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MicroRNA-15A is a Potential Circulating Biomarker of Abdominal Aortic Aneurysm with Both Diagnostic and Prognostic Properties

Abdominal Aortic Diseases

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Introduction: microRNAs (miRNAs) have been identified as post-transcriptional inhibitors of gene expression, thought to fine tune the translational output of target mRNAs. We explored the diagnostic and prognostic value of miRNAs in abdominal aortic aneurysm (AAA), a disease for which currently no established plasma biomarker exists.
Methods: Using a miRNA OpenArray platform, we profiled the expression of 754 human miRNAs in plasma from 195 patients with AAA and 184 matched non-aneurysmal control individuals with a similar cardiovascular risk profile from the Dutch Second Manifestations of Arterial disease (SMART) cohort. To evaluate the potential prognostic value of discovered miRNA candidates we used a separate longitudinal patient cohort, consisting of 31 AAA patients who underwent computed tomography AAA diameter measurements and plasma sampling at baseline ($n = 31$) and after six months ($n = 31$) and 12 months ($n = 11$) of follow up.
Results: Among 12 differentially expressed miRNAs, miR-15a (4.4 fold; $p = .041$) and -659 (6.8 fold; $p = .037$) were the most significantly upregulated miRNAs, whereas miR-1183 (0.16 fold; $p = .007$) and -192 (0.29 fold; $p = .013$) were the most significantly downregulated ones.

Further, expression of miR-15a in plasma was significantly correlated ($n = 36$, $p = .019$; R^2=0.15) with aortic diameter increase after six months. Among known targets of miR-15a are *Reck* and *Fgf2*, both known to be involved in human AAA pathogenesis. Upon administration of anti-miR- 15a *in vivo* in an elastase induced mouse model of AAA, the observed aortic diameter increase at day 7 was significantly lower compared with mice treated with scrambled control (1.22- *vs.* 1.49 fold; $p = .008$; $n = 4+5$). In a separate mouse AAA model (AngII infusion), a similar trend could be observed at several timepoints - days 7/14/28.
Conclusion: Our findings suggest that miR-15a is a potential diagnostic and prognostic AAA biomarker. Through *in vivo* studies and based on its target profile, we show that miR-15a seems to be involved in AAA pathogenesis and is thus a potential treatment strategy. *In vitro* studies are currently ongoing to better elucidate the underlying mechanisms behind these observations.
Disclosure: Nothing to disclose

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Inflammation and Microcalcification are Processes That May be Non-Invasively Identified Via Molecular Imaging as Markers for Vulnerable Plaques

Vascular Imaging

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Introduction: Inflammation and microcalcification are established hallmarks of vulnerable plaque formation. Ga-68-Pentixafor and F-18-NaF have been proposed as molecular probes to specifically target the aforementioned processes, however they have mostly been assessed in separate retrospective clinical studies. Here, their ability to correctly identify the distinct mechanism is assessed in an unitary atherosclerotic mouse model *via* preclinical µPET/CT.
Methods: ApoE knockout mice were fed a western type diet for 12 weeks in order to develop early stage atherosclerosis. To establish a mouse model that develops intimal calcification, the feed of one group (calcification group) was switched to a Warfarin + Vitamin K1 supplemented diet for an additional 12 weeks. Mice on Warfarin develop lethal bleedings, a side effect that can be avoided by adding vitamin K1 to the feed (1). For the inflammation group, the western type diet was maintained up to 24 weeks. For the control, wild type mice on normal diet for 24 weeks were used. All three groups were scanned with both Ga-68-Pentixafor and F-18-NaF µPET/CT in subsequent days. Plaque morphology was assessed *via* various *ex vivo* histological, immunohistological and micro-autoradiography techniques.
Results: The calcification group developed spotty calcifications (on µCT) in the proximal aorta, which were confirmed by Alizarin Red stainings. Tracer uptake was correlated with plaques on haematoxylin eosin and with inflammation marker positive areas or Alizarin Red for Ga-68-Pentixafor and F-18-NaF respectively.
Conclusion: To our knowledge, this is the first mention of a mouse model that develops only vascular spotty calcifications detectable by µCT. Moreover, Ga-68-Pentixafor is able to specifically target inflamed, CXCR-4 positive areas, while F-18-NaF correctly targets microcalcified atherosclerotic plaques.
Disclosure: Nothing to disclose

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Haemodynamic Crosstalk Between Carotid Arteries and Implications for Wall Shear Stress Measurements

Supra-aortic Arterial Disease

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Introduction: Atherosclerotic plaque accumulation in the carotid arteries is usually asymmetric, leading to a haemodynamically relevant stenosis limited to one side, compensated by the opposite vessel [1]. Although the impact of stenosis treatments in cerebral perfusion has been extensively investigated [2], the analysis of the intra-individual haemodynamic crosstalk between carotids remains limited. The aim of this study was to elucidate the role of a severe carotid stenosis on the local haemodynamics of the opposite carotid artery.

