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Protein biomarkers and coronary microvascular dilatation assessed by rubidium-82 PET in women with angina pectoris and no obstructive coronary artery disease

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ABSTRACT

Background and aims: While a plethora of biomarkers have been shown to be associated with coronary artery disease, studies assessing biomarkers in coronary microvascular dysfunction (CMD) are few. We investigated associations between cardiovascular protein biomarkers and non-endothelium dependent CMD assessed by positron emission tomography (PET).

Methods: In 97 women with angina pectoris and no significant obstructive coronary artery disease (<50% stenosis on invasive coronary angiography), CMD was defined as myocardial blood flow reserve (MBFR) < 2.5 by rubidium-82 PET. Blood samples were analyzed with a cardiovascular disease proteomic panel encompassing 92 biomarkers. The relation between MBFR and biomarkers was evaluated with age-adjusted regression analysis.

Results: Median age was 62 years (range 31–79), median MBFR was 2.7 (range 1.2–4.7) and 32% had non-endothelium dependent CMD (MBFR<2.5). Four biomarkers were significantly correlated with MBFR: Galectin-4 (Gal4, $p = 0.008$), growth differentiation factor 15 (GDF15, $p = 0.026$), tissue-type plasminogen activator (tPA, $p = 0.030$) and von Willebrand factor (vWF, $p = 0.018$), while 12 biomarkers showed a trend for correlation ($0.05 \leq p < 0.15$). Of the 16 identified biomarkers, 10 are involved in pro-inflammatory pathways.

Conclusions: In a panel of 92 cardiovascular protein biomarkers, 4 were significantly associated with non-endothelium dependent CMD in women: Gal4, GDF15, tPA and vWF, suggesting that inflammatory status and coagulation changes are associated with impaired microvascular dilatation. Further confirmatory studies are needed to corroborate these findings.

1. Introduction

Coronary microvascular dysfunction (CMD) has gathered increasing interest as a potential cause of angina pectoris in patients with no obstructive coronary artery disease [1,2]. A plethora of biomarkers have been linked to macrovascular coronary artery disease risk and prognosis [3], while studies investigating biomarkers in CMD are few. Potential biomarkers in CMD include interleukin-6 (IL-6) and tumor necrosis factor alfa (TNFα) [4], reparative circulating progenitor cells [5], high-sensitivity c-reactive protein (hs-CRP) [6], galectin-3 (Gal3) [7] and soluble urokinase-type plasminogen activator receptor (sUPAR) [8], which have all been associated with CMD or cardiac syndrome X in small study populations.

We performed an exploratory analysis of associations between coronary artery disease related protein biomarkers and non-endothelium dependent CMD assessed by Rubidium-82 positron

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emission tomography (PET) in women with angina-like chest pain and no obstructive coronary artery disease, aiming to identify new biomarkers associated with CMD in women, and to re-evaluate biomarkers previously shown to be associated with CMD.

Other well founded diagnostic tests in non-obstructive angina patients include spontaneous coronary slow flow phenomenon on coronary angiography [9], invasive acetylcholine provocation testing for epicardial or microvascular vasospasm [10], and evaluation of endothelium dependent CMD [1]. The present study addresses associations between only non-endothelium dependent CMD measured by PET and protein biomarkers, and consequently potential presence of the above mentioned conditions was not evaluated.

2. Patients and methods

2.1. Enrollment, inclusion, baseline data

Participants were enrolled in Eastern Denmark between March 2012 and September 2014, according to the inclusion criteria for the iPOWER study [11]: Women aged 18–80 years with angina-like chest pain referred for a coronary angiogram, with no significant obstructive coronary artery disease (<50% coronary artery stenosis), meaning both women with primarily exertional angina and/or angina at rest were included in the study. Exclusion criteria were previous myocardial infarction, valvular or congenital heart disease, heart failure with reduced ejection fraction (<45%), previous percutaneous coronary intervention, and severe asthma or chronic obstructive pulmonary disease. Patients were also excluded if any known cardiac or non-cardiac cause of chest discomfort was deemed highly likely.

Standard assessment included demographic data, symptom questionnaires, physical examination, electrocardiogram, blood samples and echocardiography [11,12]. A subgroup of the iPOWER cohort was randomly selected for evaluation with PET [13].

