 30, 31].

Candidia, Mycobacteria, and Aspergillus infections are uncommon but pose a significant risk to patients who are immunocompromised [2]. Although uncommon, they are all expected to increase in frequency because of their increasing resistance to standard prophylactic antibiotics [32].

Table 29.2 Bacteriology of prosthetic vascular graft infections from collected series (location and incidence (% of total))

Microorganism	Thoracic aorta	Graft enteric erosion/ Graft enteric fistula	Aortofemoral	Femoro-popliteal-tibial
S. aureus	22	4	27	28
S. epidermidis	25	2	26	11
Strep sp	2	9	10	11
Pseudomonas sp	14	3	6	16
Coliforms and gram negative	10	49	28	29
Other species	11	15	1	3
No growth/no culture	16	18	2	2

Modified from Back [27]

There is an association between the type of infecting organism, the type of vascular complication and the arteries that are involved in the anastomosis to the prosthetic graft. Bandyk and Bergamini [2] in a collective survey of 1258 patients who had a vascular graft infection, found that the majority of aortoenteric fistulas were the result of either *Streptococci or E. Coli* and, if the anastomosis involved the femoral artery, the thoracic aorta, the subclavian, carotid or innominate arteries *S. epidermidis or S aureus* was the likely causative organism. *E. Coli, Enterococci and Enterobacter* were the more likely organisms to be involved in aortoiliac anastomoses.

Significant interest has developed in the role of small colony variants (SCVs) [33]. Many clinical studies and observations have been published which tie recurrent, persistent staphylococcal infections, including device-associated infections to this special phenotype. It has been shown that SCV were strong biofilm (slime) formers, indicating that biofilm formation is a special feature of the pathogenesis of SCVs [34]. As with biofilm producing microorganisms, SCVs are associated with alterations in metabolism and host-pathogen interplay, resulting in chronic and relapsing, often therapy-refractory infections. SCV are difficult to identify by conventional methods with identification mostly performed by 16S rRNA gene sequencing [33].

29.6 Investigations for Detection of Prosthetic Graft Infections

The diagnosis of vascular prosthetic infections can be difficult as the presentation may be subtle especially if it is a late onset infection, the prosthesis is intra-abdominal and the micro-organism is one of low virulence. Its presentation is thus very dependent on the location of infection and the causative microorganism/s. The diagnosis is aided by multiple available microbiological and imaging testing but in general is directed more at proving the absence of infection rather its presence. It is infrequent that single modality imaging is sufficient in establishing the presence of vascular graft infection [35].

29.6.1 History and Physical Examination

Clinical signs and symptoms are typically non-diagnostic for the presence of vascular graft infections. The clinical clues suggesting graft infection especially those placed superficially include an inflammatory perigraft mass, overlying cellulitus, presence of exposed prosthetic graft, a sinus tract with persistent purulent drainage and/or bleeding and/or a palpable anastomotic pseudoaneurysm, graft thrombosis and distal septic embolization [2–4, 36]. The presence of intra-abdominal prosthetic

graft infection may be non-specific, such as fever of unknown origin, septicaemia, or abdominal pain [3]. Upper or lower gastrointestinal haemorrhage either of an acute or chronic nature may indicate a graft-enteric fistula [17, 36]. Occasionally indirect evidence, such as the presence of hydronephrosis or osteomyelitis of the spine may be indicative of perigraft inflammation.

29.6.2 Laboratory Investigations

Routine laboratory studies such as white cell count and differential, erythrocyte sedimentation rate (ESR), C Reactive Protein (CRP), and blood cultures are obtained. However, the results may be non-specific and even normal if the organism is *S. epidermidis* [2]. Wherever possible, pus, exudates, tissue specimens, blood and wound cultures should be analysed microbiologically to aid in microorganism identification and to allow the commencement of appropriate and specific chemotherapy. To aid in the diagnosis of *S. epidermidis* all solid material should be mechanically or ultrasonically disrupted [37–39].

29.6.3 Diagnostic Imaging

Various diagnostic modalities (Computerised Tomography (CT), ultrasonography, Magnetic Resonance Imaging, Leucocyte or immunoglobulin labelled scanning, Positron Emission Tomography (PET) scanning ± CT, angiography and/or endoscopy) may assist the vascular surgeon in determining the presence and extent of prosthetic graft infection. Not infrequently, a combination of diagnostic modalities to improve sensitivity and specificity are utilised to confirm the presence or absence of a vascular prosthetic graft infection. These modalities are also helpful in planning definitive surgery. The utility of CT angiography with the capability of vascular three-dimensional reconstruction has largely replaced digital subtraction angiography as the method of diagnosis and therapeutic planning. In general the features suggestive of graft infection include perigraft fluid and/or gas (Fig. 29.4), graft disruption, absence of graft incorporation, and pseudoaneurysm formation. The presence of periprosthetic gas more than 6 weeks following graft implantation is an abnormal finding and should alert the physician to the possibility of a graft infection. Oesophagogastroduodenoscopy (Fig. 29.5) and or colonoscopy is of value to exclude other gastrointestinal causes of bleeding in patients with aortic prosthesis and gastrointestinal bleeding. However, the presence or absence of a gastrointestinal lesion does not rule out prosthetic graft infection [36].

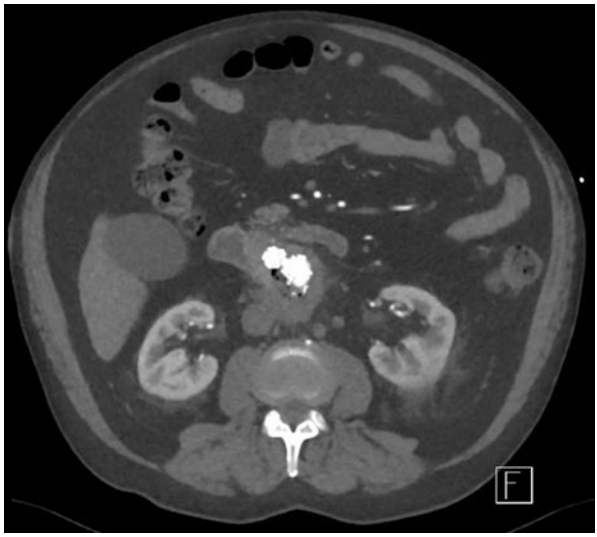

Fig. 29.4 CT scan demonstrating perigraft inflammatory mass and gas around stent graft

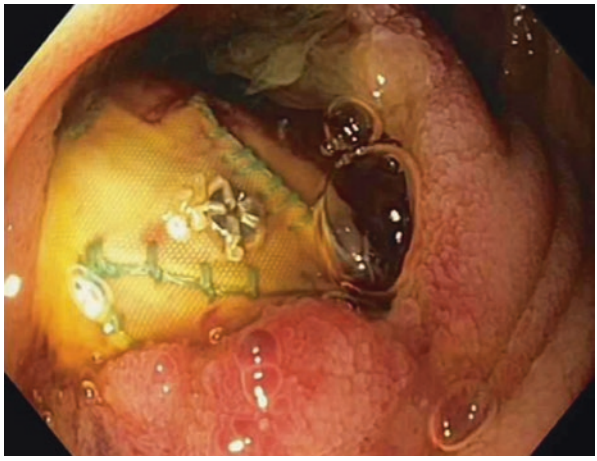

Fig. 29.5 Duodenoscopy demonstrating graft enteric fistula with graft on view in duodenum

29.7 Management of Prosthetic Graft Infections

In general, the management of prosthetic graft infections is dependent on patient clinical presentation, extent of graft involvement, site of the prosthetic graft, initial indication for the prosthesis, revascularisation options and microbiology of infecting organisms. Procedures may be undertaken as single-staged or multi- staged accounting for these variables.

Preventive measures such as the routine use of skin preparations [40], the use of a depilatory agent [41], limiting the length of preoperative hospitalisation [13],

Table 29.3 Antimicrobial impregnation of vascular grafts

Antibiotics used in experimental models	Vehicles, agents
Silver allontoin-heparin [43]	Silver [43–46]
Norflaxacin [44] Penicillin [47], oxacillin [42, 45, 48, 49]	Triododecylmethylammonium chloride (cationic surfactant) [44, 47]
Amikacin [50]	Benzalkonium chloride [47, 48]
Cephazolin [47], cefoxitin [51], ciprofloxacin [46, 52, 53] Oflaxacin [42]	Luminal coated glycosaminoglycan-keratin fibrin glue (cryoprecipitate, bovine thrombin, aminocaproic acid) [51, 54]
Tobramycin [54, 55]	N-butyl-2 cyanoacrylate [55]
Gentamicin [56]	PTFE threads [42]

operating time and intensive care stay all contribute to the reduction in wound infection and more importantly, the chance of developing resistant multiple nosocomial infections [40]. Antimicrobial prophylaxis has been shown to reduce wound infections in vascular surgery [42] and ideally should be given as close as possible prior to incision and repeated in the event of haemorrhage and lengthy operations every 4 h.

As a preventive measure, host resistance may be enhanced by the antimicrobial impregnation of grafts. A number of novel combinations of grafts and antibiotic with or without various forms of treatment have been trialled at both the *in-vitro* and *in-vivo* levels (Table 29.3) [42–56].

Rifampicin, a known anti-staphylococcal agent, particularly methicillin resistant, is a hydrophobic semi-synthetic substance with a high affinity for gelatin [57]. It inhibits DNA dependent RNA polymerase activity in bacterial cells without affecting mammalian cells [58] and has been passively incorporated into gelatin-sealed Dacron grafts as a mode of staphylococcal protection at the time of implantation. It has been shown to be resistant to experimental bacterial contamination [59–62] with in-vivo bioactivity to 22 days [63], and *in-vitro* bioactivity to 4 days [64–66]. It is these qualities plus its excellent tissue and intracellular penetration [57] that make rifampicin an ideal antibiotic to be bonded to prosthetic grafts in order to prevent subsequent graft infection.

In an *in-vitro* study [67], the effect of soaking four clinically available prosthetic grafts (Polytetrafluoroethylene (PTFE™), Gelsoft™, Thoratec™ and Fluoropassiv™) in known concentrations of rifampicin against staphylococcal (MRSA and *S. epidermidis*) infection was evaluated. Graft segments were soaked in concentrations of rifampicin of 1.2, 10 or 30 mg/mL and placed on a bacterial lawn of either MRSA or *S. epidermidis*. In all the graft types, with increasing rifampicin concentration there was a significant increase in the average zone of inhibition and the total antibacterial activity of the rifampicin-soaked graft. No significant differences were apparent between the staphylococcal species when adjusting for graft type and rifampicin concentration. The Dacron type grafts, namely Fluoropassiv and Gelsoft, were significantly better compared to the other grafts (polytetrafluorethylene or Thoratec) at all studied rifampicin concentrations.

It has been shown in an ovine model [68] in which a segment of carotid artery was replaced with a rifampicin soaked Gelsoft graft (1.2 or 10 mg/mL) and then directly inoculated with 10^8 colony forming units of either methicillin resistant staphylococcal aureus or methicillin resistant staphylococcal epidermidis, that the rifampicin soaked graft offered significant prophylaxis compared to non-rifampicin soaked grafts [69–71].

For the *S. epidermidis* arm, in the 10 mg/mL rifampicin group there was a significant reduction in graft infection when compared to both the control group ($p < 0.05$) and the 1.2 mg/mL group ($p < 0.05$) [71]. Similarly, for the MRSA group, in the 10 mg/mL treatment group there was a significant reduction in the total number of positive cultures when compared to the control group ($p < 0.05$) and the 1.2 mg/mL group ($p < 0.05$) [71].

Although there is ample in-vitro evidence supporting antibiotic impregnation of prosthetic grafts, there continues to be no evidence that prophylactic rifampicin bonding to dacron grafts reduced graft infection at either 1 month (RR 0.63, 95% CI 0.27–1.49) or 2 years (RR 1.05, 95% CI 0.46–2.40) [72].

29.8 Established Infection

29.8.1 Antibiotic Therapy

Once the diagnosis or suspicion of prosthetic vascular graft infection is made then broad-spectrum antimicrobial therapy is initiated and subsequently converted to organism specific antibiotics [3]. The length of antibiotic therapy following excision of the infected graft remains unclear. Continued therapy is generally dependant on causative microorganism/s, extent of graft infection and if any prosthetic material remains unreplaced.

29.8.2 Operative Approaches to Vascular Graft Infection

The "gold standard," although technically challenging, is the removal of all infected tissue and extra-anatomical revascularisation [73]. A number of more conservative approaches have been advocated depending on the site of the infection and the microorganism involved. The most conservative of treatments is aggressive local wound care with graft preservation providing the graft and anastomoses are intact and the patient has no systemic features of sepsis [74]. A series of patients who had graft preservation concluded that with the exception of *pseudomonas,* vascular graft infections could be managed with debridement, antibiotic therapy and wound closure [31]. The skeletonised prosthetic graft can be covered using viable regional rotational flaps [75]. The risk of such treatment is fatal graft disruption and

persistent graft infection [27]. Graft excision without revascularisation may be considered in well collateralised distal arterial beds and/or grafts that are thrombosed with no symptoms of distal ischaemia. Some groups have advocated aggressive wound debridement with vacuum assisted closure without rotational muscle flap coverage even with exposed grafts with minimal morbidity and mortality [76]. Others have proposed graft excision and replacement with cadaveric arterial allografts [77], venous autografts [78], cryopreserved saphenous vein homografts [79], autogenous arteries and/or veins [80] or prosthesis [81]. The major drawback with in-situ reconstruction is recurrent and/or ongoing sepsis. Experimental evidence for in-situ prosthetic replacement [21], was provided in an *in- vitro* model which compared the bacterial adherence of four strains of bacteria (*S. aureus*, "mucin" and "non-mucin" producing *S. epidermidis* and *E. coli*) to ePTFE, woven Dacron and velour knitted Dacron. The study demonstrated that bacterial adherence was greatest to velour knitted Dacron and least with ePTFE. In addition, "mucin" producing *S. epidermidis* adhered to Dacron in 10- to 100-fold greater numbers compared to PTFE [82].

An established ovine model [68] was used to determine if the replacement of a staphylococcal infected vascular graft with a graft impregnated with rifampicin would be considered appropriate surgical management in preventing early recurrent infection [83]. Gelsoft grafts without any antibiotic treatment were infected with overwhelming concentrations of either MRSA or MRSE. The grafts were removed at 3 weeks and replaced with either control (no rifampicin) grafts or grafts soaked in either 1.2 or 10 mg/mL of rifampicin. The replacement grafts were removed 3 weeks following placement. For MRSA [83] there were no statistically significant differences between the groups for any of the macroscopic or microbiological parameters recorded. For *S. epidermidis* [83] there were no statistical differences between the concentrations for macroscopic findings. There were however, statistically significant reductions in the number of total infected specimens in the 10 mg/mL when compared to both the control, ($p < 0.001$) and the 1.2 mg/mL ($p < 0.005$) [83]. The conclusions from the studies [83] were that established *S. epidermidis* bacterial graft infections model can be treated by the in-situ replacement of the infected prosthesis with a 10 mg/mL rifampicin impregnated Gelsoft graft. However, such management for MRSA established infections cannot be recommended from the results obtained in this particular animal model.

To date, a number of groups [77, 84, 85] have successfully managed prosthetic graft infections with rifampicin impregnated grafts with zero mortality, no requirement for limb amputation and to date no recurrence of infection. Interestingly, a prospective United Kingdom trial defining the role of extra-anatomical bypass with rifampicin bonded grafts found no evidence to support their use when compared to non- treated collagen impregnated grafts, with respect to graft infection and mortality [78]. The same group reported fewer amputations, conduit failures, and early mortalities with the use of rifampicin bonded grafts than other treatment modalities for aortic graft infection but reinfection was worst for rifampicin-bonded prostheses and lowest for autogenous veins. When considering all revascularisation options and outcomes, the in-situ reconstruction for aortic graft infections was the preferred option over extra-anatomic bypass [78].

Stent grafts have become a viable alternative in the management of not only infected prosthetic grafts but also in mycotic aneurysms in patients presenting with pseudoanastomotic active bleeding, acute fistulisation or rupture [79]. This is considered definitive management for patients with limited life expectancy (<6 months) but in those with predicted lifespan in excess of 6 months more definitive intervention should be undertaken following the initial endovascular procedure [79].