Methods: Ten consecutive patients having a >70% symptomatic or >80% asymptomatic internal carotid stenosis were enrolled in this study (Group A). Another 10 consecutive patients referred for enrolment who did not fulfil the inclusion criteria were used as a control group (Group B). All patients underwent head and neck contrast enhanced computed tomography and phase contrast magnetic resonance imaging (MRI) to retrieve the carotid diameters and flow rates in the common, internal, and external carotids (CCA, ICA, and ECA respectively). Furthermore, computational fluid dynamics (CFD) simulations were performed in the vessel opposite to the stenosis (side of interest - *SOI*) to quantify the impact of the disease in local haemodynamics using flow data retrieved from MRI. As an indicator of plaque development, Wall Shear Stress (WSS) was extracted from the simulations following the classification by Malek[3]: atherogenic threshold (WSS< 4 dyn/cm^2) and physiological flow (10< WSS< 70 dyn/cm^2); values averaged along the cardiac cycle were considered (taWSS). In order to assess the importance of accounting for actual flow imbalance by patient specific flow data, a set of CFD simulations using idealised flow boundary conditions retrieved from literature [4]were performed, as well.

Results: The selected patients of Group A were 71(7) years old, 70% male and had a median SOI stenosis of 40(27)%. The contralateral (diseased) peak systolic velocity was 285(154) cm/s and was significantly higher than Group B ($p < .001$). In group A, SOI flow was significantly higher in the CCA and the ICA compared with the contralateral carotid (CCA: 6.6(2.0) ml/s *vs.* 4.4(3.5) ml/s, $p = .002$ and ICA: 3.2(2.1) ml/s *vs.*1.6(0.9) ml/s, $p < .001$). Such a difference was not observed in Group B. Moreover, CCA flow in the SOI of Group A was significantly higher than Group B (6.6(2.0) ml/s *vs.* 5.3(2.2) ml/s, $p = .010$). Carotid flow split, defined as the proportion of CCA flow exiting from the ICA, was also significantly higher in the SOI of Group A than the contralateral stenotic vessel (54(33)% *vs.* 38(31)%, $p = .009$). The use of literature data induced an overestimation of atherogenic areas in the CCA ($p = .021$), underestimating the areas experimenting physiological taWSS ($p = .006$).

Conclusion: Carotid artery stenosis created a flow imbalance between sides that reconfigures local haemodynamics in the non-diseased vessel. In particular, such a haemodynamic crosstalk increases the flow rate in the SOI, highlighting the importance of using patient specific flow data when computing taWSS. Moreover, we speculate that haemodynamically relevant stenoses may have an atheroprotective effect on the opposite side.

Disclosure: Nothing to disclose

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Dissecting the Link Between Calcification and Macrophage led Inflammation in Atherosclerosis

Vascular Biology

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Introduction: Calcification hugely impacts the inflammatory state of the atherosclerotic plaque, and inversely, inflammation was seen to regulate calcium deposition. We aimed to assess the role that macrophage led inflammation has in human vascular calcification and remodelling and identify central cues in this process.

Methods: WGCNA was performed on transcriptomics data for a human carotid plaque cohort comparing stable and unstable regions in a paired manner. Calcification was measured by alizarin red staining of paraffin embedded tissue sections of the same plaques, and macrophage presence measured via CD68 immunohistochemistry. Both parameters were correlated to gene module expression in the cohort, defining three co- expressed gene modules. Genes in correlating modules were ranked by macrophage relevance and gene cluster centrality, and the top ten chosen. Expression was measured via RT-PCR on a panel of vascular cell types, and on peripheral blood mononuclear cell derived macrophages exposed for 24, 48 and 72 hours to either hydroxyapatite nanoparticles, high phosphate media or high calcium phosphate media. siRNA knockdown was performed for each gene and functional screening done with a panel of eight assays including phagocytosis capability and lipid uptake, using BD Pathway high content analysis.

Results: In stable plaques there was strong correlation between CD68$^+$ macrophage presence and calcification presence ($r = 0.869$, $p = 1.2\text{x}10^{-5}$), whereas in unstable plaques, calcification highly correlated to arginase 1 expression ($p = 1.2\text{x}10^{-4}$) and multinucleate giant cell presence ($p = .03$). Three gene modules in unstable plaques highly correlated to calcification (p value 0.02, 0.01, $0.7\text{x}10^{-4}$), from which ten candidates were selected. Candidate gene expression in VSMC, VEC, and M0, M1, M2 and Mox macrophages highlighted seven of the candidates with significant macrophage relevancy. In M0 macrophages after calcification stimuli exposure, six of the genes showed significant changes in expression, including upregulation of SPP1 and