ORIGINAL ARTICLE

Biomarkers of calcification and atherosclerosis in patients with degenerative aortic stenosis in relation to concomitant coronary artery disease

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KEY WORDS

aortic stenosis, atherosclerosis, fetuin-A, interleukin 4, osteoprotegerin

ABSTRACT

INTRODUCTION There is an ongoing debate regarding aortic valve degenerative processes. Some markers of calcification and atherosclerosis may be potentially useful in establishing their etiology.

OBJECTIVES The aim of the study was to assess the biochemical markers of calcification and atherosclerosis in patients with degenerative aortic stenosis (AS) in relation to the aortic valve calcium score (AVCS) and concomitant coronary artery disease (CAD).

PATIENTS AND METHODS The study involved 88 patients: 68 patients with degenerative AS (group A), including 44 patients with severe AS (A1; 25 patients with CAD) and 24 patients with moderate AS (A2; 13 patients with CAD) and 20 matched subjects as controls (18 patients with CAD). In all patients, clinical data were assessed, laboratory tests were done (including the analysis of serum interleukin 4 [IL-4], osteoprotegerin [OPG], and fetuin-A levels), coronary angiography was performed, and the AVCS was measured.

RESULTS Study groups and subgroups had comparable serum IL-4, OPG, and fetuin-A levels. There were significant differences in the AVCS between patients with severe AS, moderate AS, and controls (3605 ±2542 Agatston units [AU], 1390 ±1143 AU, 100 ±194 AU, respectively; $P < 0.001$). There were no significant correlations between the AVCS and serum IL-4, OPG, or fetuin-A levels. In moderate AS, serum OPG levels were higher in subjects with concomitant CAD (5.84 ±1.4 vs. 4.03 ±1.3 pmol/l, $P = 0.036$). In severe AS, the mean AVCS was similar in patients with and without CAD. Higher AVCS was observed only in patients with moderate AS and coexisting CAD compared with patients without CAD (1644 ±1285 vs. 902 ±789 AU, $P = 0.038$).

CONCLUSIONS There were no significant differences between patients with and without degenerative AS in selected biochemical markers. The presence of CAD in moderate AS was associated with increased AVCS and serum OPG levels suggesting the effect of atherosclerosis on early valve calcification. In patients with severe AS, there were no correlations between calcification and atherosclerotic markers.

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INTRODUCTION Degenerative aortic stenosis (AS) is the most frequent heart valve disease affecting from 2% to 7% patients over 65 years of age.[1-3] According to some authors, valve degeneration is associated with atherosclerotic process.[4-7] Other investigators consider this to be an independent process with osteoblast differentiation as an essential component of age-related cardiovascular remodeling.[8,9] Inadequate mineralization and soft tissue calcification are probably associated with primary disorders of calcium and phosphate levels and a number of the biomarkers, e.g., osteoprotegerin (OPG), interleukin 4 (IL-4), and fetuin-A.[10-12]

OPG and the OPG/RANKL/RANK system are members of the tumor necrosis factor family.[10] They regulate the activity of osteoblasts and osteoclasts and directly affect endothelial and vascular smooth muscle cells.[6,11-13] Antiatherogenic and vasculoprotective function of OPG demonstrated in in-vitro studies has not been confirmed in vivo.[11,14,15] IL-4 is a pleiotropic, anti-inflammatory cytokine that affects atherogenic and calcification processes by regulating osteoblast and osteoclast activity. Independent contribution of IL-4 and OPG has been observed only in some in-vitro studies.[9,16] Fetuin-A is associated with transforming growth factor β by blocking its capability to enhance stability of atherosclerotic plaques. Fetuin-A is an important ectopic calcification inhibitor; therefore, its low serum levels might coexist with pathological valvular and vascular mineralization.[17]

Multislice computed tomography (MSCT) is a valuable diagnostic tool in patients with AS. Contrast-free cardiac examination enables to perform quantitative evaluation of valve mineralization with the calculation of the aortic valve calcium score (AVCS).[18-20] According to the available data, 27% of the patients aged over 60 years have aortic valve calcification.[17] AVCS as a marker of calcification may correspond to the degree of valve degeneration, especially if calcification is the main cause of the disease.[18,20]

The aim of the study was to assess correlations between biochemical markers of calcification and atherosclerosis in patients with degenerative AS in relation to the AVCS and concomitant coronary artery disease (CAD).

