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Endostatin and osteopontin are elevated in patients with both coronary artery disease and aortic valve calcification

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ABSTRACT

Background: The angiostatic factor endostatin (ES) plays an important role as mediator of angiogenesis. Elevated osteopontin (OPN) was associated with valve calcification in healthy individuals. The present study aimed to investigate ES and OPN levels in patients with both coronary artery disease (CAD) and aortic valve calcification (AVC).

Methods and results: In total 224 non- or ex-smoking patients (161 male, mean age: 61.09 ± 11.02 years; 63 female: mean age: 67.49 ± 7.87 years) with angiographically verified and quantified CAD were recruited. Serum ES and plasma OPN levels were measured by ELISA and AVC was evaluated by a parasternal short axis view and quantified as non-, mild or moderate/severe. There was a stepwise increase of ES measurable with increasing severity of AVC, independent from age, BMI and CAD-severity ($p = 0.018$; $F = 4.09$). OPN also increased significantly with the grade of AVC severity ($p = 0.029$; $F = 3.61$) but was no longer significant when the co-variables ($p = 0.31$; $F = 1.18$) were inserted.

Conclusions: This is the first study showing an association of ES with AVC in CAD-patients independent from age, BMI and CAD-severity which seems to be of distinct interest when trying to understand the process of heart valve calcification. OPN also correlates with AVC-severity but is mostly dependent on the age of the patients.

1. Introduction

The balance between angiogenic and angiostatic processes, which is controlled by numerous factors, is of particular importance for a healthy vascular system. However, angiogenesis also plays an important role in pathological angiogenesis and calcification of cardiac valves although it is rarely subject of investigation [1]. Calcification of aortic valves is the most frequent valvular disease with a prevalence of about 3–9% [2]. Furthermore, it was shown that stenotic aortic valves contain 3 types of neovessels: small and medium microvessels and organized arterioles. In stenotic valves, the distribution of neovessels is significantly higher and correlates with valvular calcification grade and mast cells in the stenotic area were shown to be activated and to contain VEGF [3].

ES is a component of nearly all endothelial basement membranes in the human body and turned out to be, in the long run, a strong angiostatic factor by inhibiting proliferation [4] of endothelial cells and tube formation [5] whereby it might also have angiogenic effects [6] depending on its concentration and on the type of cell it interacts with [7]. Its circulating amounts are influenced by several secondary circumstances such as the presence of diabetes [8] or physical activity [9]. Chalajour et al. investigated the angiogenic response of valvular endothelial cells to aortic valve stenosis in an ex vivo model of aortic leaflets and showed that sprouts from stenotic valves exhibited endostatin [10]. However, whereas recombinant murine endostatin was shown to inhibit microvessel formation in rat aortic rings in an ex vivo model, human endostatin did not [11].

Although OPN is not counted among "classic" angiogenesis-factors such as ES, it was suggested as a kind of "survival factor" for different types of cells (e.g. vascular smooth muscle cells [12]) and has angiogenic potential due to activation of PI3K/AKT- and ERK pathways through VEGF in endothelial cells [13]. OPN was shown to be absent in native non-calcified human aortic valves but present in minimally and highly calcified ones [14]. Similar results were obtained for rheumatic and non-rheumatic mitral valves [15,16]. A further study revealed that OPN is not only present in living aortic valve tissue but also in calcified areas of bioprosthetic heart valves [17]. It gets synthesized mainly by macrophages (and to a small amount by endothelial and smooth muscle cells) [18] and is localized at the surface of calcified deposits [19]. A correlation of elevated plasma levels of OPN and AVC was also found in healthy elderly subjects [20] and dephosphorylation of OPN

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correlates with severe calcification [21]. On the one hand OPN is involved in the process of calcification in bones [22] but on the other hand it was also shown to stimulate bone resorption [23]. Concerning vascular calcification, Wada et al. showed in a cell culture system that exogenous OPN potently inhibited calcification by inhibition of apatite growth [24].

ES and OPN doubtlessly play important roles in CAD but at present their role in AVC is not clear.

2. Material and methods

2.1. Population

In total 161 male (mean age: 61.09 ± 11.02 years) and 63 female (mean age: 67.49 ± 7.87 years) patients, never-smoker or ex-smoker for at least 7 years, with angiographically verified CAD of different severity were recruited. The protocol was approved by the Ethical Committee of the Medical University of Vienna and informed written consent was obtained from patients.

2.2. Definition of CAD

All patients underwent a coronary angiography for diagnostic and/or therapeutic reasons on grounds of their underlying disease. The coronary artery system was divided into 17 segments and stenosis grade for each segment was measured. A simple 3-point-grading system ("Coronary Score" [25]) was developed considering both frequency and severity of CAD. The patients received 0 points for non-stenosed or only calcified segments, 1 point for each stenosis from <30–<50%, 2 points for each stenosis from 50–<70% and 3 points for each stenosis >70%.