29.8.3 Stent Graft Infection

Stent grafts are increasingly being utilised for the management of arterial occlusive and/or aneurysmal disease. Although stent graft infection is uncommon, with widespread aortic endograft placement for the treatment of aortic pathology, complications of endografts are increasingly being reported. Infection of an endograft placed during an abdominal endovascular aortic repair (EVAR) or thoracic endovascular aortic repair (TEVAR) is reported to have an incidence in small series of 0.2–5% [80, 81]. There are to date no large multi-institutional studies.

The management can be complicated and associated with significant morbidity and mortality. A recent meta-analysis [86] demonstrated superior results with conventional surgical therapies, namely explantation of the endoprosthesis and revascularisation independent of means of revascularisation and conduit type compared to conservative management. Patients in the surgical group had a higher survival rate compared with conservative group (58% vs. 33%, $P = 0.002$). Outcomes were worse with infected thoracic aortic endografts compared to abdominal aortic endografts with survival rates of 27% versus 58% respectively ($P = 0.000$) [86].

29.9 Conclusion

The future management of vascular graft infections will be reliant on a better understanding of the interaction between the micro-organism, the prosthesis and the immune system. This will allow a more directed approach towards prevention and treatment. Possibilities would include more powerful antibiotics either administered parenterally or incorporated into the prosthesis, acting as a local delivery system for prolonged periods of time. The role of the biofilm in the pathogenesis of graft infection needs further understanding from both a molecular and an immune level.

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Chapter 30
Radiation Physics and Biological Effects of Radiation in Vascular Surgery

Joseph Dawson and Stephan Haulon

Key Learning Points

- Understand how radiation interacts with the body leading to various forms of injury.
- Understand the concept of scatter radiation and its importance in daily practice.
- Define and characterise deterministic and stochastic effects of radiation.
- Explain the linear no-threshold theory.
- Describe the most common deterministic and stochastic effects of radiation.
- Understand the various ways radiation dose and exposure can be measured.
- Be familiar with the most common forms of radiation dose measurements.
- Know the maximum dose limits, annual occupational exposure limits and common diagnostic reference levels (DRLs).
- Understand the risks of radiation injury amongst interventionalists.

30.1 A Brief History of Radiation Use in Vascular Surgery

30.1.1 The Discovery of Radiation

X-rays were first discovered by Wilhelm Conrad Roentgen in Germany in 1895 when he discovered the emission of rays from a cathode-ray tube that, unlike light, passed through heavy black paper shielding [1]. One of his first images using this

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new discovery was the now-famous picture of his wife's hand. The interest in uptake of this new technology was unsurprising, and within 6 months a direct medical utility was born, with radiographs being used on the battlefields to help locate bullets in wounded soldiers [1].

Concurrently Henri Becquerel discovered another form of penetrating rays produced by uranium; which was radioactivity. His work was complemented and expanded by Marie Curie, and together they won the Nobel Prize for Physics in 1903 [1]. The concurrent discoveries of X-rays and radioactivity led to a new aspect of health care. There was initially no concern regarding any potential harmful effects, due to the apparent similarity of these X-rays to harmless light, and the delayed onset of symptoms [1]. However as early as 2 years after radiation was discovered some of these early pioneers reported what we now know were radiation-induced injuries due to overexposure; skin burns, pigmentations, eye irritations, ulceration, hair loss, limb loss and even death [2], most noticeably Marie Curie dying from aplastic anaemia [3]. Further realisation that X-rays and gamma rays, whilst similar to light in the form of electromagnetic waves, have much shorter wavelengths led to the understanding that, with a much higher inherent energy, they could penetrate material that light cannot. The disadvantage of this additional energy was the propensity to alter and break chemical bonds in tissues and cells, leading to radiation-induced injury [1].

30.1.2 Endovascular Pioneers

A handful of events occurred in early and mid twentieth century that contributed to the inception of endovascular surgery, with the true revolution occurring in the latter two decades of the century. In 1920 Dos Santos performed the first angiogram and in 1953 the Seldinger technique was developed allowing safe percutaneous access to vessels [4, 5]. Towards the end of the 1950s, Thomas Fogarty invented the first minimally invasive endovascular device, the embolectomy catheter, which gained popularity during the 1960s. In 1964 Dotter performed the first percutaneous transluminal angioplasty [6] but this was not widely accepted by surgeons and remained limited to use by radiologists. In 1978 Andreas Grüntzig, a Swiss cardiologist, published his first series of cases of percutaneous transluminal angioplasty (PTA) of the coronary vessels using a balloon he had developed. In 1985 Julio Palmaz, an Argentinian Radiologist introduced stenting to complement PTA which really 'lit the fuse' for the endovascular revolution [5]. One of his large Palmaz stents was used as the proximal segment in the first endovascular repair of an abdominal aortic aneurysm (EVAR), performed in 1990 by fellow Argentinian vascular surgeon, Juan Parodi [7]. New technology followed thereafter to advance these concepts, and by the late 1990s all of the elements were in place for the endovascular explosion [5].

30.1.3 Exposure to Medical Radiation

30.1.3.1 General Population

With at least two thirds of all medical imaging involving ionising radiation [8] it now represents the greatest man-made source of ionising radiation to the general population [9]. In 1980 medical radiation only contributed 15% of the per capita all-cause radiation exposure, but by 2006 this had risen to 48% [8]. In 1987 the estimated total annual medical radiation exposure per person was 0.6 mSv, but by 2006 this has risen to 3.2 mSv, representing a five-fold increase [10] and overtaking the natural background dose per person of 3.0 mSv per year [10]. This is the equivalent of 150 chest X-rays for every member of the population [10]. When the National Council on Radiation Protection publishes contemporaneous data in 2019 it is anticipated this exposure will be even higher [10].

The causes for this pattern are multifactorial:

1. Modern medicine has a growing reliance on imaging technology [2];
2. Higher radiation doses usually accompany advances in medical imaging, for example abdominal CT delivers 500-times the radiation of a routine CXR and multiphase scans routinely double that [2];
3. Radiation doses for the same scan can vary considerably among institutions, and even within the same institution [11];
4. Many investigations are not medically justified, with 30% of CT examinations thought to be unnecessary [2];
5. Medical personnel seriously underestimate the radiation dose attributable to CT scans, and their association with increased lifetime risk of cancer [12].

This poses a real risk to individual patients, with an estimated 1.5–2.0% of all cancers in the US potentially being attributable to the radiation from CT scans [12]. It also increases the risk to occupationally exposed medical workers [10], who now have a similar radiation hazard to those working in the nuclear industry [13].

30.1.3.2 Vascular Patients

Vascular patients, and particularly those undergoing endovascular aortic repairs, demand particular mention. These patients often undergo multiple and repeated studies [2] comprising pre-operative diagnostic investigations including CT scans, nuclear stress tests and coronary interventions [2, 14, 15], complex prolonged procedures [16], multiple reinterventions [16] and often prolonged CT follow-up [14–16]. All of these modalities can help establish accurate diagnosis, stratify operative risk, reduce the risk of vascular interventions and evaluate post-operative success [2]. However the benefits of these medical procedures must outweigh any potential risk from the attendant radiation [2]. Cumulative radiation dose needs to be seriously considered in such patients, as their cancer risk is not insignificant [15],

cancer being the second most common cause of death in EVAR patients [9]. The challenge remains to link cancer risk to radiation exposure in these patients as it has a large background frequency and long lead-time [10].

The considerable radiation dose delivered at the time of endovascular aortic repair pales in to insignificance compared to that received during routine follow-up with CT [17]. Surveillance scans in the first year exceed the radiation dose during EVAR (15 mSv vs. 12.6 mSv) [18]. Similarly the follow-up CT surveillance following TEVAR contributed to a cumulative radiation exposure of 87% after 1 year, 92% at 5 years and 96% in a lifetime [19]. These calculations suggest that a 2-year radiation exposure of >100 mSv with a life expectancy of >15 years leads to a lifetime risk of radiation-induced leukaemia and solid tumour malignancy of greater than 2.7% [19]. The lifetime cancer risk for a 70 year old undergoing EVAR followed by conventional CT surveillance is 0.6%, and for a 50 year old this rises to 1.0% [20]. When considering a young patient for open repair versus EVAR, it has been suggested that the patient's lifetime attributable risk of cancer associated with lifelong surveillance and reinterventions should contribute the same weight to the decision-making and consent process as do anatomy and durability [14, 21]. Once the need to perform endovascular repair has been duly justified, the true need for follow-up with CT needs to be justified [17], with consideration of other options such as a minimised-dose 'light' protocol, or use of plain or contrast-enhanced ultrasound [21].

These risks could be better managed if the cumulative radiation dose of patients was routinely monitored [2]. An electronic national registry documenting and recording radiation doses for every medical procedure using ionising radiation has been suggested [9]. Dose information tracking systems have been developed to incorporate into institution's IT systems, performing statistical analysis and sending alerts to medical staff [22].

30.2 Radiation Physics, Measurements, Dosimetry and Units

30.2.1 X-rays

X-rays are a form of electromagnetic radiation (EMR) lying on a spectrum with light, radiowaves and microwaves. What characterises these various forms of radiation are their wavelength and frequency; the shorter the wavelength the greater the frequency and also the greater amount of energy they carry [23]. X-rays have a short wavelength and therefore carry a high amount of energy which is why they can penetrate material including tissue.

X-rays are produced by a cathode-ray tube when high speed electrons strike a solid target and rapidly decelerate releasing energy [24]. Energy is carried through space in a bundle of energy, or a packet, called a photon [23]. Photons travel at the speed of light and carry no mass or charge [10]. Their electromagnetic energy can

range from a few electron volts (eV) to millions of eV and is set by the voltage applied across the X-ray tube. The ranges typically found in fluoroscopy have a photon energy spectrum of 30–140 keV, the higher the keV, the higher the X-ray energy [10].

What differentiates X-rays (ionising radiation) from other forms of non-ionising EMR is their ability to displace an outer electron from an atom when they collide with them (ionisation). Non-ionising radiation such as light and microwaves only have enough energy to move electrons to a higher energy state without actually displacing them [23].

The other form of radiation is particulate, in which small particles such as electrons carry the energy. This is the form of radiation produced by radioactive materials and linear accelerators and doesn't have a role in angiography as it has a low penetration of matter [23].

30.2.2 Interaction with the Body

When X-rays interact with the body they are absorbed, either completely or partially. This degree of energy transfer to the tissues, or absorption, not only accounts for the difference in transparency of the subsequent shadow image obtained from various tissue densities, but also the radiation 'dose' delivered to the patient [22]. This dose is related to the photons that enter but do not leave the body [24]. If the energy transferred is large enough, or cumulative, it can cause DNA and tissue damage.

The energy exchange in this process is due to the loss of energy from the photon and increased motion in the ejected electron. If the photon is not extinguished from this interaction it can continue to travel, but at a lower energy. This weakened photon is known as scatter radiation and may collide with further tissue, potentially ionising them as well, until either all of its energy is used up, or it escapes into the environment.

30.2.3 Scatter Radiation and The Inverse Square Law

X-rays that enter a patient from the primary beam can either be fully absorbed by the body, pass straight through the patient, or be partially absorbed by the tissues and change direction. These X-rays that exit the patient after changing direction are known as 'scatter' radiation, with the highest level occurring at the entry point of the patient's skin. Although scatter causes blurring of the image, of far greater consequence is that it comprises the main source of radiation to the operator and staff, and thus controlling patient dose generally reduces scatter and subsequent operator dose [22, 25]. Scatter is affected by many variables including beam size, large distances

between the X-ray source and the image intensifier, patient BMI, gantry angulation, fluoroscopy and acquisition settings [25].

Scatter is the reason why fluoroscopy machines are set up with the primary source positioned under the patient (posteroanterior imaging) [26]. This encourages scatter to be deflected down towards the legs of the operator, an area that is easier to protect with lead drapes, rather than up towards the head and neck [27]. With an anterioposterior set-up the head, neck and upper body exposure is approximately four times greater where shielding is less effective [26].

As these scattered X-rays leave the body they travel in straight, but divergent directions. With an increase in distance there is an exponential decrease in number of photons per unit area and hence energy; doubling the distance from the X-ray source decreases exposure by a factor of 4, tripling the distance results in a reduction by a factor of 9. This 'inverse-square law' ($X = 1/d^2$, X = exposure, d = distance) has a simple yet profound impact on radiation safety and is a cornerstone in reducing radiation exposure, namely increasing the distance between yourself and the X-ray source [3, 23]. Whilst recognising that maximising distance between operator and source is a fundamental principle of radiation safety behaviour, it must also be appreciated that the inverse square law pertains to radiation in a vacuum. The interaction of the radiation beam within a working vascular suite has a profound effect on this otherwise predictable scatter pattern, producing an eccentric 'scatter cloud' which is less easy to map [28].

30.2.4 Measuring Radiation Dose

Many factors conspire to make radiation dosimetry confusing to understand;

1. Two different nomenclature systems (conventional and International System of Units),
2. No direct way to measure radiation absorption in the body,
3. Several surrogate methods for estimating dose,
4. Terms such as exposure and dose are often used interchangeably within the literature without specific meanings,
5. Different units used to describe the radiation exposure for the same procedure making comparisons between studies challenging [9, 10].

Dose terminology can be categorised into;

1. Conceptual Measurements; those that are impossible or difficult to measure (e.g. Absorbed dose, Integral dose, Equivalent dose, Effective Dose, Isodose),
2. Direct Measurements; possible to measure but labour intensive and not real-time (Peak Skin Dose),
3. Indirect Measurements; used in daily practice but are surrogates of the above and therefore have limitations (Cumulative Air Kerma, Dose Area Product and Fluoroscopy Time).

30.2.4.1 Conceptual Measurements

Absorbed Dose: Unit = Gray (Gy)

This is the concentration of energy deposited by radiation into the absorbing tissue and gives an index of the potential biological risk [24]. Only a fraction of the radiation energy that reaches the body is absorbed, depending on the tissue's mass, composition, type of radiation and exposure time [29]. One Gy represents 1 J/kg; (1 joule of energy deposited in 1 kg of irradiated tissue) and, whilst this is relatively small in terms of energy deposition (it would only raise the temperature of water by 0.00024 °C), the effects of this energy transfer on biological functions is complex and can be deleterious [22]. It is the concentration of energy deposited, not the total amount deposited. Not all the radiation that tissues are exposed to is absorbed; some will pass through without imparting any energy (thus producing an X-ray image) while some is deflected, thus imparting smaller amounts of energy [10]. In this way absorbed dose differs from exposure in that the radiation present at a given location will not deposit all of its energy there [10]. This is dependent on the energy of the radiation, the tissue type and depth from the skin [10]. The 'Integral Dose' is the cumulative dose absorbed by all the tissues corresponding to the amount of potential tissue damage. It is extrapolated from exposure because there is no way to measure radiation absorption in the body [23]. The Gray has replaced the older unit of 'rad' where 1 Gy = 100 rad.

Equivalent Dose: Unit = Sievert (Sv)

Different forms of radiation have different tissue-injuring potential, some causing more biological damage than others [23]. The Equivalent Dose concept allows the biological effects of different forms of radiation to be compared with each other. Equivalent Dose is calculated by multiplying the Absorbed Dose (in Gy) by a weighting factor (W_R) specific to each form of radiation. Fortunately X-rays have a W_R of 1, so from the point of view of medical radiation (X-rays and gamma rays) the Equivalent Dose and the Absorbed Dose are equal, with 1 Sievert being equivalent to 1 Gray [10, 23, 24]. The Equivalent Dose is more useful when assessing for potential tissue injury due to highly ionising particles such as alpha particles, protons and neutrons, rather than X-rays, gamma radiation and electrons. The Sievert has replaced the older unit of rem (roentgen equivalent in man), where 1 Sv = 100 rem.

Effective Dose: Unit = Sievert (Sv)

In the same way that various forms of radiation have different capacities for biological damage, different tissues and organs have different sensitivities to radiation damage. In addition, radiation exposure is not uniform. Some organs and tissues

receive more than others [10]. The Effective Dose (ED) for a particular organ is calculated by taking the Equivalent Dose and multiplying it by a specific tissue weighting factor (W_T) for that organ [23]. The W_T relates to the organ's specific susceptibility to cancer and genetic defects as a result of radiation exposure. For example the W_T for the breast (0.12) is ten times higher than that for the brain (0.01) [22]. The potential stochastic risk (see Sect. 30.3.3) contributions from all the various exposed organs are then added to calculate a whole-body risk, expressed as the Effective Dose [10]. In this way effective dose is a measure of the estimated potential for a biological effect on the whole body caused by a particular absorbed radiation dose [10]. It is a hypothetical uniform whole-body dose that confers the same stochastic risk as the non-uniform regional dose actually delivered, and estimates the radiation dose's contribution to stochastic risk [10, 17]. In this way it reflects overall biological risk from radiation to an average person from a specific radiation exposure scenario [10]. The Effective Dose provides the same whole-person stochastic risk as an absorbed dose for a limited portion of the body; it allows comparison of the risk among several individuals regardless of the body areas irradiated [24].