PATIENTS AND METHODS

Group A included 68 patients with severe or moderate degenerative AS with preserved ejection fraction. The group was divided into subgroups A1 and A2. Subgroup A1 included 44 patients with severe AS (age, 69.7 ±7.6 years; mean effective orifice area [EOA], 0.77 ±0.34 cm^2), New York Heart Association (NYHA) class I–III, Canadian Cardiovascular Society (CCS) class 0–4. Subgroup A2 included 24 patients with moderate AS (age, 66.7 ±7.5 years; mean EOA, 1.41 ±0.22 cm^2), NYHA class I–III, CCS class 0–4. Twenty subjects without heart valve degenerative changes matched for age, sex, and prevalence of CAD were recruited as the control group. Patients were hospitalized in the Department of Cardiology, Medical University of Silesia in Katowice, Poland.

The study was approved by the Ethics Committee of the Medical University of Silesia in Katowice (NN-6501-1/07). Patients were included into the study for 6 months in the years 2009 and 2010. All subjects provided written informed consent.

The inclusion criteria for group A were as follows: presence of degenerative AS (severe and moderate) evaluated according to the European Society of Cardiology (ESC) recommendations.[21]

The exclusion criteria for both groups were: contraindications for MSCT AVCS evaluation (heart rhythm disorders, such as atrial fibrillation, numerous ventricular extrasystoles, breath-holding problems, claustrophobia, lack of consent); contraindications for coronary angiography (severe heart failure, severe hypertension, contrast allergy, hypocalcemia, digitalis intoxication, fever, kidney failure, hyperthyroidism, anticoagulation – prothrombin time <50%), electrocardiogram (ECG) abnormalities that caused difficulties in the interpretation (atrial fibrillation, LBBB, VVI, or DDD stimulator rhythm), congenital heart diseases, left ventricular systolic dysfunction (ejection fraction <50%), acute or chronic inflammation (within the previous 3 months), autoimmune diseases, immunosuppressive treatment, neoplastic diseases, alcohol or drug abuse, clinically significant disorders of the internal organs, smoking on the day of examination, lack of consent.

Additional exclusion criteria were as follows: for group A – nondegenerative AS (rheumatic valve disease, bicuspid aortic valve), serious mitral, tricuspid, or pulmonary valve diseases; for the control group – moderate and severe cardiac valve diseases.

Methods **Clinical data** Symptoms of CAD according to the CCS, symptoms of heart failure according to the NYHA, concomitant diseases (hypertension, diabetes, peripheral artery disease), and smoking history were recorded in all patients. The following parameters were measured: body mass index, waist-to-hip ratio, systolic and diastolic blood pressure in the sitting position after a 3-minute rest.

Laboratory tests Blood samples of 10–12 ml were collected from the peripheral vein between 7 and 8.30 a.m., after at least 12-hour fast. Serum levels of total cholesterol, high- and low-density lipoprotein cholesterol, and triglycerides were measured. Serum levels of IL-4, OPG, and fetuin-A were measured with enzyme-linked immunosorbent assays (Roche, Paris, France; BioVendor, Brno, Czech Republic).

Sensitivity, intra- and interassay coefficients of variations were as follows: for IL-4 – 0.6 pg/ml, 4.8%, 5.6%, respectively; for OPG – 0.13 pmol/l, 4.5%, 5.5%, respectively; for fetuin-A – 0.35 ng/ml, 3.5%, 5.4%, respectively.

Electrocardiographic examination ECG examination at rest was performed in all subjects. Patients with ECG abnormalities that hampered data interpretation (atrial fibrillation, LBBB, VVI or DDD stimulator rhythm) were excluded from the final analysis.

Echocardiographic examination Echocardiographic examination included transthoracic examination in both groups and transesophageal examination in selected patients in group A. Examinations were performed according to the ESC guidelines, especially the European Association of Echocar-

diography/American Society of Echocardiography recommendations.[21]

To evaluate AS, we measured maximum (P_{max}) and mean (P_{mean}) transvalvular pressure gradients, EOA using linear equation and aortic valve area (AVA) using planimetry. The parameters of left ventricular geometry, function, and afterload were analyzed using standard methods. The parameters included left ventricular end-diastolic and end-systolic volume, left ventricular ejection fraction (LVEF), stroke volume, stroke volume index, interventricular septum, left ventricular mass, and left ventricular mass index. Left ventricular parameters were assessed using the two-dimensional left parasternal long-axis view.

