

MECHANISMS OF VASCULAR DISEASE:

A REFERENCE BOOK FOR VASCULAR SPECIALISTS

15

The background of the cover is a detailed illustration of a blood vessel. The vessel lumen is at the top, with a red, fibrous wall. A large, white, irregularly shaped plaque is attached to the vessel wall, partially obscuring the lumen. The plaque has a rough, porous texture. An orange, ribbon-like structure is draped over the top of the plaque, with an arrow pointing downwards towards it. Below the plaque, the vessel wall is shown in cross-section, revealing a layer of yellowish, rounded cells. The bottom part of the vessel wall is a darker, reddish-brown color.

EDITED BY ROBERT FITRIDGE AND MATTHEW THOMPSON
COMPLETELY UPDATED EDITION 2011

BARR SMITH PRESS

Mechanisms of Vascular Disease

Mechanisms of Vascular Disease:

A Reference Book for Vascular Specialists

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BARR SMITH PRESS

An imprint of
The University of Adelaide Press

Published in Adelaide by

The University of Adelaide, Barr Smith Press
Barr Smith Library
The University of Adelaide
South Australia 5005
press@adelaide.edu.au
www.adelaide.edu.au/press

The University of Adelaide Press publishes peer-reviewed scholarly works by staff via Open Access online editions and print editions.

The Barr Smith Press is an imprint of the University of Adelaide Press, reserved for scholarly works which are not available in Open Access, as well as titles of interest to the University and its associates. The Barr Smith Press logo features a woodcut of the original Barr Smith Library entrance.

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This CIP cataloguing for this work is as follows;

Mechanisms of vascular disease : a reference book for vascular surgeons / Robert Fitridge, Matthew Thompson, [editors].

1. Blood vessels, Diseases.
 2. Blood vessels, Surgery.
-
- I. Fitridge, Robert
 - II. Thompson, M. M.

For the full Cataloguing-in-Publication data please contact National Library of Australia:
cip@nla.gov.au

ISBN (paperback) 978-0-9871718-2-5

Book design: Midland Typesetters
Cover design: Emma Spoehr, based on a diagram by Dave Heinrich of the Medical Illustration and Media Unit, Flinders Medical Centre
Paperback edition printed by Griffin Press, South Australia

Table of Contents

Contributors vii

Detailed Contents xi

1. Endothelium 1
Paul Kerr, Raymond Tam, Frances Plane (Calgary, Canada)
2. Vascular smooth muscle structure and function 13
David Wilson (Adelaide, Australia)
3. Atherosclerosis 25
Gillian Cockerill, Qingbo Xu (London, UK)
4. Mechanisms of plaque rupture 43
Ian Loftus (London, UK)
5. Current and emerging therapies in atheroprotection 79
Stephen Nicholls, Rishi Puri (Cleveland, USA)
6. Molecular approaches to revascularisation in peripheral vascular disease 103
Greg McMahon, Mark McCarthy (Leicester, UK)
7. Biology of restenosis and targets for intervention 115
Richard Kenagy (Seattle, USA)
8. Vascular arterial haemodynamics 153
Michael Lawrence-Brown, Kurt Liffman, James Semmens, Ilija Sutalo (Melbourne & Perth, Australia)
9. Physiological haemostasis 177
Simon McRae (Adelaide, Australia)
10. Hypercoagulable states 189
Simon McRae (Adelaide, Australia)
11. Platelets in the pathogenesis of vascular disease and their role as a therapeutic target 201
Sandeep Prabhu, Rahul Sharma, Karlheinz Peter (Melbourne, Australia)
12. Pathogenesis of aortic aneurysms 227
Jonathan Golledge, Guo-Ping Shi, Paul Norman (Townsville & Perth, Australia; Boston, USA)
13. Pharmacological treatment of aneurysms 247
Matthew Thompson, Janet Powell (London, UK)
14. Aortic dissection and connective tissue disorders 255
Mark Hamilton (Adelaide, Australia)
15. Biomarkers in vascular disease 277
Ian Nordon, Robert Hincliffe (London, UK)
16. Pathophysiology and principles of management of vasculitis and Raynaud's phenomenon 295
Martin Veller (Johannesburg, South Africa)
17. SIRS, sepsis and multiorgan failure 315
Vishwanath Biradar, John Moran (Adelaide, Australia)
18. Pathophysiology of reperfusion injury 331
Prue Cowled, Robert Fitridge (Adelaide, Australia)
19. Compartment syndrome 351
Edward Choke, Robert Sayers, Matthew Bown (Leicester, UK)
20. Pathophysiology of pain 375
Stephan Schug, Helen Daly, Kathryn Stannard (Perth, Australia)

21. Postamputation pain 389
Stephan Schug, Gail Gillespie
(Perth, Australia)
 22. Treatment of neuropathic pain 401
Stephan Schug, Kathryn Stannard
(Perth, Australia)
 23. Principles of wound healing 423
Gregory Schultz, Gloria Chin,
Lyle Moldauer, Robert Diegelmann
(Florida, USA)
 24. Pathophysiology and principles of
varicose veins 451
Andrew Bradbury (Birmingham, UK)
 25. Chronic venous insufficiency and leg
ulceration: Principles and vascular
biology 459
Michael Stacey (Perth, Australia)
 26. Pathophysiology and principles of
management of the diabetic foot 475
David Armstrong, Timothy Fisher,
Brian Lepow, Matthew White,
Joseph Mills (Tucson, USA)
 27. Lymphoedema – Principles, genetics
and pathophysiology 497
Matt Waltham (London, UK)
 28. Graft materials past and future 511
Mital Desai, George Hamilton
(London, UK)
 29. Pathophysiology of vascular graft
infections 537
Mauro Vicaretti (Sydney, Australia)
- Index 549

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Detailed Contents

CHAPTER 1 – ENDOTHELIUM

Paul Kerr, Raymond Tam, Frances Plane

- Introduction 1
- Endothelium-dependent regulation of vascular tone 2
- Angiogenesis 7
- Haemostasis 8
- Inflammation 9
- Conclusions 10
- References

CHAPTER 2 – VASCULAR SMOOTH MUSCLE STRUCTURE AND FUNCTION

David Wilson

- Introduction 13
- Smooth muscle (vascular) structure
- Cytoskeleton 14
- Contractile myofilament
- Functional regulation of vascular smooth muscle: Neuronal, hormonal, receptor mediated 15
- Smooth muscle function 17
- Myofilament basis of smooth muscle contraction and relaxation
- Smooth muscle contraction and relaxation 18
- Ion channels important in the regulation of smooth muscle function
- Regulation of cellular Ca^{2+}
- Sources of cytosolic Ca^{2+} entry 19
- Potassium channels
- Endothelial regulation of smooth muscle vasodilatation 20