Advantages of Effective Dose [17, 30];

- Accounts for the portion of body exposed
- Accurate reflection of stochastic risk

Disadvantages of Effective Dose [17, 30];

- Impossible to measure or calculate; can only be estimated
- No real time assessment
- Cannot predict radiation risk for an individual due to the nature of stochastic risk

30.2.4.2 Direct Versus Indirect Measurements

Patient radiation dose is most accurately evaluated by the direct measurement of radiation exposure. For deterministic risks (see Sect. 30.3.2), this is best estimated by Peak Skin Dose (PSD), and for stochastic risks (e.g. cancer) this is best estimated by Effective Dose (ED). However direct measurements are either impossible or impractical in everyday practice, labour-intensive and not real-time [22]. Therefore a range of indirect measurements have been developed as surrogates to measure radiation exposure and approximate these risks. Radiation exposure is the quantity of radiation to which a tissue is subjected. PSD and ED can be calculated from these indirect measurements using equations [30].

By definition, indirect measurements don't measure dose directly, but give enough real-time information to allow monitoring during a procedure [22]. However they have potential for considerable error [17]. In a study comparing direct and indirect measures during complex aortic repairs the authors found that even though indirect measures (CAK and DAP) correlated reasonably to direct measures (PSD), they were higher than direct measurements, giving rise to three sentinel threshold events of >15Gy, which were well below this threshold on direct measurement [17].

30.2.4.3 Direct Measurements

Peak Skin Dose: Unit = Gray (Gy)

The 'Peak Skin Dose' (PSD) refers to the maximally irradiated area (including both primary beam and scatter) [22]. PSD is measured using thermoluminescent dosimeters (TLDs), radiochromic film (Gafchromic™) or optically stimulated luminescence dosimeters (OSLDs) [22]

Advantages of peak skin dose

- A reliable direct measurement of specific points of interest at the most irradiated area [17]
- Directly attributable to deterministic effects [9, 30]

Disadvantages of peak skin dose

- Expensive and labour intensive to measure using radiochromic film [9, 17, 30]
- Not real time, therefore usually used only for research purposes [17, 31]

30.2.4.4 Indirect Measurements

Cumulative Air Kerma at the Interventional Reference Point (CAK):
Unit = Gray (Gy)

Also known as;

- *Cumulative Dose*
- *Air Kerma at the Reference Point; Reference Air Kerma (RAK);*
- *Reference Point Air Kerma (RPAK)*

This is used to assess the level of radiation present at a location [10]. The 'air kerma' is used to measure radiation quantity of external radiation beams such as those used in fluoroscopy. 'Kerma' describes Kinetic Energy Released in MAtter [24]; i.e. the amount of energy released by the interaction of radiation with a unit mass of absorbing material. 'Air kerma' refers to the amount of energy released by the interaction of the radiation beam with 1 kg of air as the absorbing material [10]. One Gray represents the amount of radiation that releases 1 joule of energy when it interreacts with 1 kg of air.

The next pertinent question therefore remains 'where in relation to the patient is the 'air kerma' measured'? The answer is the 'Interventional Reference Point' (IRP). This has been chosen as a constant position in space to measure the air kerma. It is a fixed position located along the central ray of the X-ray beam at a distance 15 cm towards the X-ray tube side of the isocentre of the C-arm [24]. The IRP approximates the point where the X-rays reach the patient's skin. Therefore the 'air kerma at the IRP' provides an index of the dose reaching the skin and the risk of skin injury. In this way the 'air kerma at the IRP' is also known as the 'Entrance

Surface Dose', or more commonly the 'Entrance Skin Dose' [24]. The air kerma accumulates at a specific point (the reference point) in space relative to the fluoroscopy gantry. As the gantry rotates the reference point will change over the full surface of a 15 cm radius sphere representing a 30 cm diameter patient. The Cumulative Air Kerma (CAK) is the sum of all of the energy liberated in air at the IRP during the whole procedure. It can be appreciated that during a procedure where the C-arm remains static (e.g. cardiac catheterisation) the beam 'Entrance Skin Dose' will approximate well to the area of skin to which the beam is directed. In these situations the Entrance Skin Dose is associated with the deterministic effects of radiation [31]. However, during procedures where the C-arm is frequently moved (e.g. EVAR) the Entrance Skin Dose may actually be spread over a larger area, so no one point receives the total dose. In order to correct for some of the shortfalls of CAK, manufacturers have developed more sophisticated tools to correct air kerma at the IRP to the actual position of the patient, taking into account patient size, system geometry, backscatter and table attenuation. Maps can be displayed of dose distribution in real time giving a more accurate estimation of deterministic effects [22].

Advantages of Cumulative Air Kerma

- Easy to calculate
- Easy to interpret
- Accounts for high-dose acquisitions
- Best estimation of Peak Skin Dose to monitor deterministic effects [9, 30, 32, 33]
- Provided by most fluoroscopy units
- Recommended over FT or DAP for high-dose procedures by the National Council on Radiation Protection and Measurements (NCRP) [22]
- Since 2006 real time display of CAK became mandatory in new systems in the USA [31]

Disadvantages of Cumulative Air Kerma

- Does not represent Entrance Skin Dose if the patient's size or table height results in the IRP being inside or outside of the patient, rather than on the surface.
- Does not account for the area exposed [9, 17]
- Does not account for the patient's location in relation to the C-arm or different beam projections [17]
- Does not account for backscatter from the patient which can increase skin dose by one third
- It tends to overestimate deterministic risk compared to direct measures of PSD [22, 33]

Dose Area Product (DAP): Unit = Gray.cm^2 (Gy.cm^2)

Also known as; Kerma Area Product (KAP)

The Dose Area Product (DAP) is the air kerma multiplied by the beam cross sectional area at the point of measurement [24]. One disadvantage of CAK is that it

doesn't take into consideration the area exposed, and therefore the volume. Whilst CAK is a good surrogate for dose at the surface, deeper structures receive smaller doses. For every 5 cm of tissue that X-rays pass through they are attenuated by a factor of 2, thereby decreasing exponentially with depth from the entrance [10]. Therefore to estimate the dose to a particular body part within the path of an X-ray beam adjustments need to be made for the beam's absorbance; this is the DAP [10]. The DAP is the cumulative sum of the product of the air kerma and X-ray cross sectional beam area. It provides a good measure of the total energy output of the X-ray tube and therefore a good approximation of the total energy absorbed by the patient [24, 34]. In this way it provides the basis of estimated Effective Dose from a procedure [10].

Advantages of Dose Area Product

- Modern machines have an integral DAP meter built in with mandatory real-time display since 2006 [24, 31]
- Correlates to the amount of scatter during a procedure, and therefore the stochastic risk to staff [3, 24, 30, 33, 34].
- Surrogate measure for the entire amount of energy delivered to the patient, and therefore stochastic risk to patient [22]
- Widely used allowing comparisons of doses between procedures and institutions [22]
- Best correlates with Effective Dose and can be used to estimate Effective Dose per organ or anatomical region using conversion factors [22, 30]
- Provide a measure of the efficacy of radiation protection practices within a unit [24]

Disadvantages of Dose Area Product

- Not as intuitive as CAK [30]
- Doesn't account for different projections [17]
- Estimation of absorbed skin dose can be inaccurate—a large dose delivered to a small area yields the same DAP as a small dose delivered to a large area [15].

Fluoroscopy Time: Units = minutes (min)

Fluoroscopy time (FT) is the total time the X-ray beam is activated [17, 30]. However some machines record total pedal time and others total fluoroscopy time, which can differ if using pulsed fluoroscopy.

Advantages of fluoroscopy time

- Provided by all fluoroscopy units
- Easy to measure and interpret
- Correlates with the complexity of a procedure [22]
- Can be used as a quality assurance tool for assessing the efficiency of an interventionalist at completing a procedure

Disadvantages of fluoroscopy time

- Poor correlation to dose, biological risk and clinical outcomes [22]
- Doesn't account for system settings, field size, dose contributions of various modes such as acquisitions, beam quality [17], output rate, differences in equipment, technique, patient BMI and angulation of gantry [15, 31].
- Ambiguous as some machines measure total pedal time, others limited to the sum of X-ray pulses [22, 33].
- Not recommended for routine use unless other parameters unavailable, or as a correlation with procedural complexity [22]

30.2.4.5 Documenting Dose, Reporting Threshold Limits and Diagnostic Reference Levels

In daily practice the CAK, DAP and FT should be recorded in the operative report. This will encourage interventionalists to become familiar with their procedural doses. Maximum dose limits for a single case should stimulate documentation, prolonged patient follow-up for deterministic effects, and a review of operator and institutional radiation safety behaviours and systems. Maximum dose limits are: FT >60 min, PSD >3 Gy, CAK >5 Gy, DAP >500 Gy.cm^2 [22]. Diagnostic Reference Levels (DRLs) differ in that they have been determined for specific diagnostic or interventional procedures and are indicative benchmark doses which aren't expected to be exceeded in normal circumstances.

30.3 The Biological Effects of Radiation

30.3.1 Interaction of X-rays with Tissue

When X-rays enter the body they can (i) pass straight through with no interaction, (ii) transfer varying degrees of energy to the tissues with a resultant deviation in their path (scatter), or (iii) completely transfer all their energy to the tissues (photoelectric effect) [22]. This transfer of energy has the ability to form ions within tissues by ejecting electrons from the atoms that makes up molecules, which is the basis for the term 'ionising' radiation [10]. This can subsequently modify the chemical, physical or biological effects of the tissues and cause radiation-induced injury in two ways;

1. Direct cellular damage due to molecular alterations, leading to a change in structure and function
2. Indirect cellular damage from hydrolysis of intra-cellular water leading to unstable and highly destructive free radicals which go on and interact with other molecules, proteins or nucleic acids, causing a cascade of injury [9, 10, 22].

The most susceptible cells to damage are immature or dividing cells. At low doses the body's repair mechanisms can mitigate direct cellular damage and can bind proteins to free radicals forming stable complexes. However higher doses overwhelm these repair mechanisms leading to apoptosis and the resulting clinical manifestations of radiation injury, namely 'deterministic' and 'stochastic' effects [22, 23].

30.3.2 Deterministic Effects

Deterministic effects, also known as tissue reactions, are predictable injuries that will occur in all patients subjected to a sufficient radiation dose [10, 24]. Once a threshold dose has been exceeded, cellular death will occur if the molecular damage exceeds the cell's own repair mechanisms. The severity of the injury typically correlates to the exposure in a dose-dependent manner [10, 22], in the same way that sunburn occurs as a result of a threshold of sun exposure, and worsens with increased exposure [33]. Although the corollary is that one is safe from such effects under threshold values, subclinical injury may occur if a sufficient proportion of cells are able to maintain function despite a certain number undergoing necrosis [10]. Another important point is that there may be a significant time lag between the damage occurring and clinical manifestation of injury. Examples of deterministic injuries include skin burns, hair loss, cataracts and sterility [23, 35].

30.3.2.1 Skin Injury

This is the most common deterministic injury and is estimated to occur between 1:10,000 and 1:100,000 examinations [22]. Skin injuries can present as mild skin erythema resembling sunburn hours, days or even weeks after injury [35]. It is probably underreported due to a combination of: delays in presentation, often located on the back, a failure to link burns to previous radiation exposure both by patient and physician and failure to follow-up patients [22, 24]. Typically they resemble the rectangular shape of the X-ray beam and will be present at the site of beam entrance, usually the back. They can vary in severity from erythema, epilation, hyperpigmentation, telangiectasia, desquamation, dermal atrophy and ulceration [10].

Thresholds for skin injury are

1. <2 Gy; no observed injury,
2. 2–5 Gy; transient temporary erythema,
3. 5–10 Gy; erythema and epilation with dermal atrophy towards 10 Gy,
4. 10–15 Gy; prolonged erythema, possible desquamation, permanent epilation and dermal atrophy,
5. >15 Gy; desquamation, dermal atrophy, ulceration and necrosis of subcutaneous tissues [36].

It must be remembered when treating vascular patients that these thresholds are for single first dose exposures, and repeated doses, especially those in close temporal proximity, may lead to injuries at lower doses than expected, due to inadequate time for DNA repair to occur [22]. The typical patient to suffer a skin injury is a smoking, obese, diabetic who has undergone 1 or more long-duration fluoroscopically-guided procedures over a period of few months [10]. Another group at risk of multiple interventions are dialysis patients, with rising numbers of patients and increasing endovascular interventions for dialysis access [33].

Interestingly, in a study comparing direct versus indirect measures of skin dose during complex aortic repairs investigators failed to identify any skin changes in the half of their cohort that had a measured skin dose >2 Gy [17]. Whilst this is not cause for complacency or lowering of the thresholds, this study does highlight the importance of distributing the dose over a relatively large area during aortic repairs, compared to coronary procedures which tend to be more static leading to a higher Peak Skin Dose in one area.

Additional risk factors for radiation-induced skin injury include obesity, extreme degrees of angulation of the C-arm, connective tissue disorders (scleroderma, systemic lupus erythematosus and ataxia telangiectasia), diabetes, hyperthyroidism and some chemotherapy agents [10, 15, 22, 24, 35]. Extraneous body parts within the radiation field of view such as limbs, breasts and overlapping skin folds will all lead to increased radiation dose due to the X-ray machine's automatic brightness control (ABC), thus increasing skin injury risk [31, 35, 37].

The global obesity epidemic demands an appreciation of the profound effect obesity has on elevating patient skin dose, and operator scatter exposure [24, 38]. Due to overlapping skin folds and excess soft tissue, more energy is required to penetrate tissue. Radiation dose decreases by a factor of 2 for every 5 cm travelled in soft tissues, therefore twice as much radiation is required to penetrate 10 cm of fat than 1 cm [3]. ABC settings force the dose up to obtain an adequate image [3, 14, 32, 39], with subsequent doses reaching 4–10 times higher in obese patients [3, 38]. For example during EVAR, obese patients received up to 3× PSD [40] and 2× DAP [40, 41] than those with normal BMI, with BMI predicting radiation burden in a similar manner to fluoroscopy time [42].

At the end of a procedure the CAK should be recorded and used to estimate the patient's entrance skin dose to provide an index of the risk of deterministic effects [24]. Current recommendations suggest that patients receiving >5 Gy CAK should be counselled regarding the possibility of skin injury and followed-up closely, in addition to involving institutional and local radiation regulatory authorities [10, 31].

30.3.2.2 Bone Injury

Whilst bone requires a higher dose than skin to undergo necrosis, the calcium content of bone leads to a greater capacity to absorb X-rays and thus a higher absorbed dose than the overlying skin [10]. Occasionally this may manifest as osteonecrosis of superficial bones such as ribs at doses inadequate for skin necrosis [10].

30.3.2.3 Eye Injury

Unlike skin injuries, cataracts comprise a deterministic effect far more relevant to interventionists than patients. Cataracts describe a permanent clouding of the lens of the eye which leads to loss of acuity and even sight. They are classified according to their location in the lens:

1. Nuclear (centre)
2. Cortical (edge) and
3. Posterior subcapsular (back) [43].

Radiation exposure can lead to direct protein and DNA damage, and indirect oxidative stress through free radicals. The dividing epithelial cells are particularly susceptible, and when damaged migrate to the posterior of the lens resulting in opacities.

A higher incidence of cataracts were first noted in atomic bomb survivors, astronauts, radiation workers and radiotherapy patients, but more recently have been identified in interventionalists [44]. For example, cardiologists were found to have three times the incidence of posterior subcapsular cataracts (PSC) than matched non radiation-exposed doctors [44].

Already known to be one of the most radiosensitive tissues, emerging evidence suggests that the lens is much more radiosensitive than previously thought, and thresholds for the development of cataracts are far less than previously assumed, (and may not exist at all) [35, 44]. The International Commission on Radiation Protection (ICRP) has stated that such a threshold is 4Gy for fractionated exposure, but single dose thresholds of 0.5 Gy [45] to 1 Gy [35] may be enough to induce cataracts. The delay between exposure and onset of symptoms is many years, making it difficult to demine if they develop as a result of continued accumulation of small doses over time, but the latent period between exposure and formation seems to be inversely related to radiation dose [10].

