

## **A human arterial transcriptomic signature predicts major adverse cardiac events and identifies novel, redox-related therapeutic targets within the vascular wall**

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**Background:** The transcriptomic profile of the human vascular wall is implicated in a range of pathologies. RNA sequencing technologies allow for interrogation of gene expression patterns that are associated with clinical outcomes and can guide future research and drug development.

**Purpose:** To apply discovery network transcriptomics to internal mammary arteries (IMAs) obtained from patients undergoing cardiac surgery, in order to identify redox-related molecular pathways within the vascular wall that can be treated therapeutically.

**Methods:** Arm 1 included 377 patients in whom segments of IMA were used for *ex-vivo* quantification of NADPH-stimulated superoxide production by lucigenin-enhanced chemiluminescence. Arm 2 included 205 patients in whom bulk RNA sequencing was performed in RNA isolated from IMA, and the WGCNA package used for the analyses. The association with future incidence of major adverse cardiac events (MACE: cardiovascular death, non-fatal myocardial infarction, and stroke) was assessed using Cox regression models (adjusted for age, sex, hypertension, dyslipidaemia, diabetes mellitus, body mass index, smoking, and plasma TNFa).

**Results:** Over a median follow-up of 4.84 years [IQR: 2.03-7.14], 38 (11.2%) MACE occurred in Arm 1. High arterial NADPH-stimulated superoxide was independently associated with MACE risk (Adj. HR[95%CI]: 2.62 [1.13–6.07] high group,  $p=0.02$ ). Unsupervised transcriptomic analysis in Arm 2 allowed identification of 10 coexpressed gene ‘modules’. Eigengenes summarising modular coexpression signatures were then correlated with NADPH-stimulated superoxide revealing the red module (**a**) as the most significant ( $\rho=0.19$ ,  $p=0.01$ ). In survival analysis the red module showed significant correlation with MACE (Adj. HR[95%CI]: 1.40 [1.00–1.95] per SD,  $p=0.04$ ). For an optimal cut-off, patients with high eigengene values for the red module showed a 4-fold higher risk of MACE (**b**), and significantly higher arterial oxidative stress (**c**). Enrichment analysis (performed with Enrichr) of genes in the red module revealed ‘Electron Transport Chain’, ‘Oxidative phosphorylation’, ‘Striated Muscle Contraction Pathway’, and ‘Glycolysis and Gluconeogenesis’ amongst the top enriched pathways (**d**).

**Conclusion:** We present for the first time a novel human arterial transcriptomic signature reflecting changes in redox state, which identifies long-term cardiovascular risk. Targeting pathways in the vasculature related with the mitochondrial electron transport chain, the contractile mechanism, or glucose metabolism may lead to the development of novel therapeutics in cardiovascular disease.