Smooth muscle proliferation and vascular remodeling 20

Summary 22

References

CHAPTER 3 – ATHEROSCLEROSIS

Gillian Cockerill, Qingbo Xu

Introduction 25

Atherosclerotic lesions 26

Fatty streaks

Plaque or atheroma

Hypercholesterolemia and oxidised-LDL 27

High-density lipoproteins role in atheroprotection 28

Hypertension and biomechanical stress 29

Biomechanical stress-induced cell death 30

Biomechanical stress and inflammation 31

Biomechanical stress-induced smooth muscle cell proliferation 32

Infections and heat shock proteins

Infections

Heat shock proteins 33

Infections and HSP expression

Infections, sHSP and innate immunity 34

Immune responses 36

MHC class II antigens and T cells

Oxidised LDL as a candidate antigen

HSP60 as a candidate antigen 37

B2-glycoprotein Ib as a candidate antigen

Inflammation

C-reactive protein	38
CD40/CD40L	
Summary and perspectives	39
References	

CHAPTER 4 – MECHANISMS OF PLAQUE RUPTURE

Ian Loftus

Introduction	43
Evidence for the ‘plaque rupture theory’	44
Coronary circulation	
Cerebral circulation	
The role of individual components of the arterial wall	
The endothelium	45
The lipid core	47
The cap of the plaque	49
Smooth muscle cells and collagen production	50
Macrophages and collagen degradation	51
The vessel lumen	56
The role of angiogenesis in plaque rupture	
The role of infectious agents in plaque rupture	57
Risk prediction of plaque instability	58
Imaging	
Blood markers	59
Therapy aimed at plaque stabilisation	
HMG Co-A reductase inhibitors	60
MMP inhibition	
Tissue inhibitors of metalloproteinases (TIMPs)	61
Synthetic MMP inhibitors	
Doxycycline	
ACE inhibitors	
Summary	62
References	63

CHAPTER 5 – CURRENT AND EMERGING THERAPIES IN ATHEROPROTECTION

Stephen Nicholls, Rishi Puri

Background	79
Pathology	
Risk factor modification	80
Statins, LDL lowering and C-reactive protein	
The complexity of HDL	84
The controversy of triglycerides	87
Hypertension	
Risk factor modification in the diabetic patient	89
Glycaemic control	
Global risk factor reduction in diabetics	91
The metabolic syndrome	92
Future targets	93
Conclusion	
References	94

CHAPTER 6 – MOLECULAR APPROACHES TO REVASCULARISATION IN PERIPHERAL VASCULAR DISEASE

Greg S McMahon, Mark J McCarthy

Introduction	103
Mechanisms of vascular growth	
Vasculogenesis	
Angiogenesis	104
Neovessel maturation	105
Microvascular network maturation	106
Arteriogenesis	
Therapeutic induction of vascular growth	107
Delivery of molecular activators of vascular growth	
Angiogenic activators	108
Arteriogenic activators	109
Clinical trials for angiogenic therapy of peripheral vascular disease	
Conclusions	110
References	

CHAPTER 7 – BIOLOGY OF RESTENOSIS AND TARGETS FOR INTERVENTION

Richard Kenagy

Introduction 115

Mechanisms of restenosis

Thrombosis 116

Remodelling

Intimal hyperplasia 123

Sequence of events after injury

Origin of intimal cells 125

Inflammation 126

Role of ECM production 127

The contribution of specific factors to restenosis

Growth factors/cytokines

Inhibitors 128

Coagulation and fibrinolytic factors 129

Matrix metalloproteinases

Extracellular matrix/receptors

Targets for intervention 130

Intracellular signalling molecules

mTOR and microtubules

Transcription factors

miRNA 131

Inflammation targets

Brachytherapy

Extracellular targets and cell-based therapies

Angiotensin pathway

Cell-based therapies 132

Differential effects on endothelium and SMCs

Delivery devices

Prevention versus reversal of restenosis

Conclusions 133

References 134

CHAPTER 8 – VASCULAR ARTERIAL HAEMODYNAMICS

Michael Lawrence Brown, Kurt Liffman, James Semmens, Ilija Sutalo

Introduction 153

Laplace's law of wall of tension 154

Newtonian fluid 155

Non-Newtonian fluid

Poiseuille flow 158

Bernoulli's equation

Young's modulus and pulsatile flow 159

Mass conversion 161

Reynold's number

Arterial dissection, collateral circulation and competing flows 163

Shear stress and pressure 164

Forces on graft systems 165

Case 1 – The cylindrical graft 168

Case 2 – The windsock graft

Case 3 – The curved graft 169

Case 4 – The symmetric bifurcated graft

Computational modelling 170

Recent development and future directions 171

Conclusions 172

References 173

CHAPTER 9 – PHYSIOLOGICAL HAEMOSTASIS

Simon McRae

Introduction 177

Primary haemostasis

Platelets

Platelet adhesion

Platelet activation and shape change 179

Platelet aggregation 180

Interactions between primary and secondary haemostasis 181

Secondary haemostasis

The coagulation cascade 182

Initiation 183

Amplification

Propagation 184

Normal inhibitors of coagulation

Fibrinolysis 185

Conclusions 186

References

CHAPTER 10 – HYPERCOAGULABLE STATES

Simon McRae

Introduction 189

Classification of thrombophilia

Inherited thrombophilia 190

Type 1 conditions

Antithrombin deficiency

Protein C and Protein S deficiency

Type 2 conditions 191

Factor V Leiden

The prothrombin (G20210A) gene mutation

FVL/PGM compound heterozygotes

Other inherited conditions

Acquired thrombophilia 192

Antiphospholipid antibodies

Heparin induced thrombocytopenia

Myeloproliferative disorders 193

Potential reasons for performing thrombophilia testing

Patients with venous thrombosis and their relatives

Providing an understanding of the aetiology of a thrombotic event

Determining risk of recurrence and therefore optimal duration of anticoagulation 194

Determining the need for primary prophylaxis in asymptomatic family members 195

Making decisions regarding the use of the oral contraceptive pill 196

Determining the need for thromboprophylaxis during pregnancy

Patients with arterial thrombosis

Potential detrimental effects of thrombophilia testing 197

Conclusion

References

CHAPTER 11 – PLATELETS IN THE PATHOGENESIS OF

VASCULAR DISEASE AND THEIR ROLE AS A THERAPEUTIC TARGET

*Sandeep Prabhu, Rahul Sharma,
Karlheinz Peter*

Introduction 201

Platelet function – Adhesion and activation

Platelet adhesion 202

Platelet activation 203

Mediators of platelet activation and ‘outside in’ signalling

Thrombin and collagen 204

Adenosine diphosphate (ADP)

Thromboxane A2 (TXA2)

Adrenaline 206

Second messenger systems 207

Physiological consequences of platelet activation

The GP IIb/IIIa receptor and ‘inside-out’ signalling

Granule exocytosis 208

Activation-induced conformational change of platelets

Platelets and atherosclerosis 209

Role of platelets in the initiation of the atherosclerosis

Role of the platelets in the progression of the atherosclerosis

Role of platelets in vulnerable plaques and plaque rupture

Current and future anti-platelet agents 210

Aspirin (salicylic acid)