PSC represents the most common type of radiation-induced cataract, followed by cortical cataract [35, 43]. This compounds the problem for interventionalists because in the general population PSCs are uncommon, difficult to detect and treatment is suboptimal. Because PSCs are uncommon, ophthalmologists may not even consider the possibility of radiation-induced cataracts. Routine eye chart testing is not sensitive for their detection, as they affect contrast prior to acuity, and most clinics won't have the sophisticated equipment required for their detection. Finally, even after diagnosis and treatment, 'successful' cataract surgery can still result in reduced visual acuity; 20/25 vision being considered an excellent surgical outcome [44].

30.3.2.4 Impaired Fertility

Impaired fertility appears to be a deterministic effect of prolonged radiation exposure [3], although foetal abnormalities in offspring can represent stochastic effects [22].

30.3.3 Stochastic Effects

Stochastic effects are less predictable as any level of exposure can cause them, but not every exposure will, and as such there is no threshold dose [35]. These describe genetic mutations and cancer, due to a cumulative radiation dose that overwhelms the individual's ability to repair DNA damage [42]. Tissues that have a rapid turnover such as bone marrow and breast tissue are more susceptible than more quiescent tissues [23].

30.3.3.1 DNA Damage

Stochastic effects are due to unrepairable DNA damage and consequential genetic mutations. The type of damage, the body's repair mechanisms and the person's age will determine whether radiation-induced DNA damage leads to genetic mutations that cause cancer, the most feared stochastic effect, or go by unnoticed. Stochastic effects lead to the development of solid tumours and blood malignancies months or even years following exposure [22].

Sublethal damage to non-coding areas of DNA may be inconsequential, and repaired successfully, avoiding a stochastic event [35]. However damage to coding areas, regulatory regions, or double-stranded breaks that are harder to repair, could affect genetic products or regulation, turning a normal gene into a cancer-inducing oncogene [10, 46]. Thereafter the clinical development of cancer still depends on many other biological factors including genetics and age. Children and young adults are at a greater risk of stochastic events simply due to a longer life-expectancy in which a long latent cancer may develop, with those less than 30 years having greatest dose sensitivity [10]. Solid organs that are most sensitive to radiation are those with a high mitotic activity such as colon, thyroid, breast, lung and bladder. Haemopoietic tissues are highly sensitive for the induction of leukaemia [10].

30.3.3.2 The Linear No-Threshold Theory

Stochastic effects differ from deterministic effects in that they do not conform to a dose relationship. Whereas deterministic effects do not occur below a certain threshold and thereafter follow a dose-dependent severity, stochastic effects are not associated with a threshold and there is no correlation with dose. They can occur at any dose, and there is no dose at which they will never occur. However, the likelihood of development is probabilistic, with the probability increasing with total dose, but the severity of effect is not related to the dose that induced it [24].

This linear relationship of dose and stochastic risk has led to the concept of the linear no-threshold theory. Although stochastic risk increases with dose exposure, there is no threshold below which stochastic risk is zero [10]. This is because, in theory, a single X-ray photon could ionise a critical portion of DNA creating an

oncogene [10]. Therefore even the lowest exposure of radiation increases stochastic risk [22]. This forms the foundation of the ALARA principal (As Low As Reasonable Achievable) [47] which maintains that radiation exposure should always be minimised as much as possible within the limits of clinical need [10].

30.3.3.3 Predicting Stochastic Risk

Despite the linear no-threshold theory, stochastic risk can still be estimated. At small doses it is difficult to distinguish from zero [10] but the Biological Effects of Ionizing Radiation VII Committee (BEIR VII) has suggested an estimate of lifetime attributable risk of cancer due to low dose exposure based on mathematical models [22]. For example the estimated risk of lifetime fatal malignancy in the US population is 21% and this risk increases by 5% for each 1Sv of Effective Dose radiation one is exposed to, assuming a normal life-expectancy [33]. In this way Effective Dose provides an index for stochastic risk [24]. This has implications for younger patients who may undergo multiple medical exposures in their lifetime, and career interventionalists regularly exposed to low dose radiation.

30.3.4 What Is the Risk for Interventionalists?

Recent health issues in prominent interventionalists have highlighted the long-held concerns regarding the risk of occupational radiation exposure [48]. Most data on human stochastic effects are gleaned from epidemiological studies on survivors of atomic bombs in Nagasaki and Hiroshima, or nuclear power plant accidents such as Chernobyl [10]. However these relate to large doses received over a short period of time, whereas occupational radiation workers are subject to small cumulative doses over longer periods. There are some observational data from nuclear plant operators and some longitudinal studies in healthcare workers suggesting that chronic exposure increases the risk of cancer compared to the general population [35]. However one of the problems with developing any causal link between occupational radiation exposure and stochastic risk is the long latency of effects [9], coupled with the fact that radiation-induced malignancies are often indistinguishable from cancers developing de novo [2].

Nonetheless since the first case report published in 1912 highlighting leukaemia in radiologists [44], and a case series in 1942 reporting a ten-fold increase in the incidence of leukaemia in this specialty [49], there have been numerous studies reporting several-fold increases in the incidence of potential radiation-injuries amongst angiography suite staff [50]. These include skin cancer [51], cataracts [52], solid organ and haematological cancers [53], and brain and neck cancers [54]. However, outside of cataract formation, it is currently impossible to draw definite conclusions from the existing evidence that these associations are due to occupational exposure [22, 55]. Whilst there is no conclusive evidence that radiation due to

medical imaging can induce cancer, radiation remains one of the most extensively studied carcinogens [2].

30.3.4.1 Statistical Risk of Cancer in Interventionalists

The BEIR VII statistical models based on the linear no-threshold theory suggest a small but measurable risk for the development of cancer in medical radiation exposed healthcare workers. In one scenario an 18-year old worker is exposed to the upper limits dose of 10 mGy/year, for a career ending aged 66 years old. This model suggests an additional risk of cancer incidence of 3.06% (male) or 4.29% (female) and cancer mortality of 1.7% (male) or 2.39% (female). This obviously needs to be put into the context of the background risk of developing cancer in the general population of 46% overall and 23% fatal [10].

30.3.4.2 Recommended Occupational Exposure Limits

Based on the BEIR VII models the current ICRP recommended limits for occupationally exposed medical workers are [56, 57];

- Total Body = 20 mSv/year (averaged over 5 years with no single annual exposure >50 mSv)
- Lens of Eye = 20 mSv/year (averaged over 5 years with no single annual exposure >50 mSv)
- Skin = 500 mSv/year
- Hands and Feet = 500 mSv/year (averaged over the most irradiated 1 cm^2 area of skin)

The total body dose is an Effective Dose relating to stochastic risk throughout the body, and the specific tissue doses are Equivalent Doses pertaining to deterministic risk in those tissues [25].

30.3.4.3 Occupations Limits in the Context of a Career in Vascular Surgery

A 3 mSv/year effective dose throughout a career translates to a less than 1:10000 risk of radiation-induced cancer [58]. Based on the linear no-threshold theory this risk increases with dose so that a lifetime cumulative exposure of 400 mSv correlates to an increased cancer risk of 1:250, with a cumulative dose of >100 mSv still being statistically relevant [59]. To put this into context it has been estimated that an interventionalist performing 100 peripheral angiographic procedures a year will receive an annual dose of 30 mSv to the eyes and head, and 40 mSv to the hands [60]. Over a career of 40 years, this cumulative dose will approach the threshold for deterministic injury [35]. Although Effective Dose under lead has been estimated to

be 0.55 mSv for a vascular surgeon performing 50 elective non-complex EVARs over the course of a year, the equivalent dose to unprotected areas may exceed 10 mSv/year [61]. The consequences of receiving 10 mSv/year over a 30–40 year career is as yet unknown [10]. A similar estimation for a busy interventionalist adhering to ALARA principals is that their effective dose is unlikely to exceed 10 mSv/year, and more likely to be in the range of 1–4 mSv/year [25, 62]. However the exposure in vascular surgery may be greater, with the endovascular case mix exposing the vascular surgeon to higher doses [46]. The per-case dose for EVAR was found to be almost six-fold higher than that of percutaneous coronary interventions, with peripheral endovascular interventions also being associated with higher operator exposure than coronary interventions [63]. This results in vascular surgeons being classified as Category A radiation exposed workers (those likely to receive Effective dose >6 mSv/year, equivalent dose of >15 mSv to the lens) [22].

30.3.4.4 Brain Cancer and Dementia

Studies have demonstrated significant doses to the unprotected left side of the head during endovascular procedures [64] and published case reports of brain tumours occurring in interventionalists have raised concerns. This has been compounded by the finding that in one study of 31 interventionalists a disproportionate number were left sided (85%), suggesting a causal relationship [54]. In addition the majority of the malignant neoplasms reported have been associated with radiation exposure [55]. However large epidemiological studies looking at cause of death in workers exposed to fluoroscopically guided procedures, including interventionalists, failed to find any demonstrable increased radiogenic mortality in these groups [65]. The overall prevalence of malignant brain tumours is low, and in a group of 10,000 interventionalists, 25 may be expected to develop fatal brain tumours outside of any occupational risk, and 4 may develop fatal brain tumours from occupational exposure, giving a risk of 0.04% [66].

In addition to brain cancer the question of radiation-induced dementia has been raised. Animal models have demonstrated that low-dose radiation downregulates the same neural pathways responsible for cognitive dysfunction seen in normal aging and Alzheimer's disease [67] but again a causal link has not been definitively identified.

30.3.4.5 Cataracts

Numerous studies have demonstrated an increased incidence in cataracts in radiation workers, particularly PLC, up to six times in some studies [44, 50]. The lens is one of the most radiosensitive tissues in the body, and cataracts can develop despite low dose exposures [68]. As such dose limits to the eye have been dropping since they were first evaluated by the ICRP in 1977, from 300 mSv/year, dropping to 150 mSv in 1984, and again to 20 mSv/year in 2012, now identical to the

whole-body effective dose limit [43]. Epidemiological evidence suggests an even lower threshold dose may be appropriate, or even a no dose threshold, for radiation-induced cataracts [69].

Many endovascular procedures deliver considerable doses to the eye [70], with an estimated 23 hours of fluoroscopy time resulting in the 20 mSv annual threshold [70]. Further studies highlighted EVAR, embolization procedures, lower limb and renal interventions as delivering substantial doses to the eye [61, 62]. Again, extrapolating these doses to an annual workload exceeded the ICRP dose limit recommendations [71], with the completion of 50 EVARs over a year resulting in eye exposure exceeding 30% of the IRCP limit, a threshold which in the UK would classify the surgeon as a radiation worker [61].

30.3.4.6 Genetic Susceptibility to Radiation Injury

When considering dose thresholds, one must bear in mind that they are based on average sensitivities to radiation injury from population data [44]. At an individual level, biological responses to injury vary, governed largely by genetically mediated mechanisms, resulting in natural variation with regard to one's individual sensitivity to radiation exposure [44]. Animal studies have demonstrated a genetic variation to radiation repair genes [72], and in a recent study measuring DNA damage markers in the circulating lymphocytes of vascular interventionalists exposed to radiation, it was found that significant individual variation existed regarding the induction of DNA repair markers to this exposure [46]. Certainly there is growing awareness of personal radiation exposure amongst interventionalists; in a survey of over 600 European cardiac catheter laboratory workers, 11% had ceased working in the laboratory due to personal concerns regarding radiation exposure [73]. In the future, data regarding an individual's genetic susceptibility to radiation injury may help guide their career choice, but until that time this further uncertainty at an individual level highlights the importance of adherence to ALARA principals at all times.

30.4 Conclusion

A sound understanding of radiation physics and biology provides a solid foundation on which to apply the practical methods available to reduce exposure in daily practice. Although radiation dosimetry can be confusing, familiarity with the most common forms of dose measurement (CAK and DAP) will allow a deeper understanding of the literature. In addition, auditing one's own dose reports in the context of DRLs will inevitably drive exposure down; after all "what gets measured gets managed". Currently there is no definitive evidence linking occupational radiation exposure to stochastic effects in interventionalists. However, a significant body of anecdotal evidence would suggest that keeping radiation doses to a minimum is, at the very

least, highly prudent. Stronger evidence links deterministic cataract formation to occupational exposure, and adequate eye protection for interventionalists is essential, in addition to the other techniques available to minimise radiation exposure, as discussed in Chap. 31.

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Chapter 31
Radiation Stewardship: Radiation Exposure, Protection and Safety in Contemporary Endovascular Practice

Joseph Dawson and Stephan Haulon

Key Learning Points

- Understand the underlying reasons governing the variation in radiation exposure during different endovascular procedures.
- Describe the principles of ALARA and provide practical examples of ALARA in daily practice.
- Describe how staff position and distance in relation to the patient table affects occupational radiation exposure and what steps can be taken to reduce this.
- Describe the specific considerations and actions that are required for pregnant operators.
- Understand the uses and limitations of dosimeters and ways to improve their utility.
- Understand how manipulating machine controls, table position and gantry angle can influence radiation exposure.
- Understand the difference in radiation dose associated with fluoroscopy versus digital subtraction angiography.
- Describe all of the available shielding techniques available to operators and the importance of their combined use.

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31.1 Radiation Stewardship

Over the last 50 years there has been a proliferation in fluoroscopically-guided minimally invasive procedures, and endovascular interventions have frequently become the preferred choice with both patients and surgeons [1–3], due to a lower morbidity, mortality, reduced length of hospital stay [4], and favourable outcome data in almost every vascular territory [5].

This endovascular 'revolution' has now been superseded by 'evolution' where not only are the number of procedures increasing, but so is the complexity [1, 6]. The advent of the hybrid suite, combined with a pro-endovascular approach [4], has resulted in prolonged screening times with a substantial and significant increase in accompanying radiation dose [4, 6, 7]. To date, the majority of attention has been dedicated to developing and perfecting endovascular techniques to tackle increasingly complex pathology, with far less focus on the potential hazards that this cumulative radiation burden poses [8]. However there has been a growing concern regarding this increasing radiation exposure, not only to the patient, but to the whole endovascular team [2, 9, 10]. With more patients being treated by more complex procedures, we will likely see this exposure continuing to rise dramatically in the future [11]. Whilst radiation exposure to patients has been studied in some detail, there is a relative paucity of data looking at occupational exposure for interventionalists [3, 4], although lessons learned from other specialties such as cardiology can be readily translated into the vascular field [12].

Whist it is now accepted that ionising radiation is an inherent feature and accepted component of modern vascular practice [1, 13, 14] (the X-ray beam being described as the "modern vascular surgeon's scalpel" [15]), the considerable clinical benefit of minimally invasive interventions must be tempered by the attendant disadvantage of a reliance on ionising radiation. Unfortunately the risks of radiation exposure are not universally recognised by vascular operators [9], and perhaps underappreciated by those that learned their trade in the operating theatres of old [11]. As radiation is an invisible threat, protection can be challenging for the surgeon to understand and enforce [16]. It is not as intuitive as, for example, blood-borne virus protection, a comparative safety issue of equal magnitude for the surgeon [16]. One of the hurdles identified in establishing an effective radiation protection culture is a lack of proper understanding of radiation risks by key players; knowledge and understanding of the real radiation risks relative to their benefits is critical [17].

With vascular surgeons now principal operators in the angiography suite, and no longer merely guests [15], their responsibilities must evolve. While continuing to attend to the procedural and clinical needs of the patient, they must now also shoulder the responsibility of radiation protection for the patient, themselves, their trainees and team; we term this essential new leadership role 'Radiation Stewardship'.

The aim of this chapter is to aid in this task; to build on the concepts of Chap. 30 and describe the numerous methods available to protect against radiation in practice. In this way, it is hoped that vascular surgeons feel empowered to implement Radiation Stewardship within their hospitals; to apply a heightened level of

knowledge and responsibility regarding ionising radiation so they can protect themselves, their colleagues and their patients [18].

31.2 Radiation Exposure during Endovascular Interventions

The majority of literature regarding radiation exposure and safety describes interventional procedures pertaining to cardiac interventions with an under-representation of peripheral vascular interventions [1, 19]. Meaningful comparisons of the data that do exist are hampered by significant methodological and outcome heterogeneity in small, non-randomised studies. Other limiting factors include significant differences regarding anatomical lesions, procedural complexity, technique, equipment, patient factors, operator experience, units of measurement and reporting [6, 20–22].