The procedure was performed using the Toshiba Aplio equipment, with a 2.5 MHz sector ultrasound transducer and 2–7 MHz transoesophageal transducer.

64-multislice spiral computed tomography For quantitative AVCS analysis, MSCT was performed with Toshiba Aquilion 64 scanner (Toshiba, Japan) using standard retrospective ECG gating protocol without contrast media. The detector collimation was 32 × 0.5 mm. The tube current ranged from 170 to 300 mA; the tube voltage was set at 120kV and pitch was 0.4. Axial images were reconstructed at 60% of the RR-interval, to achieve least motion artifacts, with an effective slice thickness of 3 mm. Calcification lesions were identified by detection of at least 3 contiguous pixels (voxel size = 1.03 mm^3) of peak density ≥130 Hounsfield units (HU). The lesion-specific scores were computed as the product of the area of each calcified focus and peak computed tomography number (scored as 1 if 131 to 199 HU, 2 if 200 to 299 HU, 3 if 300 to 399 HU, and 4 if ≥400 HU) according to the Agatston method.[22] AVCS was gauged cumulatively for the valvular annulus and cusps. Effective radiation dose reached about 2 mSv for each patient.

Coronary angiography Coronary angiography using the Seldinger method was performed in some patients from group A (subjects initially scheduled for surgery – subgroup A1) and in all control subjects. CAD was diagnosed if there were changes in coronary artery lumen with lumen diameter narrowing of at least 50%; critical changes were defined as lumen diameter narrowing of at least 70% in 1, 2, or 3 major epicardial coronary arteries.

Statistical analysis Statistical analysis was performed using Statistica 7.0 and MedCalc 9.0. The following techniques were used: the Shapiro-Wilk's *W* test, *t* test, Mann-Whitney test and Kruskal-Wallis test, the analysis of variance, Fisher exact test, regression and correlation analysis using the Pearson and Spearman correlation coefficients, and multiple stepwise regression. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical data of group A and controls

Demographic data, laboratory test results, concomitant diseases, and smoking history in group A (including subgroups A1 and A2) and controls are presented in TABLE 1. There were no significant differences between patients except for smoking activity and serum high-density lipoprotein cholesterol. Statins were administered in 52 patients (76%) from group A, (subgroup A1: 34 [77%] and A2: 18 [75%]) and in 16 controls (80%); β-blockers in 58 patients (85%) from group A (A1: 37 [84%]; A2: 21 [87%]) and in 18 controls (90%); angiotensin-converting enzyme inhibitors in 52 patients (76%) from group A (A1: 31 [70%]; A2: 21 [87%] and in 18 controls (90%).

Echocardiographic parameters of geometry, function, and left ventricular afterload

Echocardiographic findings showing AS severity are presented in TABLE 2. Echocardiographic parameters of geometry, function and left ventricular afterload are shown in TABLE 3.

Serum levels of interleukin 4, osteoprotegerin, and fetuin-A and the aortic valve calcium score

Group A and controls had similar serum IL-4, OPG, and fetuin-A levels. The mean AVCS in group A (2704 ±2348 AU) was significantly higher than in controls (100 ±194 AU, $P < 0.001$) (TABLE 4).

Mean serum levels of biochemical markers in subgroups A1, A2, and the control group were similar (TABLE 4).

The AVCS was significantly higher in subgroups A1 (3605 ±2542 AU) and A2: (1390 ±1143 AU) compared with controls: 100 ±194 AU ($P < 0.001$; post-hoc analysis: $P < 0.01$) (TABLE 4).

There were 38 patients (56%) with concomitant CAD in group A, (25 [57%] in subgroup A1 and 13 [54%] in subgroup A2). There were 18 patients (80%) with concomitant CAD in the control group.

In AS patients with CAD, serum IL-4 levels were 1.10 ±0.5 pg/ml, OPG – 5.31 ±2.7 pmol/l, and fetuin-A – 176.9 ±45.7 μg/ml. In patients without CAD, serum IL-4 levels were 0.86 ±0.2 pg/ml ($P = 0.04$), OPG – 5.06 ±2.2 pmol/l, and fetuin-A – 178.3 ±41.7 μg/ml. In control subjects with concomitant CAD, serum IL-4 levels were 1.24 ±1.7 pg/ml, OPG – 4.97 ±1.6 pmol/l, and fetuin-A – 171.9 ±34.4 μg/ml. Due to a small number of control subjects without CAD, the above parameters were not analyzed in this group.