Thienopyridines 211

Clopidogrel

Prasugrel 213

Ticlopidine

Ticagrelor

GPIIb/IIIa Antagonists

Other anti-platelet agents and promising new developments 214

Platelet function testing 215

Light-transmission aggregometry

Whole blood aggregometry 217
 VerifyNow® Assay
 Flow cytometry 218

References

CHAPTER 12 – PATHOGENESIS OF AORTIC ANEURYSMS

*Jonathan Golledge, Guo-Ping Shi,
 Paul E Norman*

Introduction 227

Differences between thoracic and abdominal aortic aneurysms 228

Summary of current theories and stages of AAA evolution

Atherosclerosis and AAA

Immune mechanisms in AAA 229

Extracellular matrix dysfunction 232

Infection 233

Biomechanical forces

Angiogenesis

Intra-luminal thrombus

Extracellular matrix proteolysis 234

Genetics 236

AAA rupture 237

Biomechanical factors in aneurysms rupture

The role of enzymes in AAA rupture

Role of intraluminal thrombus in aneurysm rupture 238

Future research

References

CHAPTER 13 – PHARMACOLOGICAL TREATMENT OF ANEURYSMS

Matthew Thompson, Janet T Powell

Background 247

Screening programmes

Pathophysiology 248

Therapeutic strategies

Beta blockade

Modification of the inflammatory

response 249

Non-steroidal anti-inflammatories

Matrix metalloproteinase (MMP)

inhibition

Anti-chlamydial therapy 250

Drugs acting on the renin/angiotensin axis

HMG Co-A reductase inhibitors 251

The future – Data from recent

experimental studies

References

CHAPTER 14 – PATHOPHYSIOLOGY OF AORTIC DISSECTION AND CONNECTIVE TISSUE DISORDERS

Mark Hamilton

Introduction 255

Embryology of thoracic aorta and arch vessels

Haemodynamics of thoracic compared to abdominal aorta 257

Sizes of normal aorta

Classification of aortic syndromes

Acute/Chronic

DeBakey classification of class 1 dissection – Type 1, 2, and 3

Stanford classification 258

European task force

Pathogenesis of thoracic aortic dissection

Classical thoracic aortic dissection (class 1 dissection) 260

Intramural haematoma (class 2 aortic dissection) 261

Penetrating aortic ulcer (class 4 aortic dissection) 262

Complications of acute aortic syndromes 263

Visceral ischaemia /malperfusion syndromes

Fate of the false lumen

Aneurysmal degeneration and rupture 264

Connective tissue disorders and acute aortic syndromes

Marfan syndrome
 Fibrillin and Marfan syndrome 265
 The role of transforming growth factor
 beta in development of the vascular
 system in health and disease 266
 Ehlers-Danlos syndrome 267
 Diagnosis of Ehlers-Danlos syndrome
 268
 Loeys-Deitz syndrome 270
 Familial thoracic aortic aneurysm disease
 271
 Bicuspid aortic valve 273
 Turners Syndrome
Summary 274
Reference list

CHAPTER 15 – BIOMARKERS IN VASCULAR DISEASE

Ian M Nordon, Robert J Hinchliffe

Introduction 277
What is a biomarker?
Types of biomarkers
 A classical clinical example 278
**Potential value of biomarkers in vascular
 disease 279**
Biomarker discovery steps 280
AAA biomarkers
 Circulating extracellular matrix markers
 281
 Matrix-degrading enzymes 283
 Proteins associated with thrombosis
 Markers of inflammation 284
Biomarkers of AAA rupture 285
Biomarkers following endovascular repair
 Inflammation 287
 Lipid accumulation
 Apoptosis
 Thrombosis
 Proteolysis 288
Challenges in biomarkers discovery
Future work
Conclusion 289
References

CHAPTER 16 – PATHOPHYSIOLOGY AND PRINCIPLES OF MANAGEMENT OF VASCULITIS AND RAYNAUD'S PHENOMENON

Martin Veller

Vasculitides 295
Introduction
Classification of vasculitides 296
Clinical presentation of vasculitides
Investigations of vasculitides
**Principles of treatment of vasculitides
 297**
**The vasculitides of specific interest to
 vascular surgeons 298**
 Giant cell arteritis
 Takayasu's arteritis 299
 Thromboangitis obliterans (Buerger's
 disease) 300
 Behcet's disease 301
 Polyarteritis nodosa 302
 Vasculitides secondary to connective
 tissue diseases 303
 Systemic lupus erythematosus (SLE)
 Antiphospholipid antibody syndrome
 (APS) 304
 Rheumatoid arthritis 305
 Scleroderma
 Infective vasculitides 306
 Human immunodeficiency virus (HIV)
**Pathophysiology and principles of
 Raynaud's phenomenon 307**
 Prevalence of Raynaud's phenomenon
 308
 Clinical findings in Raynaud's
 phenomenon 309
 Diagnosis of Raynaud's phenomenon
Prognosis 310
Treatment
Recommendations 311
References 312

CHAPTER 17 – SIRS, SEPSIS AND

MULTIORGAN FAILURE*Vishwanath Biradar, John Moran***Epidemiology 315****Historical perspectives and definition 316****Risk factors for sepsis 317**

Causative agents

Pathophysiology of sepsis

innate immunity and toll-like receptors (TLRs) 319

Proinflammatory response

Coagulation cascade

Multorgan dysfunction syndrome (MODS) 320

Epithelial and endothelial dysfunction

Immune suppression and apoptosis

Sepsis, circulatory failure and organ dysfunction

Management 322

Steroids 323

Recombinant human activated protein C (rhAPC) 324

Glucose control 325

Renal replacement therapy

3-hydroxy-3-methylglutaryl-coenzyme reductase inhibitors (HMG-CoA) 326

Other adjuvant therapies in sepsis

Cytokines and anticytokine therapies

Pooled immunoglobulin (IVIG)

Acute respiratory distress syndrome (ARDS) 327

References**CHAPTER 18 –
PATHOPHYSIOLOGY OF
REPERFUSION INJURY***Prue Cowled, Rob Fitridge***Introduction 331****Ischaemia**

ATP and mitochondrial function

Gene expression during ischaemia 332

Reperfusion 333

Reactive oxygen species

Eicosanoids 334

Nitric Oxide 335

Endothelin 336

Cytokines

Neutrophil and endothelial interactions 338

Complement activation 340

Tissue destruction 341

Proteases and metalloproteinases

Apoptotic cell death during ischaemia-reperfusion injury

No-reflow phenomenon 342

Therapeutic approaches to IRI

Ischaemic preconditioning

Ischaemic post-conditioning 343

Conditioning effects of volatile anaesthetics

Pharmacological treatments 344

Summary 345**References****CHAPTER 19 – COMPARTMENT
SYNDROME***Edward Choke, Robert Sayers, Matthew Bown***Definition 351****Acute limb compartment syndrome**

