plaques. Growth factors and inflammatory cytokines are potential serum biomarkers to identify patients at risk of stroke.

Materials and methods Immunohistochemistry and quantitative PCR (Q-PCR) were used to establish localisation and expression of angiogenic growth factors within carotid endarterectomy specimens from symptomatic and asymptomatic patients. Stable or unstable microvessels were distinguished by CD31 and CD105 staining. Systemic levels of circulating angiogenic growth factors and inflammatory cytokines were measured in venous blood using Bio-Plex arrays.

Results Hepatocyte growth factor (HGF) and its receptor c-Met were detected in CD31-positive endothelia, and α-SMA-positive cells, respectively. Q-PCR demonstrated upregulation of the angiogenic factors CD105, HGF ($p<0.001$) and c-Met ($p=0.011$) in symptomatic versus asymptomatic plaques. A significantly greater neovessel density was detected in symptomatic plaques ($p=0.042$), associated with elevated expression of HGF and c-Met. Suspension arrays demonstrated elevated HGF ($p=0.002$) and decreased platelet-derived growth factor (PDGF; $p=0.036$) serum levels in symptomatic versus asymptomatic patients. Twenty-seven cytokines were examined; seven endarterectomy patients demonstrated significantly increased levels in comparison with controls. No differences were observed between preoperative and postoperative serum.

Discussion Plaque instability may be mediated by HGF-induced formation of microvessels, and decreased PDGF. We will investigate the effects of inflammatory cytokines with a view to comparing symptomatic versus asymptomatic patients. Targeting surgery to those who will benefit would eliminate unnecessary risk.

BAS/BSCR3 PARTIAL RECONSTRUCTION OF MYOCARDIAL METABOLIC PATHWAYS FOLLOWING ANALYSIS OF PERIPHERAL SERUM USING METABOLOMICS IN EARLY CARDIAC ISCHAEMIA

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Cardiac metabolism and cardiac function are inextricably linked, with changes in cardiac metabolism during cardiac ischaemia contributing to the development of cardiac arrhythmias. Using metabolomics, we aim to identify metabolite changes occurring during cardiac ischaemia through analysis of peripheral serum to reconstruct myocardial metabolic pathways that contribute to the development of cardiac arrhythmias. Peripheral venous samples from 25 patients (and 20 patients in a validation cohort) were analysed in an untargeted fashion using LC-MS following cardiac ischaemia induced by transient coronary artery occlusion during PCI at baseline, 1 and 5 min. Following validation, 99 and 126 metabolite peaks were significantly different at 1 min and 5 min after coronary occlusion compared with baseline ($p<0.05$). Predominantly metabolic pathways involving lipids were perturbed with changes in diacylglycerols (DG), lysophophatidylcholines (LPC), phosphatidylcholine (PC) and free fatty acids (FFA). Myocardial metabolic pathways involving the synthesis of PC from DG and their subsequent breakdown by phospholipase A2 into LPC and FFA such as arachidonic acid (AA) stimulating the oxidation of adrenaline to form the arrhythmogenic metabolite adrenochrome were reconstructed. We are able to reconstruct metabolic pathways involving lipid metabolism within the myocardium during cardiac ischaemia through analysis of the peripheral serum using metabolomics. Our unbiased approach has identified metabolic pathways involved in the production and release of metabolites with pro-arrhythmic properties (AA, LPC and adrenochrome) and metabolites with anti-arrhythmic properties (omega-3 fatty acids: eicosapentaenoic and docosahexanoic acid). This suggests that arrhythmogenesis may be a delicate balance between the endogenous formation of pro-arrhythmic and anti-arrhythmic metabolites.