2.2. Myocardial blood flow reserve evaluated by rubidium-82 PET

Myocardial blood flow reserve (MBFR) was assessed with a Siemens Biograph computed tomography/PET 128-Slice scanner (Siemens Healthcare, Knoxville, Tennessee, USA) shortly after completion of the baseline examination. Maximal non-endothelium dependent myocardial hyperemia was induced over 6 min with adenosine (0.84 mg/kg), which primarily induces vasodilatation and increased myocardial blood flow by activating adenylate cyclase in vascular smooth muscle cells in the microvasculature via binding to adenosine A_2 receptors. This results in increased intracellular cyclic adenosine monophosphate concentration, in turn leading to vascular smooth muscle cell hyperpolarization and relaxation [14,15]. Each participant received 1110 MBq (±10%) rubidium-82 supplied from a CardioGen-82 strontium-82/Rubidium-82 generator (Bracco Diagnostics Inc., Princeton, New Jersey, USA) during the scan, with a maximum radiation exposure of 5.2 mSv. Infusion of the tracer Rubidium-82 during hyperemia was initiated 2.5 min after initiation of adenosine infusion. MBFR was calculated as the ratio between myocardial blood flow during maximal hyperemia and myocardial blood flow at rest. Myocardial blood flow quantification was performed using Syngo software based on a single-compartment model for Rubidium-82 tracer kinetics, as described by Lortie et al. [16]. We have previously published a paper detailing PET MBFR assessment in the present patient cohort [13].

2.3. Protein biomarkers

All blood samples were analyzed with the Olink cardiovascular disease panel III (CVD III, Olink Proteomics, Uppsala, Sweden), measuring 92 protein biomarkers related to cardiovascular or other diseases by real-time polymerase chain reaction (Table 1) [17].

The samples were analyzed with the Proximity Extension Assay technique, using the Proseek Multiplex INF 96 × 96 panel covering the 92 protein biomarkers and 4 internal controls. All data were quality controlled, normalized and delivered as normalized protein expression values. Results below the limit of detection (LoD) were replaced with the LoD value. Further details regarding LoD, reproducibility and validity are given at http://www.olink.com.

2.4. Statistical analyses

Baseline patient characteristics were analyzed for possible associations with MBFR treated both as a continuous variable using linear regression, and MBFR treated as a dichotomized categorical variable (<2.5 and ≥ 2.5, respectively) using t-tests and χ^2-tests for continuous and categorical baseline variables, respectively.

The primary scope of biomarker analyses was exploratory. The relation between each of the 92 biomarkers and the primary outcome, MBFR, was explored using age-adjusted linear regression analysis after plotting each biomarker against MBFR for possible non-linear associations. Model assumptions were assessed graphically for all biomarkers and were best satisfied with log-2 transformed biomarker values (covariate) and untransformed MBFR values (outcome). We also assessed the relation between MBFR as a dichotomized categorical variable (<2.5 and ≥ 2.5, respectively) and all biomarkers, as well as the association between resting myocardial blood flow and all biomarkers.

The large number of biomarkers and the relative small patient sample prevented us from employing false discovery rate or family wise error controlling procedures, e.g. Bonferroni, which would have required a p-value < 0.00054 for significance in our population. As a result, all identified significant biomarkers were critically evaluated with meticulous attention to prior research and physiological profile (see Discussion).

All analyses were performed using STATA/IC 13.1 (StataCorp LP, College Station, TX, USA).

2.5. Ethics

The study was performed in accordance with the Helsinki Declaration and was approved by the Danish Regional Committee on Biomedical Research Ethics (H-3-2012-005). All participants gave written informed consent, after receiving oral and written information about the study.

3. Results

3.1. Study population

Between March 2012 and September 2014, 963 women were included in the iPOWER study [11,12], and from this population, 112 women were randomly included in the PET substudy. Five patients were excluded during the PET examination due to claustrophobia or side effects to adenosine infusion. Blood samples were unavailable in 10 additional patients, leaving 97 patients available for biomarker analysis in the present study.

Median age was 62 years (range 31–79), median MBFR was 2.7 (range 1.2–4.7), and 32% had MBFR<2.5 indicating non-endothelium dependent CMD. Patients with CMD had more hypertension ($p < 0.01$), and there was an insignificant association