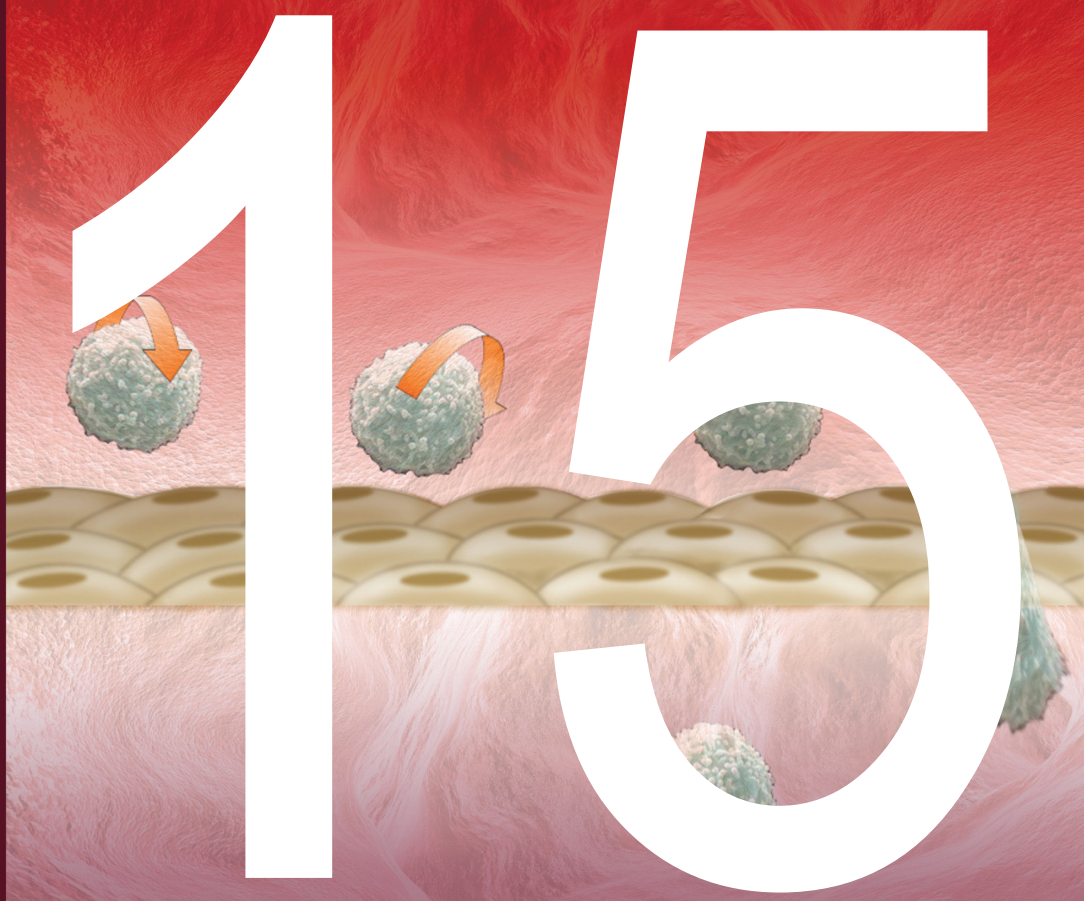
Mechanisms of Vascular Disease:

A Reference Book for Vascular Specialists



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A Reference Book for Vascular Specialists

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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	Adrenocorticotropic 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
β2-GPI	β2-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	Calcitonic 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-1α	Interleukin-1 alpha
IL1-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 *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
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
TαCE	TNFα 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]

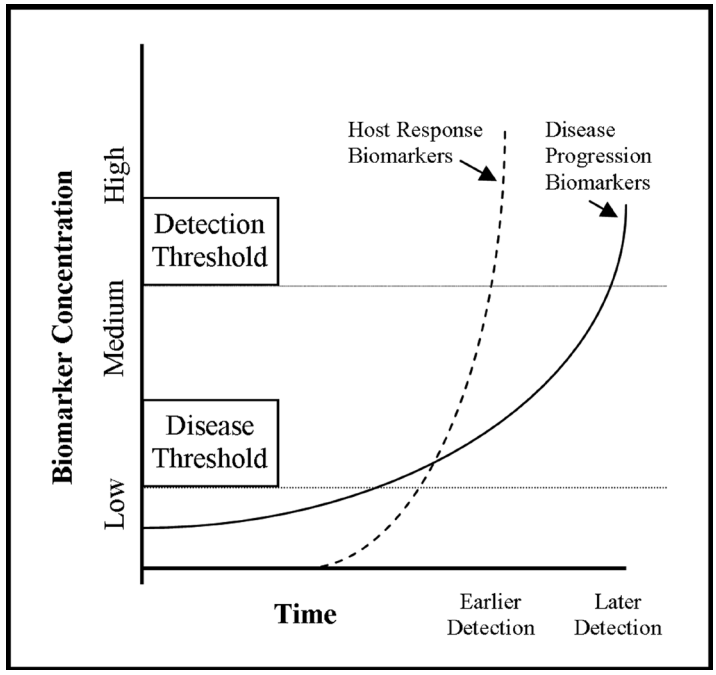

FIGURE 15.1: Comparison of Host Response and Disease Progression Biomarkers.