2.3. Echocardiographic analysis

Echocardiographic data were obtained with the use of the commercially available ultrasound systems (GE Medical Systems Vivid 7 Dimensions, Horton, Norway). Transthoracic echocardiography was performed by experienced echocardiographer without knowing levels of ES or OPN and was therefore "blinded". The severity of AVC was assessed by two-dimensional echocardiography in a parasternal short axis view. Patients with bicuspid aortic valves were excluded from the evaluation of AVC because bicuspid aortic valves are associated with valvular calcifications per se.

The degree of calcification of the aortic valve (Fig. 1) was scored as follows: 1: no calcification; 2: mild calcification (small isolated spots); and 3: moderate/severe calcification (multiple larger spots or extensive thickening and calcification of all cusps).

2.4. Endostatin and osteopontin analysis

ES was analyzed in serum, OPN in plasma by Enzyme-linked Immunosorbent Assay (ELISA) according to the instructions of the manufacturer.

2.5. Statistical analysis

Statistical analysis was done with SPSS 20.0. Continuous and normally distributed data is described by means ± standard deviation (SD) and group differences were tested by independent sample t-test. Univariate analysis of variance (ANOVA) was used to test the influence of AVC severity on ES and OPN. In a next step variables significantly related to AVC were entered as covariates. Ordinal logistic regression was used to model significant outcome variables onto AVC.

All tests are performed two-sided and p-values ≤0.05 were considered significant.

3. Results

Important anthropometric data, blood pressure and results from ECG are shown in Table 1. Female patients were about 6.5 years older, had a significantly lower BMI ($p = 0.017$) and diastolic blood pressure ($p < 0.001$) and were less commonly ex-smokers ($p = 0.003$) compared to men. No further significant sex-specific differences occurred. Atrial fibrillation was observed in 23 patients, consequently, the PQ-interval refers to the remaining 221 patients.

The coronary score representing the severity of CAD was significantly higher in male compared to female patients ($p = 0.017$). The most frequently affected coronary segments were the medial and proximal

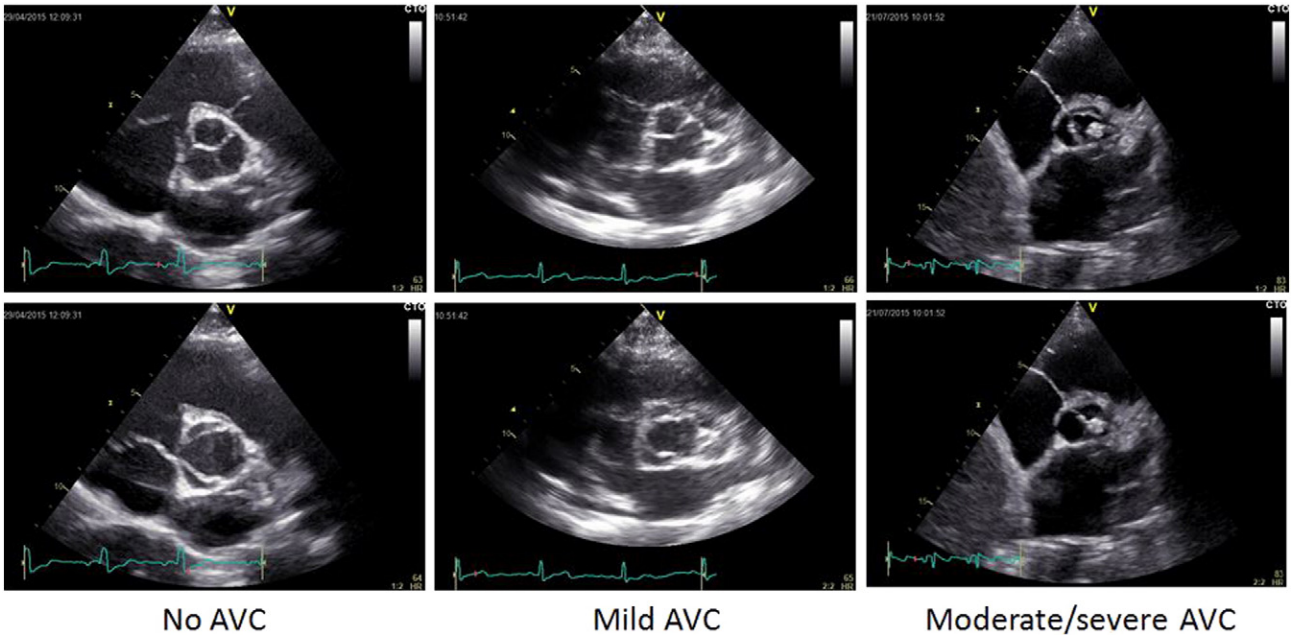

Fig. 1. Severity of AVC.