The analysis of serum IL-4, OPG, and fetuin-A levels in subgroups A1 and A2 in relation to the presence of CAD showed significant differences only for OPG. Serum OPG levels in patients with severe AS were 5.07 ±2.36 for those with CAD vs. 5.86 ±2.2 pmol/l for those without CAD (nonsignificant). Serum OPG levels in patients with moderate AS were 5.84 ±1.46 for those with CAD vs. 4.03 ±1.38 pmol/l for those without CAD ($P = 0.036$).

The comparison between subjects with severe and moderate AS without concomitant CAD also

TABLE 1 Characteristics of the study group, subgroups, and controls

Parameters	Group A (n = 68)	Subgroup A1 (n = 44)	Subgroup A2 (n = 24)	Controls (n = 20)
age, y	68.6 ±7.7	69.7 ±7.6	66.7 ±7.5	66.1 ±7.7
weight, kg	79.8 ±12.9	79.7 ± 13.4	79.9 ±12.6	75.4 ±11.3
height, cm	166 ±8	166 ±9	167 ±8	167 ±8
WHR, cm	0.97 ±0.07	0.98 ±0.06	0.95 ±0.06	0.94 ±0.05
BMI, kg/m^2	28.7 ±4.2	28.8 ±4.0	28.6 ±4.5	27.0 ±3.3
BSA, m^2	1.92 ±0.19	1.91 ±0.20	1.9 ±0.18	1.87 ±0.17
SBP, mmHg	133.9 ±21	136.9 ±15.8	134 ±15.6	133.7 ±19.8
DBP, mmHg	80.7 ±12.4	83.5 ±10.7	80 ±7.9	79.7 ±9.5
MAP, mmHg	98.9 ±10.8	99.8 ±11.7	97.8 ±8.95	98.7 ±11.4
men/women, n (%)	39 (57) / 29 (43)	26 (59) / 18 (41)	13 (54) / 11 (46)	12 (60) / 8 (40)
coronary artery disease, n (%)	38 (56)	25 (57)	13 (54)	18 (80)
diabetes, n (%)	16 (24)	11 (25)	5 (21)	6 (30)
hypertension, n (%)	58 (85)	38 (86)	20 (83)	17 (85)
current smoking, n (%)	4 (6)[a]	0[b]	4 (17)[b]	5 (25)[a]
smoking history, n (%)	26 (38)	20 (45)	6 (25)	6 (30)
no smoking history, n (%)	38 (56)	24 (55)	14 (58)	9 (45)
TC, mg/dl	187 ±52	193 ±56	177 ±43	178 ±53
HDL-C, mg/dl	49 ±16[a]	47 ±14	52 ±19	40 ±9[a]
LDL-C, mg/dl	111 ±46	118 ±49	99 ±39	102 ±47
TG, mg/dl	135 ±57	139 ±53	127 ±62	169 ±155
Ca, mg/dl	9.3 ±0.52	9.4 ±0.52	9.1 ±0.48	9.1 ±0.44
leukocytes, 10^3/ml	7.1 ±2.1	7.3 ±2.3	6.9 ±1.5	6.8 ±1.7

Data are presented as mean ± SD or number (percentage).

Comparison between group A and controls: independent samples *t* test, Mann-Whitney test, χ^2 test; **a** $P = 0.015$ (HDL-C), $P = 0.044$ (current smoking); comparison between subgroup A1, A2, and controls: analysis of variance, Kruskal-Wallis test, χ^2 test; **b** $P = 0.005$

SI conversion factors: to convert cholesterol values to mmol/l multiply by 0.0259 and TG values by 0.0113.

Abbreviations: BMI – body mass index, BSA – body surface area, Ca – calcium, DBP – diastolic blood pressure, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, MAP – mean arterial pressure, SBP – systolic blood pressure, SD – standard deviation, TC – total cholesterol, TG – triglycerides, WHR – waist-to-hip ratio

TABLE 2 Echocardiographic parameters of aortic stenosis severity in subgroups A1 and A2

Parameters	Subgroup A1 (n = 44)	Subgroup A2 (n = 24)	*P*
EOA, cm^2	0.77 ±0.34	1.41 ±0.52	<0.001
AVA, cm^2	0.84 ±0.31	1.51 ±0.42	<0.001
V_{max}, m/s	4.3 ±1.0	2.8 ±0.6	<0.001
P_{max}, mmHg	77.7 ±35.6	33.4 ±14.6	<0.001
P_{mean}, mmHg	44.5 ±22.7	17.7 ±8.3	<0.001
ELI, cm^2/m^2	3.33 ±4.56	8.37 ±13.6	<0.001

Data are presented as mean ± SD.