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 asses 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 *C. Pneumoniae*	Aneurysm progression correlated with IgG *C. Pneumoniae* 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 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.

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 >400ng/ml for D-Dimer had an adjusted odds ratio (OR) of 12.1 (95% CI, 7.1-20.5) and >900ngml represented an OR of 24.7 ((%% 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 1 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 assymptomatic	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 assymptomatic 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 tail 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.

KEY REFERENCES

Vasan RS. Biomarkers of vascular disease: Molecular basis and practical considerations. *Circulation* 2006; **113**: 2335–2362.

Golledge J, Tsao PS, Dalman RL, Norman PE. Circulating markers of abdominal aortic aneurysm presence and progression. *Circulation* 2008; **118**: 2382–2392.

Hermus L, Lefrandt JD, Tio RA, Breek J-C, Zeebregts CJ. Carotid plaque formation and serum biomarkers. *Atherosclerosis* 2010; [in press].

Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MSV, Go AS, Harrell FE, Howard BV, Howard VJ, P.Y. H, Kramer CM, McConnell JP, Normand S-LP, O'Donnell CJ, Smith SJ, Wilson PWF. Criteria for evaluation of novel markers of cardiovascular risk. *Circulation* 2009; **119**: 2408–2416.

Nordon IM, Brar R, Hinchliffe RJ, Cockerill GW, Loftus IM, Thompson MM. The role of proteomic research in vascular disease. *J Vasc Surg* 2009; **49**: 1602–1612.

REFERENCES

1. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; **69**(1): 89–95.
2. Fox N, Growdon JH. Biomarkers and surrogates. *Neuro Rx* 2004; **1**: 181.
3. Katus HA, Remppis A, Neumann FJ, Scheffold T, Diederich KW, Vinar G, Noe A, Matern G, Kubler W. Diagnostic efficiency of Troponin T measurements in acute myocardial infarction. *Circulation* 1991; **83**: 902–912.
4. Babuin L, Jaffe AS. Troponin: the biomarker of choice for the detection of cardiac injury. *CMAJ* 2005; **173**(10): 1191–1202.
5. Weintraub NL. Understanding abdominal aortic aneurysm. *N Eng J Med* 2009; **361**(11): 1114–1116.
6. Brady AR, Thompson SG, Fowkes FGR, Greenhalgh RM, Powell JT. Abdominal aortic aneurysm expansion: risk factors and time intervals for surveillance. *Circulation* 2004; **110**(1): 16–21.
7. Bown MJ, Sutton AJ, Bell PR, Sayers RD. A meta-analysis of 50 years of ruptured abdominal aortic aneurysm repair. *Br J Surg* 2002; **89**: 714–730.

8. Nicholls SC, Gardner JB, Meissner MH, Johansen HK. Rupture in small abdominal aortic aneurysms. *J Vasc Surg* 1998; **28**(5): 884–888.
9. Lederle FA, Johnson GR, Wilson SE, Ballard DJ, Jordan WJ, Blebea J, Littooy FN, Freischlag JA, Bandyk D, Rapp JH, Salam AA. Rupture rate of large abdominal aortic aneurysms in patients refusing or unfit for elective repair. *JAMA* 2002; **287**(22): 2968–2972.
10. Eugster T, Huber A, Obeid T, Schwegler I, Gurke L, Stierli P. Aminoterminal propeptide of type III procollagen and matrix metalloproteinases-2 and -9 failed to serve as serum markers for abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 2005; **29**(4): 378–382.
11. Zweers MC, Peeters AC, Graafsma S, Kranendonk S, van der Vliet JA, den Heijer M, Schalkwijk J. Abdominal aortic aneurysm is associated with high serum levels of tenascin-X and decreased aneurysmal tissue tenascin-X. *Circulation* 2006; **113**(13): 1702–1707.
12. Lindholt JS, Heickendorff L, Henneberg EW, Fasting H. Serum elastin peptides as a predictor of expansion of small abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 1997; **14**(1): 12–16.
13. Lindholt JS, Ashton HA, Heickendorff L, Scott RA. Serum elastin peptides in the preoperative evaluation of abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2001; **22**(6): 546–550.
14. Lindholt JS, Vammen S, Fasting H, Henneberg EW, Heickendorff L. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms. A preliminary study. *Eur J Vasc Endovasc Surg* 2000; **20**: 281–285.
15. McMillan WD, Pearce WH. Increased levels of metalloproteinase-9 are associated with abdominal aortic aneurysms. *J Vasc Surg* 1999; **29**(2): 122–127.
16. Takagi H, Manabe H, Kawai N, Goto S-N, Umemoto T. Circulating matrix metalloproteinase-9 concentrations and abdominal aortic aneurysm presence: a meta-analysis. *Interact Cardiovasc Thorac Surg* 2009; **9**(3): 437–440.
17. Vega de Ceniga M, Esteban M, Quintana JM, Barba A, Estallo L, de la Fuente N, Viviens B, Martin-Ventura JL. Search for serum biomarkers associated with abdominal aortic aneurysm growth – pilot study. *Eur J Vasc Endovasc Surg* 2009; **37**(3): 297–299.
18. Lindholt JS, Jorgensen B, Klitgaard NA, Henneberg EW. Systemic levels of Cotinine and Elastase, but not pulmonary function, are associated with the progression of small abdominal aortic aneurysms *Eur J Vasc Endovasc Surg* 2003; **26**: 418–422.
19. Al-Barjas HS, Ariens R, Grant P, Scott JA. Raised plasma fibrinogen concentration in patients with abdominal aortic aneurysm. *Angiology* 2006; **57**(5): 607–614.
20. Golledge J, Muller R, Clancy P, McCann M, Norman PE. Evaluation of the diagnostic and prognostic value of plasma D-Dimer for abdominal aortic aneurysm. *Eur Heart J* 2010; [in press].
21. Moroz P, Le MT, Norman PE. Homocysteine and abdominal aortic aneurysms. *ANZ J Surg* 2007; **77**(5): 329–332.