Some of these variables may explain the wide variations observed in the published literature. One reported a 1000-fold difference in minimum and maximum radiation doses for a range of cardiac interventions [23]. The same authors found operator dose varied up to three orders of magnitude for the same fluoroscopically guided intervention [22]. Similarly in a study of 149 mixed endovascular procedures, it was found that the range of annual body Effective Dose was lower for vascular surgeons (0.13–0.27 mSv) compared to other published reports in cardiologists (1.9–37 mSv)) and interventional radiologists (0.37–10.1 mSv) [6]. These low doses are in contrast to earlier reports of EVAR operators experiencing under-lead doses of 1.52 mSv, and over-lead doses of 13.7 mSv [19]. As increasing radiation awareness can lead to significant reductions in occupational dose, it has been suggested that large variations in operator dose could be substantially reduced with improved radiation safety practices [22].

31.2.1 Radiation Exposure During EVAR

EVAR remains the most common quintessential complex endovascular case, and as such, is the most investigated procedure regarding radiation dose in vascular surgery [8, 19, 20, 24–27]. In a systematic review reporting radiation during EVAR and other complex aortic repairs, the mean DAP for standard infrarenal EVAR was 79 Gycm^2, but as expected increased proportionally with procedural complexity [20]. Although some studies report relatively low radiation doses for the majority of aortic repairs [28], others have found that one third of EVAR patients received an Entrance Skin Dose in excess of 2 Gy [27], the threshold for deterministic effects, with some patients receiving up to 6 Gy [27]. As previously discussed these variations in dose are due, in part, to different operators and variations in technique, anatomical complexity, patient BMI and staff adherence to radiation safety principals [3]. Some investigators have gone on to investigate what part of the operator's body is most exposed during EVAR. By placing dosimeters near the eye, thyroid,

chest, abdomen, hands and feet of interventionalists, investigators found that during EVAR 90% of the cases resulted in at least one body part receiving more than 1 mSv per procedure [29], usually in the lower body where scatter is known to be highest.

31.2.2 Procedural Complexity

Unsurprisingly the more complex the procedure the higher the associated radiation dose. Complex thoracoabdominal procedures such as FEVAR and TEVAR for aortic dissection [30] generate more radiation than EVAR and standard TEVAR [31], especially if associated with challenging renal or visceral artery stenting [32, 33]. In one study one third of such repairs exceeded the 2 Gy skin dose deterministic threshold [28], and in another 21 patients, 70% of whom were undergoing FEVAR, the 6Gy CAK threshold for substantial radiation dose was exceeded [31]. Average operator doses associated with such repairs were reported at 0.17 mSv per procedure [32]. In addition to prolonged operative times, such procedures often require acute C-arm gantry angulations, further increasing the radiation dose [2].

Adverse features adding to the complexity of infrarenal EVAR associated with increased radiation doses include bilateral iliac aneurysms, AAA diameter >60 mm, neck diameter >28 mm, CIA diameter >20 mm and neck angulations >50° [13]. From this analysis the authors determined that they could predict 40% of the case radiation pre-operatively from the BMI, neck angulation, AAA diameter and aneurysm type. Having more than 2 of these anatomical risk factors was related to increased radiation exposure, with BMI and neck angulation emerging as the two most predictive variables [13]. In another study localisation of the proximal landing zone and main body deployment accounted for 24% of the total radiation dose during EVAR [34]. Other strong predictors of radiation exposure during endovascular aortic repair include number of acquisitions and acute C-arm angulation [9].

31.2.3 Other High Dose Procedures

In addition to complex aortic repairs, other endovascular procedures have emerged as contributing high radiation doses. In 318 endovascular cases, atherectomy emerged as having the longest fluoroscopy time [35]. This may explain why, in another study of aortic, carotid and peripheral interventions, atherectomy had the highest Estimated Skin Dose of all endovascular procedures, approaching 1.5 Gy [16]. This finding has been corroborated in another study looking at 2103 mixed endovascular cases reporting an ESD of 1.26 mGy associated with atherectomy [14]. These findings are significant considering the threshold values for skin injury is 2 Gy. Other procedures associated with high Peak Skin Doses include abdominal and pelvic procedures [35], particularly renal and visceral interventions [36] and embolization [2, 30, 31, 35].

It is not only total radiation dose that is important to consider, but operator proximity to the source. For example endovascular interventions for dialysis access often require the operator to be in close anatomical proximity to the area of interest, exposing them to potentially higher scatter, and inadvertent encroachment on the direct beam with their hands [2, 21]. As a result, fistulograms were found to have a two-fold higher operator Effective Dose:DAP ratio, with cerebrovascular interventions being ten-fold higher than other endovascular procedures [2]. This indicates that the operator is more exposed to a unit dose of radiation for these types of procedures than others, even if the overall radiation doses are lower than other endovascular procedures.

31.2.4 Diagnostic Reference Levels

Diagnostic Reference Levels (DRLs) were introduced in 1990 by the International Commission on Radiation Protection (ICRP) to provide an advisory reference for common procedures and to increase operator awareness of radiation safety [37]. They represent the 75% percentile radiation dose per procedure, calculated from multiple representational samples; the higher the number of samples, the more accurate the reference level [37]. We refer the reader to Hertault et al.'s comprehensive paper tabulating DRLs for a large number of previously published endovascular procedures [37].

31.3 Radiation Protection and Safety

31.3.1 Introduction

Three basic principles govern radiation safety: justification of the examination, optimisation of protection and application of dose limits [38]. This section will concentrate on optimisation of radiation protection, which in turn is shaped by knowledge, attitudes, behaviours and technical skills [39]. Despite the myriad of techniques discussed herein, they can all be reduced to three key concepts; limit fluoroscopy time, stay as far away from the source of radiation as possible, and use all available methods of shielding available [38]. These all correspond to the ALARA principles [40].

Although some methods of radiation safety are not modifiable by the interventionalist, such as architectural shielding, the majority are entirely operator dependent and can easily be implemented in everyday practice [8, 38]. In addition, because the majority of radiation received by the interventionalist is scatter, any methods used to reduce patient exposure will reduce staff exposure too—a win-win situation [16, 38].

31.3.2 Behaviour

31.3.2.1 ALARA

ALARA (As Low As Reasonably Achievable) encompasses an ubiquitous philosophy of radiation safety and is the responsibility of all operators [12]. It dictates that exposure to radiation should produce sufficient benefit to the patient to offset any risks [41]. When performing an examination the radiation doses should be minimised, but not to the detriment of being able to perform the study safely [8, 9, 39].

31.3.2.2 Team

Crew Resource Management (CRM) originated in the aviation industry in order to optimise outcomes by reducing errors with a focus on communication, horizontal leadership and decision-making. Because ALARA behaviours are the responsibility of the whole team, CRM can be applied to complex endovascular procedures to the same end, with team-based pre-procedural briefings, run-throughs and practice of high-value interactions or steps. More specifically it can identify potential problems and their solutions, define roles, confirm planning and sizing, and predict C-arm angulations [42]. Applying this to EVAR resulted in a reduction in fluoroscopy time and radiation dose [42]. In daily practice, the whole team should contribute to a 'radiation-conscious facility' [41]. For example the role of the radiographer is key in monitoring radiation doses, and prompting ALARA principals [9], particularly if the operator is focussed on the technical aspects of the procedure.

31.3.2.3 Culture

There are specific cultural factors that may impede widespread adoption of radiation safety within the medical sector, as opposed to others sectors such as the nuclear industry where radiation safety is firmly embedded [43]. These factors include;

1. A primary focus on diagnosis and treatment, with other more acute safety issues taking precedence,
2. Radiation safety being considered of secondary importance to other issues,
3. Poor understanding of radiation dose and risks
4. Highly variable education, training and supervision
5. Over-justification of medical exposures
6. Poor patient awareness of radiation safety
7. A large number of independent professional, commercial and government bodies contributing to inadequate inter-body communication [43].

The working group that identified these issues proposed a top-down and bottom-up approach to engage managers, regulators and staff utilising an easy-to-follow

10-point assessment framework constituting good radiation safety culture [43]. Key features of safety culture include the belief that everybody is personally responsible, with strong leaders providing a fundamental driving force by actively demonstrating commitment to safety. These leaders in turn need to be supported by management structures that provide adequate resources, facilities, equipment, policies and procedures through a formal radiation protection programme [44].

31.3.2.4 Leadership

It can be challenging to adopt a strong radiation safety culture within the apparent competing interests of a modern healthcare system. However, they are inextricably linked; good safety culture exemplified by priorities and patterns of behaviour inevitably leads to high quality care, safe and satisfied patients and cost effective performance [17]. Key to this is leadership from senior clinicians and managers. By championing the cause they can instil active support from their staff, permeating safety culture throughout the department [7, 17]. Trainees who observe their supervisors consistently practice ALARA principals are more likely to follow suit [40]. In a study highlighting inconsistent radiation safety practices across South Africa, a fundamental finding was an absence of senior leadership roles. The attitude of the head of unit was key to developing a culture of radiation safety, and units with inconsistent and ineffective leaders suffered [7]. Vascular surgeons are well placed to lead the charge in radiation safety [15], and the key to achieving essential 'buy-in' is to supplement strong leadership with adequate education and training [7].

31.3.2.5 Education and Training

Traditionally little time has been dedicated to radiation safety education and training for interventionalists [13, 31, 40]. The training that does exist is often heterogeneous or incomplete, with large discrepancies in the curriculum [7, 37]. A survey of vascular surgeons in the U.S.A revealed that 45% had not had any formal radiation training, 74% were unaware of their hospital's radiation safety work policy for pregnancy, 48% didn't know how to contact their hospital's radiation safety officer and 43% were unaware of the yearly recommended doses limits [45]. Cardiology fellows appeared better trained; 82% having undergone radiation safety training, with those trained displaying more knowledge of basic radiation safety, adherence to ALARA, and awareness of their personal radiation exposure from the previous year [46]. Issues that impede successful radiation safety training delivery include a lack of definition, extensive guidelines containing many aspects irrelevant to everyday practice, and a reliance on theoretical physics [39].

It is universally accepted that radiation safety education should be a mandatory and integral part of the training of a vascular interventionalist [4, 7–9, 20, 40]. The best way to ensure this is as part of the curriculum within a formal training programme [44, 47], with the requirement to obtain certification necessary for

graduation [20]. For example 15% of the interventional cardiology board examination includes questions relating to radiation safety and physics [41]. In addition to initial training, regular updates and retraining with refresher courses are imperative throughout one's career to address skills decay [37, 44, 48].

The ICRP recommends radiation safety training commensurate with the individual's use of radiation [49], with European legislation recommending expanded education for those using X-rays routinely, such as vascular surgeons [37]. The quality of education and training needs to be externally assessed in several ways;

1. Accreditation by a recognised body,
2. Periodic review to ensure it remains current,
3. Certification based on exams requiring revalidation every 3–5 years [37].

In order to determine what the most important competencies are for a radiation safety programme, an expert consensus was obtained from a wide range of European interventionalists [39]. They identified the following key components;

1. Knowledge Skills (scatter, risks for healthcare workers and management of pregnant staff),
2. Technical Skills (reducing exposure time, increasing distance from source, avoiding primary beam, use of shielding,
3. Attitudes (personal and team protection) [39].

Interestingly it was felt little was required in the way of theory and basic physics. It was felt that embracing modern and interactive training methods may improve the assimilation and practice of these skills. Alongside traditional lectures these include e-learning, simulation and game-based learning to achieve skill acquisition in a safe environment without the use of X-rays [39].

Despite these recommendations the delivery and content of such training still varies considerably from country to country [37], but there is evidence that education changes behaviour and reduces dose to patients and surgeons [4]. Vascular fellows who underwent radiation safety training were more knowledgeable regarding safety principles and adherence to ALARA principles [45]. Over a 3 year period the introduction of a comprehensive radiation safety programme led to a 40% reduction in mean cumulative skin dose [50]. A 2-day training programme consisting of 15 h education led to a 50% reduction in DAP over 3285 coronary procedures during a 2 year period [51]. An even shorter 90-minute 'mini-course' for 154 cardiologists led to a 48% reduction in DAP [52] and a 16% reduction in PSD was noted 8 months after an unspecified 'education event' taught by medical physicists [31].

31.3.2.6 Distance from Source

If there is no requirement to be in close proximity to the X-ray source or patient, particularly during high-dose acquisitions (DSA runs), then staff should position themselves as far away as is practical, as the level of radiation decreases exponentially with distance according to the inverse square law [9]. For most endovascular

procedures the working distance from the arterial access site (usually the femoral artery) to the area of interest is fixed [19]. However, this distance can be extended using power injectors for DSA runs, and extension tubing attached to catheters or sheaths for manual injections [19, 53]. For operators who routinely hand inject, 75% of their radiation exposure arises from DSA runs [54]. In cardiology, the influence of different arterial access sites on operator doses has been extensively investigated [55]. The simple but highly effective act of 'stepping away' from the patient during DSA can considerably reduce personal radiation dose [3, 9, 44]. Six-feet is considered a relatively 'safe' distance in terms of excess exposure [3], and taken to the extreme, operator dose can be effectively eliminated at 5 m [54]. If it is not practical to move 1–2 m back, even small changes can have a substantial effect, for example standing closer to the feet than the abdomen during pelvic interventions [38]. It has been suggested that 'stepping away' should be mandatory behaviour if it doesn't compromise the safety of the patient; however it was only observed in 6% of complex aortic cases [9]. These manoeuvres are of even more importance to operators of short stature whose proximity to the radiation source and patient may result in their upper body being exposed to more scatter [16]. It is also important to covey this message to anaesthetic colleagues who are often at the head of the table and close to the source. Studies have demonstrated anaesthetist's doses are far greater than necessary, with one recording a dose at the anaesthetists position significantly higher than the primary operator and 15-times that of the scrub nurse [3].

31.3.2.7 Position Around the Table

Due to the uneven distribution of scatter radiation, the position at the angiography table significantly dictates the amount of radiation exposure one receives. The highest intensity of scatter is located on the X-ray beam entrance side of the patient [44] usually under the table or in left anterior oblique (LAO) projections if one typically stands on the left. Generally doses are significantly higher for primary operators compared to assistants and scrub nurses [9, 29]. In a study reporting personal exposure during complex aortic repairs according to position around the table, it was found that the principle operator received twice the dose of the first assistant standing next to them, with the third assistant/scrub nurse position enjoying undetectable levels for almost all cases [2]. The second highest dose after the primary operator was the position required for left brachial access.

As previously discussed, the inverse square law is fundamental to understanding the importance of reducing radiation dose by 'stepping away' from the source. However it pertains to the behaviour of X-rays in a vacuum, and variation in room configurations, imaging techniques and patient characteristics create multiple variables that deviate scatter radiation from easily predictable patterns [10]. In an elegant cadaveric study which mapped the scatter around the angiography table it was found that although radiation exposure decreased with distance from the source, this decay in dose was asymmetrical, and rather than conforming to concentric circles as predicted by the inverse square law, they resembled a 'scatter cloud' [10]. With

antero-posterior (AP) imaging, the radiation fields had a bimodal symmetric dumbbell shape, with maximum exposure adjacent to the table at positions typical for the operator and assistant. Lateral projections led to peaks on the emitter side, with a full left lateral projection creating almost seven-times the radiation than a right anterior oblique (RAO) 45° projection. All of these radiation plots demonstrated non-conformity when compared to the inverse square law and therefore the authors suggested that simply taking a step back from the table may not be enough to provide the level of safety that has traditionally been ascribed to it. In concordance with other investigators they suggest emphasis should be made to move personnel away from the patient when standing on the emitter side of the table during DSA runs, as this is where the highest radiation doses were observed.

31.3.2.8 Pregnancy

Exposure to radiation in vascular training occurs in peak childbearing years, but less than 50% of women in vascular surgery surveyed had any counselling regarding radiation protection during their pregnancy [56]. With more women training in vascular surgery, specific guidelines regarding radiation safety during pregnancy are lacking [56]. The risks of intrauterine radiation on the developing foetus include miscarriage, intrauterine growth retardation, small head size with associated mental retardation, and subsequent development of childhood cancers [37]. The most sensitive phases of pregnancy are those in which the interventionalist may not be aware they are pregnant [56]. These include the pre-natal phase (0–8 days before implantation), with organogenesis occurring mostly in the first trimester [56], and weeks 8–15 where mental abnormalities are more prevalent [57]. Studies suggest that doses under 100 mGy do not represent an increased risk to the embryo or foetus [58]; a dose far in excess of the 20 mSv annual occupational exposure limit set by ICRP. Nevertheless the dose limits for pregnant workers are even lower; in the USA the foetal dose recommended by the NCRP is 5 mSv/pregnancy, with a 0.5 mSv/month limit once pregnancy is declared [37, 58]. In Europe, the foetal dose limit is lower as recommended by the ICRP, at 1 mSv/pregnancy, the same dose limit for a member of the public [37, 58].