Incidence

Anatomy/physiology 352

Aetiology/pathophysiology

Clinical presentation 354

Investigation 355

Treatment 357

Complication of LCS 359

Outcome 360

Acute abdominal compartment syndrome

Incidence 361

Aetiology

Pathological effects of raised intra-abdominal pressure 362

Clinical presentation 363

Investigation

Treatment 364

Complications of surgical decompression

Outcome 367

References 368

CHAPTER 20 – PATHOPHYSIOLOGY OF PAIN

Stephan Schug, Helen Daly, Kathryn Stannard

Introduction 375

Peripheral mechanisms

Nociception/transduction

Conduction 376

Spinal cord mechanisms

Ascending systems 377

Descending control

Pain modulation 378

Peripheral sensation

Central sensitisation in the dorsal horn

Neuropathic pain 379

Mechanisms of neuropathic pain

Peripheral mechanisms

Spontaneous ectopic discharge

Altered gene expression

Spared sensory neurons

Involvement of the sympathetic nervous system 380

Collateral sprouting

Effects of bradykinin

Central mechanisms

Wind up

Central sensitization 381

Central disinhibition

Expansion in receptive field size (recruitment)

Immediate early gene expression

Anatomical re-organisation of the spinal cord

Contribution of glial cells to pain conditions 382

Symptoms of neuropathic pain

Stimulus-dependent pain

Stimulus-independent pain 383

Sympathetically maintained pain (SMP)

Neuropathic pain syndromes

Peripheral neuropathies

Central neuropathies 385

References

CHAPTER 21 – POST-AMPUTATION PAIN

Stephan Schug, Gail Gillespie

Introduction 389

Classification and incidence of post-amputation pain syndromes

Stump pain

Phantom sensation 390

Phantom limb pain

Pathophysiology of post-amputation pain syndromes

Peripheral factors

Spinal factors 391

Supraspinal factors

Current pathophysiological model of post-amputation pain syndromes 392

Prevention of post-amputation pain

Perioperative lumbar epidural blockade

Peripheral nerve blockade 393

NMDA antagonists

Evaluation of the patient with post-amputation pain syndromes

Examination

Therapy of post-amputation pain syndromes 394

Calcitonin

Ketamine

Analgesic and Co-analgesic compounds

Opioids 395

Gabapentin

Clonazepam

Lidocaine

Carbamazepine

Tricyclic antidepressants (TCA)

Selective serotonin reuptake inhibitors

Baclofen

Capsaicin

Symptomatic treatment of pain components 396

Neuropharmacological therapies

Invasive therapies
 Electroconvulsive therapy (ECT)
 Nerve blockade
 Spinal cord stimulation
 Implantable intrathecal delivery systems
 Dorsal root entry zone (DREZ) lesions
 Psychological therapy 397

Future aims

References

CHAPTER 22 – TREATMENT OF NEUROPATHIC PAIN

Stephan Schug, Kathryn Stannard

Introduction 401

Principles of treatment

Pharmacological treatment 402

Opioids

Recommendations for clinical use of opioids

Tramadol

Mechanism of action

Efficacy 403

Adverse effects

Recommendations for clinical use of tramadol in neuropathic pain

Antidepressants

Tricyclic antidepressants (TCAs)

Mechanism of action 404

Adverse effects

Selective serotonin re-uptake inhibitors (SSRIs)

Serotonin/Noradrenaline reuptake inhibitors (SNRIs) 405

Recommendations for clinical use of antidepressants as analgesics

Anticonvulsants

Mechanism of action 406

Individual medications

Clonazepam

Gabapentin

Pregabalin 407

Carbamazepine

Sodium valproate 408

Phenytoin

Lamotrigene

Recommendations for clinical use of anticonvulsants as analgesics

Local anaesthetics and antiarrhythmics 409

Mechanism of action

Lignocaine

Mexiletine

Recommendations for clinical use of lignocaine and mexiletine in neuropathic pain

N-methyl-D-aspartate-receptor antagonists (NMDA)

Ketamine 410

Other NMDA antagonists

Miscellaneous compounds for systemic use

Clonidine

Efficacy

Baclofen

Levodopa 411

Cannabinoids

Topical treatments

Lignocaine 5% medicated plaster

Capsaicin 412

Mechanism of action

Efficacy

Non-pharmacological therapy

Transcutaneous electrical nerve stimulation (TENS)

Spinal cord stimulation (SCS) 413

Sympathetic nerve blocks

Neurosurgical destructive techniques

Cognitive behaviour therapy

References 414

CHAPTER 23 – PRINCIPLES OF WOUND HEALING

Gregory Schultz, Gloria Chin, Lyle Moldawer, Robert Diegelmann

Introduction 423

Phases of acute wound healing

Haemostasis

- Inflammation 426
 - Neutrophils 427
 - Macrophages 428
- Proliferative phase 429
 - Fibroblast migration 430
 - Collagen and extracellular matrix production
 - Angiogenesis 431
 - Granulation 432
 - Epithelialization
 - Remodelling 433
- Summary of acute wound healing 435
- Comparison of acute and chronic wounds**
 - Normal and pathological responses to injury
 - Biochemical differences in the molecular environments of healing and chronic wounds 436
 - Biological differences in the response of chronic wound cells to growth factors 439
- From bench to bedside**
 - Role of endocrine hormones in the regulation of wound healing
 - Molecular basis of chronic non-healing wounds
 - Chronic venous stasis ulcers 441
 - Pressure ulcers
- Future concepts for the treatment of chronic wounds 442**
 - Bacterial biofilms in chronic wounds 443
- Conclusion 445**
- References**

CHAPTER 24 – PATHOPHYSIOLOGY AND PRINCIPLES OF MANAGEMENT OF VARICOSE VEINS

Andrew Bradbury

- Introduction 451**
- Anatomy**
- Histology 452**
- Physiology**

- Varicose veins 453**
- Valvular abnormalities**
- Muscle pump failure 455**
- Venous recirculation**
- Recurrent varicose veins**
 - New varicose veins
 - Persistent varicose veins
 - True recurrent varicose veins 456
- Cellular and molecular biology of varicose veins**
- Conclusion 457**
- References**

CHAPTER 25 – CHRONIC VENOUS INSUFFICIENCY AND LEG ULCERATION: PRINCIPLES AND VASCULAR BIOLOGY

Michael Stacey

- Definitions 459**
 - Chronic venous insufficiency
 - Leg ulceration
 - Assessment of cause of leg ulceration 460
- Epidemiology 461
- Pathophysiology
 - Venous abnormality
 - Effect of ambulatory venous hypertension on the tissues in the leg 463
 - Influence of venous disease on the wound healing process 465
 - Genetic associations with venous ulceration 466
- Assessment of venous function 467**
- Treatment of venous ulceration**
 - Compression therapy
 - Dressings 468
 - Surgery
 - Prevention of venous ulcer recurrence 470
 - Sclerotherapy and other techniques to obliterate surface and perforating veins
 - Other therapies 471
- References**