BAS/BSCR4 THE ROLE OF RECEPTOR ACTIVATOR OF NUCLEAR FACTOR κ-B LIGAND AND ITS DECOY RECEPTOR, OSTEOPROTEGERIN IN VASCULAR CALCIFICATION

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Charcot neuroarthropathy (CN) is characterised by pathological foot fractures and osteopenia in patients with diabetes, often resulting in debilitating deformity. Paradoxically, these patients show evidence of medial vascular calcification. Recently, accentuated signalling of the receptor activator of nuclear factor κ-B ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG) have been implicated in the development of diabetic CN. This study aims to investigate the role of RANKL and OPG signalling in vascular calcification in patients with diabetes and CN, compared with healthy controls. RANKL and OPG serum levels were measured using ELISA in 12 patients with CN, 10 diabetic patients and five healthy controls. Serum RANKL and OPG levels were elevated in acute CN and in diabetic patients compared with healthy controls ($p<0.05$). Immunohistochemistry identifies upregulation of RANKL in calcified tibial arterial sections versus non-calcified controls. Human vascular smooth muscle cells (hVSMC) were grown in osteogenic conditions, as our in vitro model of calcification. When hVSMCs were treated with serum from patients with diabetes and CN, we demonstrated (i) accelerated mineralisation of hVSMC, confirmed by Alizarin red staining, and elevated alkaline phosphatase activity compared with control cells and (ii) reduced mineralisation when co-incubated with OPG. These findings demonstrate that RANKL/OPG signalling is modulated in diabetic and CN patients. Furthermore, serum from these patients accelerates vascular calcification in vitro, an effect attenuated by OPG treatment. These are the first human data implicating RANKL/OPG in diabetic vascular calcification and suggest that OPG/anti-RANKL therapy may be a potential target in combating disease progression.

BAS/BSCR5 TILRR POTENTIATES INTERLEUKIN-1-INDUCED ANTI-APOPTOSIS

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The pathogenesis of atherosclerosis is determined, in part, by inflammatory responses, induced through members of the Toll-like and interleukin (IL)-1 receptor family and controlled by NF-κB. We have identified a novel IL-1RI co-receptor, TILRR, which enhances the IL-1-induced activation of NF-κB by increasing receptor-expression enhanced recruitment of the MyD88 adaptor during activation.[1] Here we investigate the role of TILRR on the anti-apoptotic effects controlled by NF-κB. The results showed that TILRR reduces caspase-3 activity and enhances IL-1-induced phosphorylation of AKT. Alanine scanning mutagenesis of the IL-1 receptor TIR domain demonstrated that TILRR amplifies inflammatory responses through the membrane proximal part of the cytoplasmic portion, the so-called box 1, while the anti-apoptotic response is regulated through the central portion of the TIR domain, the so-called box 2. Similarly,

alanine scanning mutagenesis of the TILRR core protein, targeting conserved residues with predicted effects on secondary structure, demonstrated distinct control of inflammatory and anti-apoptotic intermediates. These results demonstrate that TILRR amplification involves selective control of NF-κB-regulated inflammatory and anti-apoptotic responses, and are consistent with induction of discrete conformational changes in the IL-1 receptor complex through TILRR association.

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BAS/BSCR6 α-KETOGLUTARATE: BIOLOGICAL EFFECTS OF A NOVEL BIOMARKER OF HEART FAILURE

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Heart failure is often associated with renal impairment (cardiorenal syndrome). Using a metabolomics approach, our group identified α-ketoglutarate as a novel metabolite that was significantly elevated in patients with heart failure.[1] It was as strongly associated with heart failure as the 'gold-standard' biomarker brain natriuretic peptide. α-Ketoglutarate is a citric acid cycle intermediate, central in cardiac energy production. It is the ligand of GPR99, a G-protein coupled receptor mostly expressed in the kidney.[2] In HEK 293 cells, GPR99 acted through a Gq-mediated pathway to increase intracellular calcium. Importantly, we found that the GPR99 receptor is present in neonatal rat cardiomyocytes (NRCM). We tested the expression of 84 genes previously known as regulators of angiogenesis in NRCM treated with 1 mM α-ketoglutarate. Using real-time PCR, we found significant increase in the expression of VEGF receptor-1 and placental growth factor, suggesting a possible effect of α-ketoglutarate in the regulation of angiogenesis and growth. These findings show that binding of α-ketoglutarate to the GPR99 receptor in the heart leads to activation of the Gq pathway and causes upregulation of VEGFR1 and placental growth factor. This might have a role in vascular adaptation to hypertrophy. In addition, in vivo animal studies have previously shown that protein kinase C has a role in renal dysfunction. This leads to the novel and testable hypothesis that α-ketoglutarate also contributes to the development of the cardiorenal syndrome.