These limits are easily achievable if the operator is already in the habit of conforming to ALARA behaviours, with the vast majority of pregnant interventionalists able to continue performing endovascular procedures safely throughout their pregnancy without increased risk of foetal death or malformations [44, 56, 58].

Although foetal doses are negligible utilising standard radiation safety principals [58], there are some additional procedures that pregnant interventionalists may wish employ to ensure their foetal dose remains as low as possible. Although standard 0.5 mmPb eq lead aprons in combination with ancillary shielding are extremely likely to provide sufficient protection [58], there is the option of wearing additional lead. Ideally this is in the form of specially designed maternity gowns with a 1.0 mmPb eq insert around the abdomen and pelvis, providing an additional protection of almost 100-fold [56]. If unavailable, the option exists to wear an additional

wrap-around apron across the abdomen, or wear two standard wrap-around aprons [58]. The drawback of this additional protection is the added weight, and this needs to be balanced alongside the additional musculoskeletal strain that pregnancy already places on the operator. Foetal dose can be estimated by wearing an additional foetal dosimeter on the abdomen under lead, but it essential that the wearer receives monthly feedback of results [44, 56]. Some flexibility in rostering has been suggested to reduce the radiation dose to trainees, at least during the most crucial gestational weeks, while continuing to fully participate in their training [56]. For more details we refer the reader to "Suggested program guidelines for vascular surgery trainees" in Shaw et al. [56].

31.3.2.9 Dosimeters

Personal radiation monitoring in the form of an individual dosimeter is an essential line of defence against occupational radiation exposure [59]. The majority are thermoluminescent (TLD) [22] or optically stimulated luminescence (OSLD) dosimeters worn for weeks or months at a time. After this set period of time they are evaluated and a personal dose equivalent is calculated as the dose received in soft tissue at a certain depth under the dosimeter [37]. Because they provide an estimate of radiation dose well after actual exposure they are termed 'passive' dosimeters.

Although decades of monitoring thousands of interventionalists has demonstrated that only a minority receive concerning doses [59], dosimeters are the only real feedback available regarding personal Effective Dose and how efficacious one's radiation safety behaviour is. To quote Lord Kelvin "If you cannot measure it, you cannot improve it" and optimising radiation protection is impossible without the data provided by personal dosimeters [59]. Analysis of personal data over a few months will provide information regarding the attenuation of lead garments and efficacy of additional safety practices [58]. Individuals and institutions should maintain lifelong exposure records, and staff working at multiple sites should collate their collective exposures [41].

Location of dosimeters: Dosimeters can be worn in specific locations to measure doses at those sites (e.g. eye, hand and gonads) [44], or in one location for an overall estimate of effective dose (e.g. trunk at waist or chest) [19]. Dosimeters placed on the abdomen may give a more accurate representation of whole body dose rather than those worn on the chest [29]. Combining doses from multiple sites, usually the collar and trunk, will give more accurate information regarding occupational exposure and radiation safety behaviour [22]. A collar badge provides a direct measure of head and neck irradiation and ambient radiation fields [59]. Badges should be worn facing the radiation source, if working on the right of the patient this means wearing it on the left [19]. Dosimeters worn under lead in addition to over it will give additional information when working in heterogenous radiation fields [22].

Number of dosimeters: The wearing of two dosimeters is strongly recommended by the ICRP and American College of Surgeons to provide a more accurate estimate of personal occupational dose; one on the torso (chest or abdomen) under lead

garments in order to estimate the dose to protected organs, and one outside lead, usually attached to the collar or left shoulder, to estimate the dose to unshielded areas, especially the head, eyes and skin [1, 37, 41, 44]. Effective Dose is estimated using the following formula: ED = 0.55HW + 0.025 Hn, where ED = Effective Dose, Hw = under lead, Hn = over lead [37].

Due to the potential issues associated with multiple dosimeters (loss, location swapping, or compliance) it has been argued that one worn correctly, on the front of the torso under lead between the shoulder and waist gives a good estimate of effective dose, and is better than two worn incorrectly [44]. However, without an outside dosimeter it is impossible to monitor eye doses, and to accurately estimate Effective Dose from personal dosimeter data; the most accurate algorithms require two dosimeter readings from inside and outside lead [44]. Other causes of inaccuracy include wearing dosimeters in the wrong location, back-to-front, leaving the dosimeter in an area of radiation exposure when not physically present, and not wearing it all times whilst in the hospital [44]. They may also underestimate exposure when angulated away from the radiation source [12].

Replacement Interval: Endovascular surgeons should monitor their radiation use with the same rigour that they audit their morbidity and mortality [16]. In the same way that one could not use published, or even institutional data, to predict one's own complication rates, the same is true of personal occupational dose. In one study, there was a 60-fold whole body dose variation between interventionalists in the same institution, with equivalent lens doses varying 100-fold [38]. Therefore periodic review of one's own personal dosimeter readings is essential. Dosimeters should therefore be changed monthly to facilitate quick identification of poor practices to allow implementation of better radiation safety practices [44]. High doses may indicate poor ALARA behaviours, and very low doses should raise the question of non-compliance of dosimeter use.

Dosimeter Compliance & Radiation Safety Culture: In a survey of 615 European interventional cardiology healthcare professionals, only 64% invariably wore their dosimeter, 18% wore it most of the time, 6.5% some of the time and 4% occasionally [60]. Two thirds of individuals viewed their radiation data regularly [60], with knowledge of personal doses reflecting good radiation safety practice [44]. Data pertaining to an institution's dosimeter practices, such as collective annual dose and number of late or non-returned dosimeters reflects radiation safety culture as a whole within the organisation [17]. In a study of five UK hospital's global dosimeter data there were 30% late returns and 10% non-returns, figures unheard of in other radiation industries such as nuclear sites, reflecting very different radiation safety cultures [17].

Real-time Dosimeters: It could be argued that the role of passive dosimeters is more regulatory than educational. Even if operators analyse their data regularly and accurately, the time-lag between recording and changing a behaviour is months at best. In addition, individual case dose data is not available, and operators must therefore infer their case exposure from the indirect metrics provided by the fluoroscopy machine (i.e. FT, CAK and DAP) [2, 16]. Therefore behaviour that results in high doses during the case cannot be highlighted at the time, and remedial behaviours such as stepping back or more effective shielding use to reduce the dose cannot be instigated when it matters [2, 3]. In an attempt to provide some real-time

feedback during a case, FDA regulations dictate that CAK and DAP are easily visible on the operator monitor, and an audible signal is activated after each 5 minute block of cumulative fluoroscopy time, which has to be acknowledged by manually silencing the alarm [61]. The use of 'active' dosimeters which sit over the top of lead aprons take these concepts a step further. They provide immediate feedback on occupational radiation exposure, either in the form of an auditory tone or a visual display, as well as recording data for case-by-case evaluation [37, 62]. The deficiency of passive dosimeters to change radiation safety lies in the protracted step of data feedback which is often in the order of several months. By bringing this step into real-time, active dosimeters highlight radiation safety behaviours to the interventionalist throughout the procedure. This can be especially useful when they are focused on the technical aspects of a case, often to the detriment of radiation safety [63]. In this manner real-time dosimetry immediately alerts operators to harmful practices and allows them to change their behaviour instantaneously [3].

The use of dose-sensitive auditory dosimeters resulted in augmented safety behaviours such as utilising the inverse square law and minimising fluoroscopy, resulting in a significant reduction in staff exposure [62], with an RCT demonstrating a 30% reduction in operator dose during coronary interventions [64]. A trial of real-time dosimeters during paediatric interventions resulted in significantly reduced primary operator doses, despite no change in procedure time, fluoroscopy time or patient dose, suggesting a positive influence on operator radiation safety practices [65]. However, in keeping with introducing any new technology into an established workflow, there can be issues. Miller et al. found no improvement in radiation dose during a range of endovascular procedures, despite the introduction of real-time dosimeters [63]. They cited a number of reasons including the inability to divide attention between the dosimeter feedback screen and that of the angiographic image, and the small screen not being visible from all angles of the room. In addition, prior to the introduction of the dosimeters, staff had already undergone radiation safety training and authors felt that dose-reduction behaviours may already have been optimised. Although Baumann at al highlighted a learning curve associated with the use of active dosimeters, this was only in the order of a few weeks, and reported a 45% reduction in total staff dose associated with the introduction of these devices in their practice [66].

31.3.3 Machine Controls

31.3.3.1 Fluoroscopy Time and Last Image Hold

The time the interventionalist has their foot on the pedal is the single most important determinant of radiation exposure to both patient and staff [33] and behaviours aimed at limiting time of exposure are essential to master [67]. These include removal of wires and catheters using short taps of 'spot' fluoroscopy rather than continuous use [19, 33, 44], disengaging as soon as data acquisition is completed [37], and only using fluoroscopy when information is required such as observing objects in motion [44]. Fluoroscopic loop recordings can also be used to review

dynamic processes rather than additional time on the pedal [44]. 'Last-image hold' capabilities is an FDA requirement of fluoroscopy units [33, 61] and should be relied on liberally for study, consultation and discussion during a case without the need for ongoing irradiation [16, 30, 68]. Perhaps just as important is considering getting assistance from a colleague if the fluoroscopy time is protracted, or even aborting the case to continue on another day if there is a significant risk of deterministic radiation injury [67].

31.3.3.2 Automatic Dose Settings

Modern fluoroscopy machines have an Automatic Brightness Control (ABC) that optimises image quality by automatically increasing radiation dose if feedback from a photodiode within the image intensifier detects low light output [23, 19, 37]. While this increased dose results in increasing X-ray penetration to produce a good image quality, ABC can significantly increase radiation exposure without the operator being aware. The amount of radiation produced depends on the energy required to produce the X-ray beam [19], which in turn is determined by the milliamperage (mA) and peak kilovolts (kVp) used to generate the X-ray beam [16, 19]. The mA setting controls the number of photons produced; low mA produces a mottled image and the image quality is improved by increasing mA (at the cost of increased radiation) [19]. kVp determines the penetration of the beam and image contrast [19]. Typical scenarios to be aware of in which ABC will significantly increase dose are when treating obese patients, a field containing extraneous radiodense material (such parts of the body outside of the area of interest), and steep gantry angulation angles. Although default settings on most modern machines are usually 'low dose' [11], if lower mA and increased kVp settings can be achieved these can further reduce exposure while not necessarily impacting greatly on image quality [16, 19, 32]. For example increasing the kVp from 75 to 96 kVp can decrease entrance dose by 50% [19], and the routine use of half dose settings has been shown to reduce skin dose significantly with no reduction in image quality [69].

Modern imaging equipment offer many low-dose technological advances to reduce radiation dose [8], and despite reducing parameters such as dose rates, pulse rate and frame rates, image processing can often compensate considerably for reduced image quality due to decreased dose [44]. Terminology may differ between different machine manufacturers and it is useful to consult with your radiographer or medical physicist [44].

31.3.3.3 Fluoroscopy and Pulse Rate

Fluoroscopy can be produced in either a continuous manner, or pulsed, with X-rays generated in short bursts [1, 20, 36]. Continuous fluoroscopy is associated with blurred images due to patient and instrument movement [16]. Pulsed fluoroscopy counteracts these movements and reduces blurring whilst reducing radiation

exposure, and is therefore the default mode in modern X-ray systems [20, 33, 41]. Pulse rates are typically available at 30, 15, 7.5, 4 and 2 pulses per second. Due to initial analogue fluoroscopy being recorded at 30 frames per second, continuous fluoroscopy was produced at 30 pulses per second. However the human eye and the brain's visual reception system can only analyse up to 12 images per second, any more than this are interpreted as an illusion of visual continuity [70]. Reducing pulse rates from 30 to 15 or 7.5 pulses/s decreases fluoroscopy dose by 47% and 72% respectively [16, 36]. The lowest pulse rate possible should be chosen to produce an adequate image [16, 20, 30, 32, 36, 37], and at even three pulses a second it is possible to perform complex FEVAR [71].

31.3.3.4 Digital Subtraction Angiography and Frame Rate

Digital Subtraction Angiography (DSA) entails acquiring several images in succession in one field of view and then digitally subtracting the non-vascular structures, leaving contrast-enhanced images of the vessels. These high-quality images are often used for diagnosis and documentation purposes but at the cost of significant radiation dose compared to fluoroscopy [37].

There appears to be a general lack of awareness of the relatively high radiation associated with DSA compared to fluoroscopy [9]. Previous reports have documented the percentage of total dose during peripheral and cardiac interventions due to DSA to range between 70 and 90% [30, 54], accounting for 50–80% of the radiation dose during TEVAR and EVAR, even with low frame rates (2/s) being employed [9, 34]. Compared to fluoroscopy, DSA is associated with a 6–10 times higher dose rate per frame [18], which contributes to 66% of the total radiation dose, while only accounting for 23% of total exposure time [12]. DSA also results in an eight-fold higher staff radiation dose [30]. Therefore documentation of the procedure by DSA results in significantly more radiation than the fluoroscopy-guided intervention itself [30].

For these reasons DSA use should be minimised to key scenes and critical steps [30], as this is one of the most effective techniques for reducing radiation dose during endovascular surgery. DSA can be replaced with fluoroscopy loops if high-quality imaging isn't required [9, 10, 20, 30, 38, 41, 68], and in fact can replace most DSA runs [37]. The operating surgeon needs to balance how much suboptimal imaging is permissible to effectively diagnose, treat and assess the condition [16].

The total number of images acquired during a procedure significantly affects radiation dose [44]. This in turn depends on number of pictures acquired per second (frame rate), time per run, and number of runs. The operator should reduce all of these factors to the minimum required to meet clinical need [44]. When DSA is required, reducing the frame rate will reduce dose in the same way as reducing pulse rate during fluoroscopy [9, 30, 32, 44], with number of frames correlating highly with total radiation dose [30]. Reducing frame rates to 7.5 fps results in a 90% reduction in image numbers with an equivalent reduction in radiation dose [37]. Reducing frame rates to 2 fps for pelvic and upper leg interventions and 1 fps for

lower leg and foot interventions appears adequate [30]. Other features of modern machines such as the ability to return the table to the exact position and overlay a fade of a previous DSA will reduce unnecessary repeated DSA examinations [30].

31.3.3.5 Collimation

Collimation uses filters within the X-ray source to reduce the radiation field size to the minimal required area of interest, thereby shielding the patient's body from radiation outside of the area of interest [38]. By shaping the beam and absorbing low-energy photons not useful in image generation, collimation not only produces a sharper image, but also reduces radiation exposure to the patient in proportion to the reduced image size, and consequently scatter is reduced [1, 16, 30, 32, 33, 37, 72]. During cardiac procedures the use of collimation reduced patient and staff radiation by 40% [73], and meticulously collimating on a modern machine reduced DAP by a factor of more than 10 compared to wide-open shutters [59]. In a cadaveric study, investigators found that horizontal and vertical collimation significantly reduced scatter and were independent of each other [74]. By increasing horizontal and vertical collimation independently from 0 to 10 cm (5 cm each side) they reduced scatter to the operator, assistant and anaesthetist by 86%, 80% and 96% for horizontal collimation and 88%, 89% and 92% for vertical collimation respectively [74]. Collimation reduces scatter by focusing the radiation field to a smaller area on the patient, resulting on a larger volume of the patient's tissues available to attenuate scatter before exiting the patient and reaching the staff [74]. The price for this is increased patient skin entrance dose [74], and care must be taken not to perform prolonged and highly collimated studies in one gantry position. Virtual collimation (where the collimation blades are projected on the monitor) eliminates the need for fluoroscopy to adjust collimation leave position [37, 44]. Even when a full field is required the collimator blade edges should be just visible on the monitor thereby reducing radiation extending outside of the image receptor [38].

31.3.3.6 Magnification

Image intensifiers (II) come in a range of sizes, known as input field of views (FOV). Using the largest FOV without magnification the output spatial resolution is lower, image distortion higher and in general radiation dose lowest. By using magnification a smaller area of the II input phosphor is irradiated, giving the effect of enlarging the image, enabling improved visibility [75]. If the FOV is halved the spatial resolution is doubled. However only a quarter of the input II is being irradiated as the area is proportional to the square of the FOV. If all other parameters were kept constant halving the FOV would reduce the image brightness to a quarter of the original FOV, rending it unusable [75]. Therefore the machine's automatic brightness controls quadruple the radiation to compensate and deliver a bright image [76]. In general the smaller the FOV the larger the magnification and the higher the

patient dose [75]. Because collimation is automatically applied during magnification, in the same way that increased collimation increases entrance skin dose but reduces scatter, a smaller FOV (increased magnification) increases CAK but decreases DAP, thereby increasing the risk of deterministic effects to the patient, but reducing stochastic effects and scatter experienced by the staff [3]. However the general advice is to use the highest FOV as possible with judicious use of magnification [19, 67, 68]. When additional detail is required but magnification is to be avoided, the combination of digital zoom and large monitors can produce a similar effect [68].