22. Norman PE, Spencer CA, Lawrence-Brown MM, Jamrozik K. C-reactive protein levels and the expansion of screen detected abdominal aortic aneurysms in men. *Circulation* 2004; **110**(7): 862–866.
23. Rohde LE, Arroyo LH, Rifai N, Creager MA, Libby P, Ridker PM, Lee RT. Plasma concentrations of interleukin-6 and abdominal aortic diameter among subjects without aortic dilatation *Arterioscler Thromb Vasc Biol* 1999; **19**(7): 1695–1699.
24. Golledge J, Muller J, Shephard N, Clancy P, Smallwood L, Moran C, Dear AE, Palmer LJ, Norman PE. Association between osteopontin and human abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol* 2007; **27**(3): 655–660.
25. Moran CS, McCann M, Karan M, Norman PE, Ketheesan N, Golledge J. Association of osteoprotegerin with human abdominal aortic aneurysm progression. *Circulation* 2005; **111**(23): 3119–3125.
26. Golledge J, Clancy P, Jamrozik K, Norman PE. Obesity, adipokines, and abdominal aortic aneurysm; Health in Men study. *Circulation* 2007; **116**: 2275–2279.
27. The UK Small Aneurysm Trial Participants. Smoking, lung function and the prognosis of abdominal aortic aneurysm. *Eur J Vasc Endovasc Surg* 2000; **19**: 636–642.
28. Wilson WRW, Anderton M, Choke E, Dawson J, Loftus IM, Thompson MM. Elevated plasma MMP1 and MMP9 are associated with abdominal aortic aneurysm rupture. *Eur J Vasc Endovasc Surg* 2008; **35**(5): 580–584.
29. Sangiorgi G, D'Averio R, Mauriello A, Bondio M, Pontillo M, Castelvecchio S, Trimarchi S, Tolva V, Nano G, Rampoldi V, Spagnoli LG, Inglese L. Plasma levels of metalloproteinases-3 and -9 as markers of successful abdominal aortic aneurysm exclusion after endovascular graft treatment. *Circulation* 2001; **104**(12 Suppl 1): I288–295.
30. Hermus L, Lefrandt JD, Tio RA, Breek J-C, Zeebregts CJ. Carotid plaque formation and serum biomarkers. *Atherosclerosis* 2010; [in press].
31. Schillinger M, Exner M, Mlekusch W, Sabeti S, Amighi J, Nikowitsch R, Timmel E, Kickinger B, Minar C, Pones M, Lalouschek W, Rumpold H, Maurer G, Wagner O, Minar E. Inflammation and carotid artery – risk for atherosclerosis study (ICARAS). *Circulation* 2005; **111**(17): 2203–2209.
32. Rost NS, Wolf PA, Kase CS, Kelly-Hayes M, Silbershatz H, Massaro JM, D'Agostino RB, Franzblau C, Wilson PWF. Plasma concentration of C-reactive protein and risk of ischaemic stroke and transient ischaemic attack: the Framingham study. *Stroke* 2001; **32**(11): 2575–2579.
33. Koutouzis M, Rallidis LS, Peros G, Nomikos A, Tzavara V, Barbatis C, Andrikopoulos V, Vassiliou J, Kyriakides ZS. Serum interleukin-6 is elevated in symptomatic carotid bifurcation disease. *Acta Neurol Scand* 2009; **119**(2): 119–125.
34. Mannheim D, Herrmann J, Versari D, Gossl M, Meyer FB, McConnell JP, Lerman LO, Lerman A. Enhanced expression of Lp-PLA2 and lysophosphatidylcholine in symptomatic carotid atherosclerotic plaques. *Stroke* 2008; **39**(5): 1448–1455.