CHAPTER 26 –
PATHOPHYSIOLOGY AND
PRINCIPLES OF MANAGEMENT
OF THE DIABETIC FOOT

*David Armstrong, Timothy Fisher, Brian
Lepow, Matthew White, Joseph Mills*

- Introduction** 475
- Pathophysiology of the diabetic foot** 476
- Neuropathy
 - Structural abnormalities/gait abnormalities
 - Angiopathy 478
- Diagnosis**
- History and rapid visual screening
 - Neurological examination 479
 - Monofilament testing
 - Vibration testing
 - Dermatologic examination 480
 - Anatomy of occlusive disease – vascular examination
 - Prediction of wound healing: assessment of perfusion 481
 - Arterial imaging
 - Soft tissue imaging 482
- Classification systems** 483
- Diabetes mellitus foot risk classification
 - University of Texas wound classification system
- Clinical problems and principles of management** 484
- Ulceration
 - Epidemiology and risk factors
 - Offloading
 - Non-vascular surgical treatment 485
 - Class I – Elective 486
 - Class II – Prophylactic
 - Class III – Curative
 - Class IV – Emergency (urgent)
 - Post-operative management
 - Infections 487
 - Charcot arthropathy
- Prevention** 490
- Conclusion** 492
- References**

CHAPTER 27 – LYMPHOEDEMA
– PRINCIPLES, GENETICS AND
PATHOPHYSIOLOGY

Matt Waltham

- Introduction** 497
- Classification of lymphoedema**
- Classification of primary lymphoedema 498
- The genetics of lymphangiogenesis in primary lymphoedema** 500
- Milroy's disease
 - Lymphoedema – distichiasis syndrome 501
 - Hypotrichosis – lymphoedema – telangiectasia syndrome 502
 - Meige disease (primary non-syndromic lymphoedema)
 - Other primary lymphoedema disorders 503
- Structure and development of the lymphatic circulation**
- Clinical aspects of lymphoedema** 505
- Summary**
- References**

CHAPTER 28 – GRAFT
MATERIALS PAST AND FUTURE

Mital Desai, George Hamilton

- The pathophysiology of graft healing** 511
- The peri-anastomotic area
 - Healing of prosthetic grafts 512
 - The healing process of the anastomosis
 - Graft porosity and permeability
- Physical properties of prosthetic materials** 514
- Tubular compliance
 - Anastomotic compliance mismatch
 - The compliance hypothesis of graft failure
- Synthetic grafts** 515
- Newer developments of Dacron grafts
 - Modifications and newer developments of PTFE grafts 517
 - Polyurethane grafts

Newer developments of polyurethane vascular grafts	518
Biological vascular grafts	519
Newer developments of biological vascular grafts	520
Prosthetic graft modifications	
Modifications to reduce graft infection	
Modifications to improve patency	521
Nanocomposite grafts	
Endothelial cell seeding	522
Single stage seeding	
Two stage seeding	
Vascular tissue engineering	
Non-degradable polymer and cell seeding	523
Bioresorbable and biodegradable polymers	
Combined bioresorbable and tissue engineered grafts	524
Mechanical conditioning of seeded vascular cells	
Alternative scaffolds	
Tissue-engineered grafts	525
Graft materials for aortic endografts	526
The future	
References	527

CHAPTER 29 – PATHOPHYSIOLOGY OF VASCULAR GRAFT INFECTIONS

Mauro Vicaretti

Introduction	537
Natural history of prosthetic vascular graft infections	
Mechanism of graft contamination at operation	538
Pathogenesis of graft infections	
Bacteriology of vascular graft infections	
Investigations for detection of prosthetic graft infections	539
History and physical examination	
Laboratory investigations	
Diagnostic imaging	540
Management of prosthetic graft infections	
Prevention	
Reduction of prosthetic vascular graft infection with rifampicin bonded gelatin sealed Dacron	541
Established infection	
Antibiotic therapy	
Operative management	
Conclusion	542
References	

Acknowledgements

The Editors gratefully acknowledge the outstanding contributions of each Author involved in this reference book. We would also like to acknowledge the invaluable efforts of Ms Sheona Page who has worked tirelessly on this project. We would also like to thank Prue Cowled PhD and Ms Cayley Wright for their assistance.

Abbreviation List

a1-PI	a1-protease inhibitor
5-HT	5-Hydroxytryptamine/Serotonin
AAA	Abdominal aortic aneurysm
AAS	Acute aortic syndrome
AAV	Adeno-associated viruses
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ACS	Abdominal compartment syndrome
ACTH	Adrenocorticotrophic hormone
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADP	Adenosine diphosphate
AIDS	Acquired immune deficiency syndrome
ALI	Acute lung injury
AMP	Adenosine monophosphate
AMPA	α -amino-3 hydroxy-5-methylisoxazole
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
AOD	Aortic occlusive disease
AP1	Activated protein 1
APC	Activated protein C
APC	Antigen presenting cell
APLAS	Antiphospholipid antibody syndrome
ApoAI	Apolipoprotein AI
ApoE	Apolipoprotein E
APS	Antiphospholipid antibody syndrome
APTT	Activated partial thromboplastin time

ARDS	Acute respiratory distress syndrome
AT	Antithrombin
ATP	Adenosine triphosphate
AVP	Ambulatory venous thrombosis
β 2-GPI	β 2-glycoprotein Ib
bFGF	Basic fibroblast growth factor
BKCa	Large conductance calcium activated potassium channel
BMPs	Bone morphogenetic proteins
BMS	Bare metal stent
CAD	Coronary artery disease
CaM	Calmodulin
CAM	Cell adhesion molecule
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
cGMP	Cyclic guanine monophosphate
CD	Cluster of differentiation
CD40L	Cluster of differentiation 40 ligand
CEA	Carotid endarterectomy
CETP	Cholesteryl ester transfer protein
CFD	Computational fluid dynamics
CG	Cationized gelatin
CGRP	Calcitonin gene regulated peptide
CHD	Coronary heart disease
CI	Confidence interval
CIMT	Carotid intimal-media thickness
c-JNK	c-Jun N-terminal kinase
CK-MB	Creatinine kinase (Myocardial specific)
CNCP	Chronic noncancer pain
cNOS	Constitutive nitric oxygen synthase enzyme
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CROW	Charcot restraint orthotic walker
CRRT	Continuous renal replacement therapy

CRP	C-reactive protein
CRPS	Complex regional pain syndromes
CT	Computational tomography
CTA	Computed tomographic angiography
CTD	Connective tissue disorders
CTGF	Connective tissue growth factor
CYP	Cytochrome P450
CVD	Cardiovascular disease
CVI	Chronic venous insufficiency
DAG	Diacylglycerol
DES	Drug-eluting stent
DRG	Dorsal root ganglion
DNA	Deoxyribonucleic acid
DSA	Digital subtraction arteriography
DTS	Dense tubular system
DVT	Deep vein thrombosis
EC	Endothelial cell
ECM	Extracellular matrix
EDCF	Endothelium-derived contracting factor
EDH	Endothelium-dependent hyperpolarisation
EDS	Ehlers-Danlos syndrome
EET	Epoxyeicosatrienoic acids
ELAM-1	Endothelial-leukocyte adhesion molecule-1
ELG	Endoluminal grafts
ELISA	Enzyme linked immunosorbent assay
E_K	Equilibrium potential
E_M	Membrane potential
eNOS	Endothelial nitric oxide synthase enzyme
EPC	Endothelial progenitor cells
EPCR	Endothelial protein C receptor
ePTFE	Expanded polytetrafluoroethylene
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate

ET	Essential thrombocytosis
ET-1	Endothelin 1
EVAR	Endovascular aortic aneurysm repair
EVLA	Endovenous LASER ablation
FDA	Food and drug administration
FDPs	Fibrin degradation products (soluble)
FGF	Fibroblast growth factor
FGF-2	Fibroblast growth factor 2
FMN	Flavin mononucleotide
FVL	Factor V Leiden
GABA	Gamma-aminobutyric acid
GABA B	Gamma-aminobutyric acid subtype B
G-CSF	Granulocyte colony stimulating factor
GMCSF	Granulocyte-macrophage colony stimulating factor
GP	Glycoprotein
GPCR	G-protein coupled receptor
GSV	Great saphenous vein
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HIF	Hypoxia inducible factor
HIT	Heparin induced thrombocytopenia
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMG Co-A	Hydroxymethylglutaryl coenzyme-A
HMW	High molecular weight
HPETE	Hydroperoxyeicosatetraenoic acid
HETE	Hydroxyeicosatetraenoic acids
HR	Hazard ratio
hsCRP	High-sensitive C-reactive protein
HSP	Heat shock protein
HUV	Human umbilical vein
IAH	Intra-abdominal hypertension

IAP	Intra-abdominal pressure
IAPP	Intra-abdominal perfusion pressure
ICAM-1	Inter-cellular adhesion molecule-1
ICAM-2	Inter-cellular adhesion molecule-2
ICP	Intra-compartmental pressure
ICU	Intensive care unit
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IHD	Ischemic heart disease
IL	Interleukin
IL-1	Interleukin-1
IL-1 α	Interleukin-1 alpha
IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ILT	Intraluminal thrombus
IKCa	Intermediate conductance calcium-activated potassium channels
IMH	Intramural haematoma
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase enzyme
IP(3)	1,4,5-inositol triphosphate
IRI	Ischemia reperfusion injury
IVIG	Intravenous pooled immunoglobulin
IVUS	Intravascular ultrasound
KGF	Keratinocyte growth factor
KGF-2	Keratinocyte growth factor-2
LAP	Latency associated peptide
LCS	Limb compartment syndrome
LDL	Low density lipoprotein
LDS	Loeys-Dietz syndrome
LLC	Large latent complex
LEC	Lymphatic endothelial cells

LFA-1	Lymphocyte function-associated antigen-1
LO	Lipoxygenase
LOX	Lysyl oxidase
LOPS	Loss of protective sensation
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
LTGFBP	Latent TGF binding protein
MAC-1	Macrophage-1 antigen
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor
MFS	Marfan syndrome
MHC	Major histocompatibility
MI	Myocardial infarction
MIP-1	Macrophage inflammatory protein-1
MLC ₂₀	Myosin light chain ₂₀
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MMP	Matrix metalloproteinase
MODS	Multiple organ dysfunction syndrome
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MRSE	Methicillin resistant <i>Staphylococcus epidermidis</i>
MRTA	Magnetic resonance tomographic angiography
MTHFR	Methylenetetrahydrofolate reductase
MT-MMP	Membrane-type MMP
MVPS	Mitral valve prolapse syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate
NGF	Nerve growth factor

NFκB	Nuclear factor kappa B
NiTi	Nitinol
NJP	Non-junctional perforators
NMDA	N-methyl-D-aspartate
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase enzyme
NSAID	Non-steroidal anti-inflammatory drug
NV	Neovascularisation
OCP	Oestrogen/progesterone contraceptive pill
OPN	Osteopontin
OPG	Osteoprotegerin
OR	Odds ratio
OxLDL	Oxidised low density lipoprotein
PAD	Peripheral arterial disease
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease activated receptor
PAR-1	Protease activated receptor-1
PAR-4	Protease activated receptor-4
PAU	Penetrating aortic ulcer
PC	Protein C
PCA	Poly (carbonate-urea) urethane
PCI	Percutaneous coronary intervention (angioplasty)
PCWP	Pulmonary capillary wedge pressure
PDGF	Platelet-derived growth factor
PDGFβ	Platelet-derived growth factor-β
PDS	Polydioxanone
PECAM-1	Platelet-endothelial cell adhesion molecule-1
PEDF	Pigment epithelium-derived factor
PES	Paclitaxel-eluting stent

PET	Positron emission tomography
PF4	Platelet factor 4
PGI ₂	Prostacyclin
PGG ₂	Prostaglandin G ₂
PGH ₂	Prostaglandin H ₂
PGEI ₂ /PGI ₂	Prostaglandin I ₂
PGN	Peptidoglycan
PHN	Postherpetic neuropathy
PHZ	Para-anastomotic hyper-compliant zone
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PLOD	Procollagen lysyl hydroxylase
PMCA	Plasma membrane Ca ²⁺ APTases
PMN	Polymorphonuclear leukocyte
POSS	Polyhedral oligomeric silsesquioxanes
PPAR	Peroxisomal proliferation activating receptor
PPI	Proton pump inhibitor
PRV	Polycythaemia rubra vera
PS	Protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothombin time
PTCA	Percutaneous coronary angioplasty
PTFE	Polytetrafluoroethylene
PTS	Post-thrombotic syndrome
PUFA	Polyunsaturated fatty acid
PVI	Primary valvular incompetence
rAAA	Ruptured AAA
Rac	Ras activated cell adhesion molecule
RANTES	Regulated upon activation, normal T cell expressed and secreted
RAS	Renin angiotensin system
RCT	Randomised controlled trial

RF	Rheumatoid factor
RFA	Radiofrequency ablation
rhAPC	Recombinant human activated protein C
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Relative risk
RSD	Reflex sympathetic dystrophy
S1P	Sphingosine-1-phosphate
SAPK	Stress-activated protein kinase
SCF	Stem cell factor
SCS	Spinal cord stimulation
ScvO2	Superior vena cava venous oxygen saturation
SDF-1	Stromal-cell-derived factor-1
SERCA	Sarco/endoplasmic reticulum CaATPases
SEP	Serum elastin peptides
SES	Sirolimus-eluting stent
SEPS	Subfascial endoscopic perforator surgery
SFA	Superficial femoral artery
SFJ	Sapheno-femoral junction
SIRS	Systemic inflammatory response syndrome
SKCa	Small conductance calcium-activated potassium channels
SLE	Systemic lupus erythematosus
SMA	Smooth muscle alpha actin
SMC	Smooth muscle cell
SMP	Sympathetically maintained pain
SNARE	Soluble N-ethylmaleimide-sensitive factor activating protein receptors
SNP	Single nucleotide polymorphisms
SNRI	Serotonin/Noradrenaline reuptake inhibitors
SPJ	Sapheno-popliteal junction
SPP	Skin perfusion pressure
SR	Sarcoplasmic reticulum
SSRIs	Selective serotonin re-uptake inhibitors
SSV	Small saphenous vein