31.3.3.7 Imaging Chain Geometry

Imaging Chain Geometry refers to the combined linear arrangement of the X-ray source-to-patient, and the patient-to-detector (image intensifier) [44]. Both have a significant independent combined influence on scatter.

The X-ray source is located under the patient to ensure that maximum scatter is dispersed under the table with a drop-off in dose as one moves towards the operator's head [44]. The distance between the X-ray source and the patient is set by the table height. As with collimation and magnification, a balance needs to be met between radiation dose delivered to the patient and that to the operator. Ensuring maximal table height from the X-ray source will reduce patient dose [41, 44, 68], but at the cost of significantly increased scatter, with maximal dose at around 5 foot (1.5 m) from the floor, exposing the operator's head, eyes and neck [68, 74]. Therefore the table needs to be as far away from the operator's chest and head as possible, increasing the distance from the patient and the main source of scatter [37]. This is of particular importance for shorter interventionalists.

The next component of the imaging chain geometry is the distance from the patient to the detector. The detector should be as close to the patient as possible [41, 44]. Unnecessary distance causes increased dispersion of the X-ray beam, with a subsequent reduction in signal reaching the II, and a resultant increase in dose initiated by the machine's ABC in order to compensate [33, 37]. Minimising the patient-to-detector distance has multiple benefits;

1. It reduces the amount of energy required to produce the image and thereby reduces scatter,
2. Increases scatter absorption by the detector itself,
3. Results in a sharper image [19, 74].

31.3.3.8 C-Arm Angulation

Appreciating the influence of the C-arm, or gantry angulation, on radiation dose complements good imaging chain geometry. It is important to avoid steep C-arm angulation [8, 10, 16, 20] for three reasons;

1. It creates more scatter to the operator, particularly above the table,
2. It requires more radiation to produce images through the lateral torso than in an anterior-posterior (AP) projection as patient thickness is increased, thereby increasing skin dose and scatter,
3. It places the X-ray source closer to the skin, increasing skin dose and risk of deterministic injuries.

Frequent changes in gantry angulation have been recommended in prolonged exposures to reduce skin dose by changing the pattern of radiation [31, 32, 67], but steep cranial and lateral angulation should never be used for this purpose [31], as it has the same effect as obesity in creating more tissue for X-rays to penetrate [33]. The machine's ABC then detects a thicker tissue path and increases dose to achieve adequate penetration [33]. The use of steep angulation in the obese compounds the risks [77] and should be used sparingly [33]. Due to the increased proximity of the X-ray source to skin that occurs with steep beam angulation, one review reported 83% of radiation skin injuries occurred with steep beam angulation [77]. Exposure to the operator increases exponentially with lateral angulation over 30° and cranial angulation exceeding 15° [37], reaching a maximum at full lateral projection [9]. On a phantom model, AP projections resulted in 5 mSv/h operator exposure rising to 11 mSv/h at a 45° projection, and 69 mSv/h at 90° [10]. Steep angulation such as that required during complex aortic repairs lead to significantly higher scatter exposure to the operator, particularly at the head level [78]. Cranial left anterior oblique projections cause the most exposure [9, 41, 44, 78–80], because the radiation source is usually on the same side as the operator in this configuration, leading to maximum backscatter towards the operator [9, 74]. The source being on the same side as the operator can lead to six times more radiation exposure [1]. When steep angulation is essential for the case, it should be for the shortest period of time with application of adequate collimation [37].

31.3.4 Imaging Equipment and Workflow

31.3.4.1 Flat Panel Detectors

Flat panel detectors reduce radiation dose [52]. They have a high sensitivity to X-rays, high signal to noise ratio, wide dynamic range, limited geometric distortion and high uniformity across the field of view. This results in 30–50% less dose than traditional image intensifiers [81, 82]. Due to this sensitivity, 'low-dose' or 'extra-low dose' modes should be chosen over the 'normal' modes, as these are associated with adequate imaging quality with much lower radiation requirements [82].

31.3.4.2 Fixed Systems vs. Mobile C-Arms

Endovascular surgery has evolved from procedures performed using a fluoroscopy machine with a mobile C-arm in a traditional operating room, into dedicated hybrid suites with fixed angiographic machines. Compared to fixed systems, C-arms have

been associated with inferior imaging quality, overheating, higher operator doses due to a lack of table and ceiling-mounted shields, worse chain-geometry and less ability for the operator to move away from the source. Despite these limitations, they have been associated with a reduced overall radiation dose of about 3.5 times lower than fixed systems [21, 26, 83–86]. It has been suggested that, for standard EVAR, mobile C-arms are of sufficient quality to perform the task with less radiation than that in hybrid suites [83], with fluoroscopy times and outcomes being similar for EVAR performed with C-arm versus a fixed system [87, 88]. However it must be considered whether it is safe to perform complex procedures with inferior imaging capabilities, particularly in an ever-growing obese population [86], and also foregoing the additional efficiencies and safety features that hybrid suites afford [87]. Modern fixed systems have more powerful X-ray systems operating at higher energies with larger beam sizes and detectors which contribute to a three- to ten-fold higher radiation dose compared to mobile C-arms [86, 89]. However improved imaging allows replacement of DSA and magnification with other methods. Combined with dose-reducing software capabilities, this enables a reduction in radiation use despite the higher quality imaging [90]. It has been shown that by strictly adhering to ALARA principles and utilising modern radiation reducing technologies, such as image fusion and fluoroscopy loops replacing DSA, moving from a C-arm to a hybrid suite can actually be associated with a halving of the median radiation dose associated with EVAR from 30 to 12 Gy cm^2. Similarly another group found radiation exposure associated with TEVAR was significantly reduced from 14.9 to 8.6 mSv with the move from mobile C-arm to a fixed system [91]. Other groups have described significant increases in operator radiation exposure during a range of endovascular procedures with the move from mobile to fixed systems [88, 89], noting that surgeons will use the best imaging available to them. Indeed the fact that the surgeon no longer has to worry about an overheating mobile unit may result in more liberal use with fixed systems [85]. This underscores the importance of utilising all the radiation-reducing capabilities that a hybrid suite offers to offset the increased exposure that accompanies superior imaging. These include dose-reducing software, low-dose settings, ceiling-mounted shielding, operator-controlled imaging and of course always adhering to ALARA and utilising the inverse square law.

31.3.4.3 Operator-Controlled Imaging

One of the advantages of moving from mobile systems into a hybrid suite is the ability to control imaging by the surgical team [84] using tableside operator-controlled imaging (OCI). OCI removes potential misunderstanding between the operator's instructions and the radiographer's actions, thereby reducing unnecessary exposure [84]. Discrepancies in language, ambiguous words (e.g. "up", "rotate" and "turn") and confusion at how best to describe certain C-arm movements can all lead to unnecessary radiation exposure [92]. In a retrospective study of OCI during EVAR, investigators reported a significant reduction in fluoroscopy time (16.2 versus 20 min) and radiation exposure (DAP 4.9 vs. 6.9 $mGym^2$) [93]. Interestingly the

range of DAP was significantly reduced (1.9–95 vs. 1.25–13.3 mGym^2), perhaps reflecting more accurate patient positioning prior to high-dose acquisition runs. If OCI is unavailable then improving communication between surgeon and radiographer can significantly reduce the time taken to move the C-arm and unnecessary radiation exposure [94].

31.3.4.4 Advanced Dose-Reduction Software

Modern imaging equipment produces excellent quality images, but at the cost of increased radiation dose. This has prompted imaging equipment vendors to focus now on radiation safety strategies, particularly hardware and software modifications to reduce radiation dose whilst maintaining imaging quality [95]. Whilst all vendors have their own proprietary approach, two studies reported on upgrading to the Philips AlluraClarity system ClarityIQ (Philips Healthcare) system [86, 96]. This system combines;

1. Machine controls (smaller focal spots, shorter pulses, lower tube current and additional beam filtration),
2. Image-processing algorithms (automatic pixel shifting, temporal averaging of consecutive imaging, spatial noise reduction, motion compensation and image enhancement),
3. Hardware configurations to reduce entrance dose (optimising acquisition chain for different anatomical regions) [86, 96].

Studies utilising the upgraded system software have reported an approximate halving of radiation associated with EVAR, 39% reduction with visceral embolization and up to 70% reduction in lower extremity interventions [63, 86, 96–98].

31.3.4.5 Pre-operative Planning Software

As previously discussed, meticulous case planning based on pre-operative CT imaging post-processing software prior to complex interventions can negate unnecessary diagnostic runs thereby reducing radiation dose [37, 42]. Iliac bifurcation profiling during EVAR often requires 20–30 degrees of lateral angulation and the neck 5–15 degrees of cranial angulation [99]. DSA runs in this position contribute to some of the highest radiation doses and operator scatter exposure during EVAR [100]. One study using SyngoVia® (Siemens) post-processing software to remove unnecessary diagnostic runs demonstrated a three-fold reduction in mean DAP during EVAR [100]. Several other studies have demonstrated similar utility with the open-source software Osirix® (Pixmeo Labs, Switzerland); whilst not measuring radiation doses they have demonstrated a reduction in operating time by one third by predicting C-arm angles pre-operatively [101, 102].

31.3.4.6 3D-Image Fusion Software

3D-image fusion (3D-IF) amalgamates pre-operative CT-A images with live fluoroscopy, resulting in a three-dimensional volume-rendered angiogram that can be used as a virtual roadmap during interventions, particularly useful during complex aortic repairs [103]. By co-registering bony landmarks on both pre-op and live images, the fused 3D model automatically follows the table and gantry movements [37], negating the need for repeated DSA and fluoroscopy during stent deployment and target vessel cannulation, thereby reducing procedure time, contrast use and radiation exposure [9, 104, 105]. Investigative and real-world studies using 3D-IF report significant reduction in radiation during a full range of standard and complex fenestrated and branched aortic interventions by up to 70% [26, 71, 106, 107].

One limitation of 3D-IF is the additional radiation required to co-register the images at the beginning of the case. Initial systems required a full or partial spin, such as cone-beam CT (CBCT), which could add approximately 5% of the total radiation dose [103]. This radiation dose can be reduced up to ten-fold by replacing CBCT with two orthogonal AP and lateral fluoroscopic shots [71, 108]. More sophisticated registration systems have been developed which preclude the requirement for pre-operative co-registration X-ray [109].

The remaining limitation is inaccuracy of overlay, particularly vessel deformation following the passage of stiff wires, which can render the overlaid pre-op images inaccurate [9]. Recent advances in 3D-IF technology using a cloud-based system significantly improved accuracy and functionality of the overlay system, with a consequential reduction in radiation exposure, fluoroscopy time and procedural time [110].

31.3.5 Shielding

Along with ALARA principles, shielding is one of the main pillars of protection against scatter for the operator. Shielding comes in many forms including architectural (built into the walls and viewing windows of angiography suites), equipment-mounted lead (e.g. table drapes), ceiling mounted leaded acrylic, large floor-supported mobile shields and leaded personal protection equipment (e.g. gown, glasses, thyroid shield). As the patient is the main source of scatter, most shielding is designed to act as a barrier between the patient and operator, and as such, shielding should be arranged to leave minimal gaps between the patient and shielding [59]. A combination of all available modalities should be routinely used as it dramatically reduces operator exposure [44]. For example, just combining ceiling and table-mounted shields in a phantom model has been shown to reduce radiation dose to the upper body by at least 80% [111]. However in clinical practice the maximum potential attenuation is not always achieved due to incorrect shield

positioning, patient position, change in tube angles, operator position, misunderstanding of optimal position or non-use of shielding. Large transparent floor-mounted mobile shields of 1–1.5 mmPb LE are useful for circulating staff during high-dose DSA [113].

31.3.5.1 Personal Lead Garments

Personal lead garments ('gowns' or 'aprons') come in a plethora of designs, thicknesses, materials and configurations. If the garments are sourced from communal collections then physical condition adds further variability; shared garments are often maltreated with rough handling and improper storage, leading to damaged lead lining and diminished protection [19, 56].

For angiography use, most national standards require a minimum of 0.35 mm of lead equivalence (mmPb LE) protection at the front and 0.25 mmPb LE at the back. Accepting that testing and reporting is highly variable, garments of 0.35 mmPb LE have been reported to attenuate 93% of radiation [9], with 0.5 mmPb LE associated with protection between 95 and 99% of scatter and protecting 80% of active bone marrow [19, 41].

Although lead provides the best protection against radiation, its disadvantage is its weight, and long-term use can lead to career-shortening chronic musculoskeletal conditions [112, 114]. A vest and kilt design allows some of the weight to be carried by the hips rather than solely on the shoulders [113]. Although backless aprons are much lighter they should only ever be worn if always facing the patient and radiation source [112], which can seldom be guaranteed. In response, manufacturers have developed lighter weight garments made from materials other than lead, such as antimony and bismuth. These lead-free or low-lead composite garments are associated with a 25–40% reduction in weight [113]. However significant concern exists regarding their ability to provide adequate protection against the wide range of radiation energies typically encountered in endovascular practice [112, 115]. Current product testing and reporting methods leave users uncertain as to the level of protection they are receiving [116], with large variations in performance compared to manufacturers stated lead equivalence [115, 117]. Some studies have reported composites transmitting as much as double the radiation equivalent of lead only aprons [118] and a recent study reported a 38-fold difference in scatter protection in garments labelled as having the same protection [115].

The current recommendation for vascular interventionalists is to choose your own personal garment and look after it. It needs to fit properly, without large gaps at the arm holes which can lead to high axilla exposures [119] and, if it is of an overlapping design, it needs to overlap adequately as the overlap design usually ensures the minimum protective thickness [44, 113]. It should predominately be made from lead that provides 0.5 mmPb LE or greater anteriorly from each posterior axillary line, being careful to check for undisclosed labelling of double-panel thickness [56, 112]. When not in use it should be stored on hangers and periodically checked for cracks with fluoroscopy [44, 113].

31.3.5.2 Leaded Eye Glasses

Although scatter levels diminish as one moves from the feet to the head, the eyes are one of the most radiosensitive tissues in the body and susceptible to cataract formation (see Chap. 30, Sects. 30.3.2.3 and 30.3.4.5). Without eye protection deterministic thresholds may be exceeded in a matter of years [120], especially with unfavourable conditions commonly encountered such as a steep gantry angle and obesity, which significantly increase eye exposure [44]. The importance of the routine use of leaded eye glasses cannot be overstated, as they dramatically reduce the eye dose by a factor of 5–10 [121, 122], or to 2–3% of baseline dose [67], thereby reducing lens exposure far below occupational limits [2].

However, as with lead garments, the situation is somewhat more complicated. There has been a wide variation of radiation attenuation in glasses reported with the same lead equivalence (35–95%), and lens thickness. Radiation incident angle and frame fitting also play a role in proportion of radiation which is attenuated [68]. Eye doses differ between right and left depending on the proximity of the X-ray source [29]. However the most important 'invisible' factor is that of absent lateral protection. Leaded glasses provide excellent protection front on, but the protective effect markedly diminishes when the head is turned to the side [4, 68, 113, 122, 123]. Up to 21% of ocular exposure is due to scatter from the operator's head, suggesting that side-shields or a wrap-around frame with added lead protection is necessary to provide adequate protection [19, 44].

It is therefore recommended that eye protection is routinely worn, made from 0.75 mmPb LE leaded glass in a well-fitted frame with no gaps at the nose and cheek, with an additional lateral leaded shield [68, 113], combined with ceiling-mounted shields to achieve full protection [4, 29, 113]. This combination can result in an undetectable lens dose [121]. In clinical practice the addition of ceiling mounted shields to leaded glasses reduced radiation dose from 2.1- to 5.7-fold [122]. In a separate experiment, leaded glasses combined with a host of addition shielding including ceiling shields and table skirts reduced eye doses in excess of 25-fold [121].

Despite the overwhelming evidence that the eye is highly vulnerable to radiation-induced injury, and that a combination of leaded glasses and adjunctive shielding dramatically reduces lens exposure, such protection remains grossly underutilised [68]. In one study, the use of eye protection during cases among vascular surgeons varied considerably from 0, 9, 10.6 and 100% of the time [6]. In another study only 25% of interventionalists and 36% of cardiologists utilised eye protection [124]. Such behaviour has been most kindly described as illogical [68].