35. Kietselaer BL, Reutelingsperger CP, Heidendal GA, Daemen MJ, Mess WH, Hofstra L, Narula J. Noninvasive detection of plaque instability with use of radiolabelled annexin A5 in patients with carotid artery stenosis. *N Engl J Med* 2004; **350**(14): 1472–1473.
36. Sayed S, Cockerill GW, Torsney E, Poston R, Thompson MM, Loftus IM. Elevated tissue expression of thrombomodulatory factors correlates with acute symptomatic carotid plaque phenotype. *Eur J Vasc Endovasc Surg* 2009; **38**(1): 20–25.
37. Sabeti S, Exner M, Mlekusch W, Amighi J, Quehenberger P, Rumpold H, Maurer G, Minar E, Wagner O, Schillinger M. Prognostic impact of fibrinogen in carotid atherosclerosis: nonspecific indicator of inflammation or independent predictor of disease progression? *Stroke* 2005; **36**(7): 1400–1404.
38. Loftus IM, Naylor AR, Goodall S, Crowther M, Jones L, Bell PR, Thompson MM. Increased matrix metalloproteinase-9 activity in unstable carotid plaques. A potential role in acute plaque disruption. *Stroke* 2000; **31**(1): 40–47.
39. Alvarez B, Ruiz C, Chacon P, Alvarez-Sabin J, Matas M. Serum values of metalloproteinase-2 and metalloproteinase-9 as related to unstable plaque and inflammatory cells in patients with greater than 70% carotid artery stenosis. *J Vasc Surg* 2004; **40**(3): 469–475.
40. Nakamura M, Tachieda R, Niinuma H, Ohira A, Endoh S, Hiramori K, Makita S. Circulating biochemical marker levels of collagen metabolism are abnormal in patients with abdominal aortic aneurysm. *Angiology* 2000; **51**(5): 385–392.
41. Satta J, Haukipuro K, Kairaluoma MI, Juvonen T. Aminoterminal propeptide of type III procollagen in the follow-up of patients with abdominal aortic aneurysms. *J Vasc Surg* 1997; **25**(5): 909–915.
42. Lindholt JS, Erlandsen EJ, Henneberg EW. Cystatin C deficiency is associated with the progression of small abdominal aortic aneurysms. *Br J Surg* 2001; **88**(11): 1472–1475.
43. Halazun KJ, Bofkin KA, Asthana S, Evans C, Henederson M, Spark JI. Hyperhomocysteinaemia is associated with the rate of abdominal aortic aneurysm expansion. *Eur J Vasc Endovasc Surg* 2007; **33**(4): 391–394.
44. Yamazumi K, Ojiro M, Okumura H, Aikou T. An activated state of blood coagulation and fibrinolysis in patients with abdominal aortic aneurysm. *Am J Surg* 1998; **175**(4): 297–301.
45. Lindholt JS, Ashton HA, Scott RA. Indicators of infection with chlamydia pneumoniae are associated with expansion of abdominal aortic aneurysms. *J Vasc Surg* 2001; **34**(2): 212–215.
46. Mallat Z, Corbaz A, Scoazec A, Besnard S, Leseche G, Chvatchko Y, Tedgui A. Expression of interleukin18 in human atherosclerotic plaques and relation to plaque instability. *Circulation* 2001; **104**(14): 1598–1603.
47. Sugioka K, Naruko T, Matsumara Y, Shirai N, Hozumi T, Yoshiyama M, Ueda M. Neopterin and atherosclerotic plaque instability in coronary and carotid arteries *J Atheroscler Thromb* 2010; [in press].

48. Handberg A, Skjelland M, Miichelsen AE, Sagen EL, Krohg-Sorensen K, Russell D, Dahl A, Ueland T, Oie E, Aukrust P, Halvorsen B. Soluble CD36 in plasma is increased in patients with symptomatic atherosclerotic carotid plaques and is related to plaque instability. *Stroke* 2008; **39**(1): 3092–3095.

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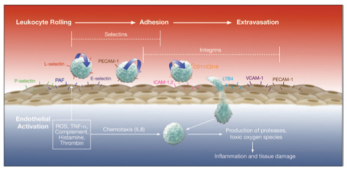

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Mechanisms of Vascular Disease

Edited by Robert Fitridge and Matthew Thompson

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