SVT	Superficial thrombophlebitis
STIM1	Stromal interacting molecule 1
T α CE	TNF α converting enzyme
TAAD	Thoracic aortic aneurysm disease
TAD	Thoracic aortic dissection
TAFI	Thrombin-activatable fibrinolysis inhibitor
Tc-99 MDP	Technetium-99 methylene diphosphonate
TCA	Tricyclic antidepressant
TCC	Total contact cast
TCR	T-cell receptor
TENS	Transcutaneous electrical nerve stimulation
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF	Transforming growth factor
TGF- α	Transforming growth factor-alpha
TGF- β	Transforming growth factor-beta
TGL	Triglycerides
Th	T helper
TIA	Transient ischemic attack
TIMP	Tissue inhibitors of metalloproteinase
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TNF- α	Tumour necrosis factor-alpha
tPA	Tissue-type plasminogen activator
TRP	Transient receptor potential
TRPC	Transmembrane receptor potential canonical
TRPV1	Transmembrane receptor potential Vanilloid-type
TXA2	Thromboxane A2
uPA	Urokinase
UT	University of Texas
VCAM	Vascular cell adhesion molecule
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

VEGF-R	Vascular endothelial growth factor receptor
VIP	Vasoactive intestinal peptide
VLA-1	Very late activating antigen-1
VOCC	Voltage operated calcium channels
VPT	Vibratory perception threshold
VSMC	Vascular smooth muscle cells
VTE	Venous thromboembolism
VV	Varicose veins
vWF	von Willebrand factor
XO	Xanthine oxidase

15 • Biomarkers in Vascular Disease

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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. 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 the '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.

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.

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 host as it responds to disease (e.g. Host Response Biomarkers). Disease progression biomarkers are very specific to 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. (Figure 15.1) When used in the correct clinical context both have discriminatory value.

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

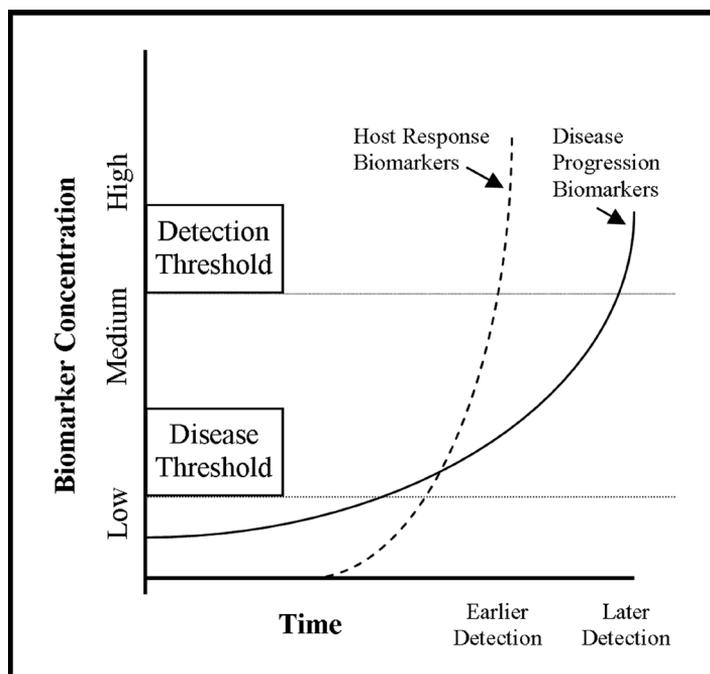


FIGURE 15.1: Comparison of Host Response and Disease Progression Biomarkers.

POTENTIAL VALUE OF BIOMARKERS IN VASCULAR DISEASE

Biomarkers have great potential to enhance all aspects of vascular care through AAA, carotid and peripheral vascular disease.

AAA development is likely to represent a product of genetic predisposition and environmental factors. AAA are characterized by local inflammation, matrix degradation and smooth muscle cell apoptosis.⁵ Once established, AAAs grow at a rate of 2.6mm/year (95% range -1.0 to 6.1mm/year).⁶ Generally this growth is insidious and asymptomatic until rupture. During this growth phase the active processes of AAA formation are on-going, both local and systemic cytokines and protein levels will be modified in response to or as a consequence of this pathology.

The principle 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 currently being rolled out 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.¹⁰ The current standard of care requires regular post-deployment surveillance to ensure the aneurysm sac is excluded from the circulation and adequately depressurized. 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. Current evidence from a Cochrane systematic review states that CEA for asymptomatic carotid stenosis reduces the risk of ipsilateral, or any stroke, by 30% over 3 years. 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 and asymptomatic patients interventions are still a matter of debate. There is growing evidence that stenosis alone is a poor guide. 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.

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 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 'omics' sciences used to trawl for novel candidates. (Table 15.2)

AAA BIOMARKERS

Candidate biomarkers have been studied based on present 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.

TABLE 15.1: Translating biomarker discovery from the laboratory to patients

Phase	Title	Explanation	Estimated numbers required (n =)
P1	Discovery	Exploratory studies to identify potential biomarkers	50
P2	Validation	Capacity of biomarker to discriminate between health & 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 [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]

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

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 numbers 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 aortic wall tissue for proteomic analysis as the availability of normal-aged aorta is limited and its method and timing of harvest and preservation will modify protein expression.

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 carboxy-terminal and aminoterminal ends of the precursor molecule are released. These two fragments represent candidate biomarkers for increased extracellular matrix remodelling and consequent 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 5-fold increase in AAA risk (OR 5.5; 95% CI, 2.0-13.8).¹¹

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 ⁴⁰
	Aminoterminal propeptide of type III procollagen (PIIINP)	Acceleration of AAA growth is reflected in serum PIIINP ($r = 0.55$)	Satta J. et al. 1997 ⁴¹
	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
Matrix degrading enzymes	Cystatin-C	Negative correlation with expansion rate ($r = -0.24$)	Lindholt J.S. et al. 2001 ⁴²
	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 ⁴³
	Thrombin-antithrombin III complex (TAT)	Elevated serum TAT levels are associated with large AAA diameter ($r = 0.57$)	Yamazumi K. et al. 1998 ⁴⁴

Related Process	Biomarker	Proposed significance	Reference
Inflammation	C-reactive protein (CRP)	CRP levels elevated in large AAAs	Norman P.E. et al. 2004 ²²
	Osteopontin (OPT)	Osteopontin level correlates with AAA presence and growth (r = 0.24)	Golledge J. et al. 2007 ²⁶
	IL-6	IL-6 level is independently associated with AAA and correlated with index diameter (r = 0.28)	Rohde L.E. et al. 1999 ²³
	Osteoprotegerin (OPG)	Osteoprotegerin associated with AAA growth	Moran C.S. et al. ²⁵
	Resistin	Serum resistin concentration is independently associated aortic diameter (r = 0.19)	Golledge J. et al. 2007 ²⁶
	Ig-G to <i>C. Pneumoniae</i>	Aneurysm progression correlated with IgG <i>C. Pneumoniae</i> infection (r = 0.45)	Lindholt et al. 2001 ⁴⁵

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.