31.3.5.3 Thyroid Shield

In order to prevent radiation-induced thyroid cancer it is recommended that all staff under 40 years old who regularly receive a monthly collar radiation dose of >4 mSv wear a lead thyroid shield [125]. However due to their small size and convenience

their use is often extended outside of these recommendations [113] resulting in almost ubiquitous use. A 0.5 mmPb LE thyroid shield substantially reduces thyroid radiation dose [22], but also has the added benefit of protecting all inferior neck structures. This includes the carotid arteries which receive 50% of the radiation detected at a left collar dosimeter [123].

31.3.5.4 Hats

Traditionally the head has not received much attention regarding radiation shielding because scatter doses are lowest at head height, the brain is of low radiosensitivity and the skull attenuates about 40% of scatter [126]. However this topic has been gaining more interest recently (see Chap. 30, Sect. 30.3.4.4).

Interventionalist's head exposure has been found to be almost 10-times higher than whole body exposure [126], with the left side of the head receiving higher doses than the right and centre of the skull [127]. Several radiopaque surgical caps are commercially available, with claims that they substantially reduce brain dose, but their efficiency in reducing brain radiation remains inconclusive. Studies based on skull surface doses in humans report significant reductions in radiation of up to 16-fold [127], but these may not reflect deeper brain doses, as the majority of brain radiation comes from scatter arising at angles not covered by caps. One study using an anthropomorphic phantom head and brain found that whilst the cap attenuated direct tangential radiation by 65–70%, it only provided 4.9% protection to the left brain, 1.8% to the right brain and 3.3% to the whole brain [123]. A similar study using a sophisticated anthropomorphic head phantom with 15 separate detectors found the caps attenuated radiation at the temporal region outer skull in interventionalists by 60% and 71% in the phantoms, but only by 7% in the upper brain with no significant attenuation in midbrain [126]. It was suggested that if the cap truly decreased brain doses, the brain levels should mirror the attenuation achieved in the temporal surface region which was 10 times higher. The conclusions of both studies were that these caps provided negligible protection to the brain and are ineffective, due to most scatter reaching the brain from a location below the physicians head, at angles outside of the cap's attenuation shadow [123, 126].

Radio-opaque surgical caps may therefore be of inadequate size and shape to provide a sufficient X-ray shadow, particularly from inferior scatter, and perhaps a different configuration may be required. A helmet design has previously demonstrated significant attenuation at the skull level [128]. A hood design made out of commercially available attenuating caps, fashioned to hang alongside the face and back of neck to the level of the chin, appears to provide much better protection than the cap [123]. In the anthropomorphic head phantom, this hood offered 70% protection to the left brain, 49% protection to the right brain, and 55% protection to the whole brain compared to 4.9%, 1.8% and 3.3% respectively with the theatre cap design [123].

31.3.5.5 Hands and Gloves

The hands are the most unprotected part of the operator's body in close proximity to the primary beam and scatter [4] and receive more radiation compared with other parts of the body [20], particularly the distal middle and ringer fingers [113]. Interestingly there is a significant correlation between DAP and eye and finger doses, with eye and finger doses correlating with each other [4]. It is important for the operator to keep their hands and fingers out of the field at all times [113]. Even when kept out of the field, extensive instrument manipulation during screening can lead to high hand doses [29] and the exposure over a career can be substantial [67]. Chronic low dose may cause microvascular damage leading to radiodermatitis [4]. In addition to behaviour, some equipment can be used to increase the distance of the hands from the radiation field, such as utilising needle holders during fluoroscopic guided punctures [129, 130], and the use of longer sheaths [37]. The use of leaded ceiling-mounted shield reduces finger exposure two-fold [38]. The use of leaded gloves should be avoided as they can paradoxically lead to higher hand doses [113]. Although they can attenuate radiation by 15–30%, if they are misused and placed within the primary beam they will not only increase forward and backscatter indefinitely within the glove, but will lead to increased radiation dose via the machine's ABC to compensate for the increased attenuation within the field, further increasing hand doses [37, 41, 44, 113].

31.3.5.6 Ceiling Mounted Shielding

The importance of the combined use of ceiling mounted shields with leaded glasses has previously been discussed [121], providing better protection than leaded glasses alone [68]. In addition, ceiling-mounted shields provide full protection to the head and neck [113], potentially reducing brain, carotid and thyroid exposure. Studies have reported a 5.7–19 fold reduction in eye dose associated with their use [8, 113]. Their lack of availability during mobile C-arm procedures was cited as contributing to an 18-fold increase in operator dose [131]. As a consequence, ceiling-mounted shields should be available in any room where fluoroscopy is performed [68], but they are often underutilised. Even in studies designed to investigate radiation protection during complex aneurysm repairs, their use was inconsistent [3] or non-existent [9].

Another important factor is their correct positioning; if they are not precisely positioned their ability to provide adequate protection is substantially compromised [68, 111, 132]. They need to be tightly applied between the patient's skin entrance of the X-ray beam and the operator, abutting the patient adjacent to the access site, rather than above the patient or pointed towards the X-ray source or receiver [2, 68]. For femoral access, this means positioning the cut out contour tight on the leg just cephalad to the access, with the use of soft lead extension flaps helping to maintain contact between the patient and the shield [111]. Rather than having the shield as

close to the scatter source as possible, it should be closer to the operator, with due attention paid to minimising the gap [111]. Moving the shield away from the patient surface or further cephalad will accentuate this gap, decreasing the efficacy of the shield [111]. In order to maintain correct positioning, the shield requires continual repositioning throughout the case, whenever there is a change to gantry angle, table position or table height [111, 132]. Therefore to reap the significant benefit of ceiling-mounted shields they need to be consistently used, precisely positioned, and frequently repositioned.

31.3.5.7 Leg and Foot Shielding

The highest amount of scatter occurs under the table where the beam enters the patient [37]. The majority of forward scatter is absorbed by the patient thereby exponentially attenuating any that reaches the operators upper body [2]. Backward scatter however exposes the legs [37] with the highest operator dose occurring at the waist and knee level [133]. Doses to the unprotected legs are similar to that received by the hands [134]. A table mounted side skirt which hangs at the side of the bed and preferably extends upwards several inches, significantly minimises this dose by a factor of 5–50 [2, 133, 134].

Leg exposure has received more attention recently with the finding that circulating markers of DNA damage in endovascular interventionalists were elevated following complex aortic repairs, despite the presence of under-table lead shielding, but completely absent in those wearing additional personal lower leg lead shielding [80]. This suggested that the majority of this DNA damage occurred in the long bones of the legs and that additional lower leg protection was essential to reduce scatter radiation-induced DNA damage [80].

Other investigators have demonstrated unequal leg and foot doses, with the highest doses recorded on the limb closest to the source, postulating that the gap between the skirt and the floor, which is variable due to table height, may be responsible [135]. Even when table-mounted side skirts reduced mean procedural foot doses from 0.19–2.61 mSv to 0.02–0.5 mSv, these investigators suggested that lower extremity annual doses could reach 110 mSv, approaching dose limits [135]. They suggested further protective measures are taken to reduce foot and leg dose, such as stepping back, or the use of a lead-lined protective clogs [135].

31.3.5.8 Drapes

Disposable sterile lead-free bismuth-barium drapes are commercially available to drape around the area of interest with the purpose of reducing scatter to the operator. Their attenuation is in the order of 0.4–0.8 mmPb LE, depending on beam energies encountered [136]. By redirecting scatter, the aim is to reduce operator-received radiation [4, 37, 68, 137], but in doing so they may increase patient dose [37, 68]. They must never enter the field of view as they will activate the machines

ABC [37, 112], and may need to be repositioned during the procedure to avoid this [113].

Their use is more common in cardiac procedures, perhaps due to the relatively focused and static set-up, and in this setting have been associated with significant reduction in operator exposure in the order of 20% [136, 137]. They have also been shown to reduce hand, eye and head doses by up to 50% [112, 113] and reduce eye dose by a factor of 1.2–24.6 [68].

In a small RCT during EVAR, two drapes were positioned around the site of femoral access and were associated with a reduction in scatter to the hands and chest of about 50% [136]. However it must be noted that the procedures were performed using a mobile C-arm in a non-dedicated theatre with no additional shielding such as table mounted skirts or ceiling mounted shields.

31.3.6 Innovations

The inherent limitations of personal shielding, principally the musculoskeletal issues, associated with the weight of lead, and the incomplete protection afforded by the combination of even the best products, have led to the development of a number of novel solutions. The holy grail of personal radiation protection is total elimination of radiation exposure to the whole body and removal of the burden of lead on the musculoskeletal system, all within a user-friendly framework that doesn't impede workflow.

31.3.6.1 Zero Gravity Suspended System

Zero Gravity (Biotronik Inc., Lake Oswego, OR) is a full body lead personal protection device which is suspended from a complex overhead motion system that eliminates any weight on the operator. As a consequence, thicker 1 mm lead can be used to provide protection from the top of the head to the calves, leaving only the right arm and left forearm uncovered [138]. It provides 5.9–46 times more attenuation over a wide range of beam energies [112], with an overall 78-fold decrease in radiation exposure compared to a 0.5 mmPb LE apron [138].

31.3.6.2 Trinity System

The Trinity Radiation Protection System (ECLS, Salt Lake City, UT) consists of fixed shields and skirts creating a complete radiation protection environment for the operator, whilst allowing full access to the patient for interventions [139, 140]. A small study comparing ten cases performed with Trinity to nine with standard shielding reported undetectable radiation associated with Trinity with complete elimination of any exposure to operators [139].

31.3.6.3 Cathpax Cabin

Cathpax® (Lemer Pax, Carquefou, France) is a single-operator mobile radiation protection cabin, designed principally for high-dose electrophysiology interventions. In a comparison to standard protection garments, its use was associated with full-body protection and negligible radiation exposure, whilst providing unimpeded access to perform complex interventions from a femoral access site [141].

31.3.6.4 EggNest Table

The EggNest (Egg Medical, Mapel Grove, MN) is a redesigned catheterisation table with more complete integrated shielding with the manufacture reporting a greater than 90% reduction in scatter [130].

31.3.6.5 Endovascular Robotics

Robotic endovascular interventions eliminate operator exposure by freeing them entirely from the vicinity of scattered radiation. The use of endovascular robotics, principally steerable catheters, have been reported in many pre-clinical and small clinical reports of EVAR, BEVAR, FEVAR, arterial embolisations, iliac and SFA cannulations, IVC filter retrieval, pulmonary artery interventions, and the management of endoleak [142–145]. Larger clinical studies are still required to determine the global applicability of this technology. The coupling of endovascular robotics with innovative navigation tracking systems such as electromagnetic tracking and fusion imaging may one day eliminate the need for fluoroscopy [146].

31.3.6.6 Non-Radiation Imaging

There are currently a number of exciting innovations that use low or no-radiation technology to guide endovascular interventions. These include augmented reality, movement tracking, electromagnetic location tracking, optical coherence tomography and magnetic particle imaging. In addition the expanded utility of already established technologies such as intravascular ultrasound should be encouraged.

31.4 Conclusion

Endovascular surgery will continue to evolve, perhaps utilising some of the novel technologies described above to eventually unshackle itself from its current reliance on ionising radiation. Until that time a basic understanding of radiation biology and

physics and a respect for radiation safety, coupled with a strict adherence to ALARA technique, using all and every shielding available, and reducing the time spent with 'foot on the pedal', will keep exposure to a minimum.

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Glossary

Apoptosis Orderly programmed (gene-directed) cell death in which a programmed sequence of events leads to the elimination of cells without releasing harmful substances into the surrounding area. Apoptosis is marked by the fragmentation of nuclear DNA and is activated either by the presence of a stimulus or removal of a suppressing agent.

Autophagy Regulated form of cell death involving lysosomal degradation and recycling of proteins.

Cell Adhesion Molecules Proteins located on the cell surface which are involved in binding to other cells or with the extracellular matrix.

Chemokines Small signalling proteins secreted by cells which have the ability to induce directed chemotaxis (movement) by nearby responsive cells.

Compliance The ratio of change in expansion and contraction of a blood vessel with changes in blood pressure.

Compliance Mismatch The difference in compliance between that of a vascular graft or stent and the host artery.

Cytokines Small secreted molecules that signal cell-to-cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma.

DAMPs Also known as danger-associated molecular patterns, danger signals, and alarmin, are host biomolecules that can initiate and perpetuate a non-infectious inflammatory response. Protein DAMPs include intracellular proteins, such as heat-shock proteins and materials derived from the extracellular matrix that are generated following tissue injury, such as hyaluronan fragments. Non-protein DAMPs include ATP, uric acid and DNA.

Diapedesis The passage of white blood cells through capillary walls into the tissues.

Efferocytosis Phagocytic clearance of dying cells, particularly by macrophages and other immune phagocytes.

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Electrospinning Fibre production method which uses electric force to draw charged threads of polymer solutions into fibres with diameters of approximately hundreds of nanometres.

Fetuin Proteins that are made in the liver and secreted into the bloodstream. They belong to a large group of binding proteins mediating the transport and availability of a wide variety of substances in the bloodstream.

Filopodia Slender cytoplasmic projections in migrating cells. They contain actin filaments cross-linked into bundles by actin-binding proteins. Filopodia have roles in sensing, migration and cell-cell interaction. To close a wound, growth factors stimulate the formation of filopodia in fibroblasts to direct fibroblast migration and wound closure.

Haploinsufficiency A situation where the total level of a gene product (protein) produced by the cell is about half of the normal level and is insufficient to permit the cell to function normally. One of the two copies of the gene may be missing due to a deletion or inactivated due to a mutation.

Integrins Transmembrane receptors that facilitate cell-extracellular matrix adhesion. Upon ligand binding, integrins activate signal transduction pathways that mediate cellular signals such as regulation of the cell cycle, organization of the intracellular cytoskeleton, and movement of new receptors to the cell membrane. Integrins signal responses to events at the cell surface (e.g. signal platelets to initiate an interaction with coagulation factors).

Ligands A molecule that forms a complex with its target protein, resulting in modification of the function of the protein.

Matricryptic Biologically active cryptic (hidden) sites within matrix molecules that are revealed after structural or conformational alteration of these molecules.

MicroRNA A small non-coding RNA molecule (containing about 22 nucleotides) that functions in RNA silencing and post-transcriptional regulation of gene expression.

Necroptosis A programmed form of necrosis, or inflammatory cell death.

Netosis Neutrophil-related cell death characterized by the secretion of large web-like structures described as NETs.

NETs Networks of extracellular fibres, primarily composed of DNA from neutrophils, which bind pathogens. NETs allow neutrophils to kill extracellular pathogens while minimizing damage to the host cells (including lipopolysaccharides, endotoxins and other bacterial and viral molecules).

PAMPs Pathogen-associated molecular patterns which are secreted in response to an infectious stimulus initiate and perpetuate the infectious pathogen-induced inflammatory response.

Planktonic Free-living motile bacteria, usually growing in cultures in the microbiology laboratory.

Secondary Heart Field The secondary heart field is initially part of the cardiogenic field but does not differentiate as myocardium until the looping stage of heart development. The secondary heart field gives rise to the last myocardium to be added to the outflow tract and is located most caudally and medially in the heart field. The second heart field consists of the progenitors that are added to

the formed heart tube at either the arterial or venous pole. The secondary heart field refers to the splanchnic mesoderm caudal to the outflow tract. It constitutes a source of cells that gives rise to both the most distal outflow tract myocardium and the most proximal smooth muscle that forms the tunica media of the arterial trunks.

Secretome A set of proteins expressed by an organism and secreted into the extracellular space. In humans, the secretome covers 13–20% of all proteins, including cytokines, growth factors, extracellular matrix proteins and regulators, and shed receptors.

SMADs Transcription factor proteins, which act as mediators of TGF-β signal transduction.

SNPs Single nucleotide polymorphisms; a substitution of a single nucleotide that occurs at a specific position in the genome. SNPs can affect the development of diseases and responses to pathogens, chemicals, drugs, vaccines, and other agents.

Th1 Type 1 T helper cells which produce interferon-gamma, interleukin (IL)-2, and tumour necrosis factor (TNF)-beta, which activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses.

Th2 Type 2 T helper cells which produce IL-4, -5, -10, and -13 and help regulate humoral immune responses to extracellular parasites and bacterial infections.

Windkessel Effect A term used to account for the shape of the arterial blood pressure waveform in terms of the interaction between the stroke volume and the compliance of the aorta and large elastic arteries.