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BAS/BSCR7 DEMONSTRATION OF GENE EXPRESSION WITHIN A THROMBUS: FURTHER REGULATION OF THE HAEMOSTATIC RESPONSE

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Vascular injury and plaque rupture activate the haemostatic response, resulting in the formation of a thrombus comprising platelets, red cells and leucocytes, incorporated into a mesh of plasma proteins. Whether cells within the thrombus act simply as structural and secretory components, or have a more active role involving gene expression is unclear. To investigate this, thrombi were produced at 37°C in a Chandler loop, using re-calcified citrated blood from healthy donors (n=6). The thrombi were removed after 2, 4 and 6 h of rotational incubation, and homogenised to extract total RNA. Following in vitro transcription, samples were hybridised to Illumina WG6 beadchips, and data normalised using Illumina Beadstudio. Differences in gene expression were assessed using a Student t test, applying fdr2d correction to eliminate false positives (R Bioconductor). Genes which demonstrated significant (>twofold) time-dependent increases included genes encoding proteins involved in chemotaxis (IL8, CCL2, CXCL1, CXCL2, CXCR4), cell adhesion (ITGAV, ITGA5, ITGB1, ALCAM), regulation of coagulation (THBD, PLAU, SERPINE1, ANXA5), wound healing (TGM2, ENDG, SPP1, LAMB3, PTGS2, TNFAIP6) and regulatory transcription factors (FOS, BMP6, IRAK2, KLRG1, PPARG). Whereas initiation of thrombosis is driven by plasma proteins and facilitated by the platelet surface, this study provides evidence that thrombus resolution may be driven by changes in gene expression within the thrombus that regulate the haemostatic response, thrombus growth and facilitate wound healing. This finding could have implications for individuals at risk of plaque rupture, where variation in gene expression may affect not just the formation of an occlusive thrombus but also the rate of resolution.

BAS/BSCR8 DOES MACROPHAGE FOAM CELL FORMATION PROMOTE EXTRACELLULAR MATRIX FORMATION OR DEGRADATION? A GENOMIC STUDY

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Foam cell macrophage (FCM) formation is an early event in atherosclerosis that precedes both fibrous cap development and subsequent cap rupture; FCM have been implicated in both events. To understand this further we compared the transcriptomes of FCM and non-foamy macrophages (NFM) produced in subcutaneous sponges implanted into fat-fed ApoE null or C57Bl6 mice, respectively (n=4 each). RNA samples of high quality by A260/280>2 were compared on Illumina bead chips. Differential expression was classified as significant ($p<0.05$ after Bonferroni correction for multiple testing) or suggestive ($p<0.001$ unadjusted). 62 genes were significantly upregulated and 59 downregulated in FCM compared with NFM. A total of 370 and 381 genes were upregulated and downregulated using the more relaxed criterion. Fold changes confirmed by quantitative RT-PCR (n=5—7) included upregulation of cathepsin C (15×), cathepsin E (19×), matrix metalloproteinase (MMP)-2 (18×) and MMP-23 (22×) but also upregulation of tissue inhibitor of matrix metalloproteinase (TIMP)-2 (4×) and TIMP-3 (8×) and downregulation of MMP-13 (5×). Surprisingly, several matrix proteins were significantly upregulated, including collagen Iα1 (55×) and VIα1 (31×), osteonectin (72×) and biglycan (19×), although thrombospondin declined (2×). Hence our genomic analysis demonstrated changes that could lead to both matrix degradation and deposition. Ingenuity pathway analysis implicated activation of LXR/RXR in FCM, in agreement with other literature, and highlighted responses to platelet-derived growth factor and transforming growth factor β. The hypothesis that interaction of these pathways accounts for the ambiguous behaviour of FCM in matrix remodelling deserves further investigation.

Contributors A C Thomas, J L Johnson and W J Eijgelaar are contributed equally.