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} Pooled analysis of these data has verified this finding,¹⁶ however the variability in the findings, sample handling and analysis highlights the principle 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 antitrypsin¹⁷ and p-elastase¹⁸ 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.

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. 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 principle 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.²⁰ This study went on to propose possible diagnostic cut-off values for AAA presence were D-Dimer to be utilized as a screening tool. In their population, a level $>400\text{ng/ml}$ for D-Dimer had an adjusted odds ratio (OR) of 12.1 (95% CI, 7.1-20.5) and $>900\text{ng/ml}$ 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 presence of an aneurysm.

Biomarkers to identify thrombosis are unlikely to translate into a universal clinical tool. The principle issue is that not all AAAs contain thrombus. Equally, in-situ thrombus is a dynamic substrate and findings from small studies may be a variable and not valid throughout the disease course.

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 (40-54mm), but no association with AAA expansion has been shown.²²

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 series $n < 100$). Also, plasma IL-6 has been correlated to aortic diameter in patients without AAA.²³ These findings are contributory to the understanding of AAA pathophysiology, supporting the role of inflammation and of macrophages in AAA progression. They lack the specificity to translate to 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 ($r = 0.24$).²⁴ 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 ($r = 0.2$).²⁵ 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 ($r = 0.19$, $P < 0.0001$).²⁶

BIOMARKERS OF AAA RUPTURE

Biomarkers capable of predicting AAA rupture would offer the greatest clinical value. Observing patients until 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.²⁷ 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.²⁸ In this cohort, a 4-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.

BIOMARKERS FOLLOWING ENDOVASCULAR REPAIR

Endovascular repair (EVAR) has become the preferred strategy for the management

of AAAs in many centers. 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.²⁹ The principle problem with any biomarker will be its ability to discriminate between benign (type II) endoleaks and more significant (type I or type III) endoleaks.

BIOMARKERS OF CAROTID PLAQUE STABILITY

One 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 has 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 specifically at the carotid plaque tissue to identify possible candidates that would be particular 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

TABLE 15.4: Substrates explored as possible biomarkers for carotid artery stenosis

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 ³¹
	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 ³¹
	IL-18	IL-18 expression found to be >3x greater in symptomatic plaques than asymptomatic	Mallat Z. et al. 2001 ⁴⁶
	IL-6	Serum IL-6 elevated in symptomatic stenosis compared to asymptomatic	Koutouzis M. et al. 2009 ³³
	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 ⁴⁷
	CD-36	Soluble CD36 elevated in patients with echolucent plaques vs. echogenic plaques	Handberg A. et al. 2008 ⁴⁸
Lipid Accumulation	Lipoprotein-associated phospho-lipase A2(Lp-PLA2)	Symptomatic carotid plaques are characterised by elevated Lp-PLA2	Mannheim D. et al. 2008 ³⁴
Apoptosis	Annexin V	Annexin V uptake associated with plaque instability	Keiselaer, B.L et al. 2004 ³⁵
Thrombosis	Tissue plasminogen activator (t-PA)	Transient increase in t-PA gene expression associated with plaque instability	Sayed S. et al 2009 ³⁶
	Fibrinogen	Elevated fibrinogen is associated with carotid disease progression	Sabeti S. et al. 2005 ³⁷
	Plasminogen activator inhibitor-1 (PAI-1)	Transient increase in PAI-1 gene expression associated with plaque instability	Sayed S. et al 2009 ³⁶
Proteolysis	MMP-9	MMP-9 level correlates with plaque instability. MMP-9 > 607ng/ml best predicted presence of unstable plaque (OR 19.2; 95% CI 3.9-94.2)	Alvarez B. et al. 2004 ³⁹

associated with plaque vulnerability. These include inflammation, lipid accumulation, apoptosis, thrombosis, angiogenesis and proteolysis.³⁰ These changes are connected to the morphological characteristics of an unstable plaque. The search for a biomarker has focused on these processes.

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 morphological features of rapidly progressive carotid atherosclerosis.³¹ CRP has also been shown to predict stroke risk in a healthy elderly population (Framingham Study).³² 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.³³

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.³⁴ No serum studies have been performed on this possible biomarker.

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. This pilot study utilized exogenous radiolabelling and only examined 4 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.³⁵

Thrombosis

Thrombotic activity on 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.³⁶ These include t-PA and

plasminogen activator inhibitor-1. To date no study has examined the possible role of these factors as biomarkers.

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 compared to the lower quartile. Fibrinogen level at follow up was also shown to be associated with progressive disease ($P = 0.004$).³⁷

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.³⁸ 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.³⁹

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 to a clinically applicable tool. The initial proteomic fervor 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 to 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 the potential can be fully realized, 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.

FUTURE WORK

The future of biomarker discovery lies in comparative proteomics combined with innovative bioinformatics and mathematical modeling. This review has demonstrated a large number of small independent scientific groups generating exciting and unique findings. The principle 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.

CONCLUSION

Biomarkers will 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 advancements in mass spectrometry and proteomic techniques combined with worldwide interest in this discovery science, a significant discovery cannot be far away. In 10 years time 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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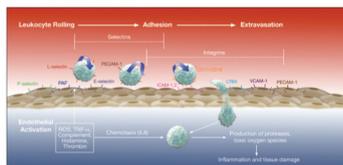
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MECHANISMS OF VASCULAR DISEASE

Edited by Robert Fitridge and Matthew Thompson

Chapter 1: Endothelium **Chapter 2:** Vascular smooth muscle structure and function **Chapter 3:** Atherosclerosis **Chapter 4:** Mechanisms of plaque rupture **Chapter 5:** Current and emerging therapies in atheroprotection **Chapter 6:** Molecular approaches to revascularisation in peripheral vascular disease **Chapter 7:** Biology of restenosis and targets for intervention **Chapter 8:** Vascular arterial haemodynamics **Chapter 9:** Physiological haemostasis **Chapter 10:** Hypercoagulable states **Chapter 11:** Platelets in the pathogenesis of vascular disease and their role as a therapeutic target **Chapter 12:** Pathogenesis of aortic aneurysms **Chapter 13:** Pharmacological treatment of aneurysms **Chapter 14:** Aortic dissection and connective tissue disorders **Chapter 15:** Biomarkers in vascular disease **Chapter 16:** Pathophysiology and principles of management of vasculitis and Raynaud's phenomenon **Chapter 17:** SIRS, sepsis and multiorgan failure **Chapter 18:** Pathophysiology of reperfusion injury **Chapter 19:** Compartment syndrome **Chapter 20:** Pathophysiology of pain **Chapter 21:** Postamputation pain **Chapter 22:** Treatment of neuropathic pain **Chapter 23:** Principles of wound healing **Chapter 24:** Pathophysiology and principles of varicose veins **Chapter 25:** Chronic venous insufficiency and leg ulceration: Principles and vascular biology **Chapter 26:** Pathophysiology and principles of management of the diabetic foot **Chapter 27:** Lymphoedema – Principles, genetics and pathophysiology **Chapter 28:** Graft materials past and future **Chapter 29:** Pathophysiology of vascular graft infections



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