Comparison between group A1 and A2: Mann-Whitney test, independent samples *t* test.

Abbreviations: AVA – aortic valve area, ELI – energy loss index, EOA – effective orifice area, P_{max} – maximum transvalvular pressure gradient, P_{mean} – mean transvalvular pressure gradient, V_{max} – maximum transvalvular velocity, others – see **TABLE 1**

revealed a significant difference, namely, serum OPG levels were higher in severe AS ($P = 0.042$).

Patients with AS and CAD and those without CAD had similar mean AVCS (3091 ±2436 vs. 2419 ±2257 AU).

The analysis of subgroups A1 and A2 in relation to the presence of CAD revealed that the AVCS was similar in subjects with severe AS and CAD and those without CAD (3748 ±2663 vs. 3340 ±2292 AU). In subgroup A2, the AVCS was significantly higher in subjects with CAD compared with

TABLE 3 Echocardiographic parameters of left vantricular geometry, function, and afterload in group A, subgroups A1 and A2, and controls

Parameters	Group A (n = 68)	Subgroup A1 (n = 44)	Subgroup A2 (n = 24)	Controls (n = 20)	*P*	*P'*
LVEDV, ml	110 ±48	117 ±57	99 ±26	97 ±28	NS	NS
LVESV, ml	46 ±29	51 ±34	38 ±12	38 ±18	NS	NS
LVEF, %	59 ±10	58 ±11	62 ±7	60 ±8	NS	NS
SV, ml	64 ±25	65.8 ±30.0	61.0 ±17.6	57.6 ±16.2	NS	NS
SVI, ml/m^2	33 ±12	33.8 ±14.3	31.3 ±8.0	31.0 ±8.7	NS	NS
IVS, mm	14.1 ±2.9	15.0 ±3.1	12.4 ±1.9	10.6 ±1.3	<0.001	<0.001
LVM, g	298 ±91	321 ±104	260 ±49	214 ±72	0.0005	<0.001
LVMI, g/m^2	154 ±48	165 ±60	137 ±19	114 ±34	0.0012	0.001

Data are presented as mean ± SD.

Comparison between group A and controls: Mann-Whitney test, independent samples *t* test (*P*); comparison between subgroups A1, A2, and controls: analysis of variance, Kruskal-Wallis test (*P'*).

Abbreviations: IVS – interventricular septum, LVEDV – left ventricular end-diastolic volume, LVEF – left ventricular ejection fraction, LVESF – left ventricular end-systolic volume, LVM – left ventricular mass, LVMI – left ventricular mass index, NS – nonsignificant, SV – stroke volume, SVI – stroke volume index

TABLE 4 Serum levels of interleukin 4, osteoprotegerin, fetuin-A, and the aortic valve calcium score in group A, subgroups A1 and A2, and controls

Parameters	Group A (n = 68)	Subgroup A1 (n = 44)	Subgroup A2 (n = 24)	Controls (n = 20)	*P, P'*
IL-4, pg/ml	1.55 ±2.7	1.04 ±0.60	2.55 ±4.37	2.24 ±5.03	NS
OPG, pmol/l	5.24 ±2.4	5.44 ±2.30	4.86 ±2.66	5.22 ±1.89	NS
fetuin-A, μg/ml	177.6 ±43.9	177.3 ±46.4	178.6 ±39.2	168.6 ±34.4	NS
AVCS, Agatston units	2704 ±2348	3605 ±2542	1390 ±1143	100 ±194	<0.001

Data are presented as mean ± SD.

Comparison between group A and controls: Mann-Whitney test (*P*); comparison between subgroups A1, A2, and controls: Kruskal-Wallis test (*P'*).

Abbreviations: AVCS – aortic valve calcium score, IL-4 – interleukin 4, OPG – osteoprotegerin, others – see TABLE 3

those without CAD (1644 ±1285 vs. 902 ±789 AU, *P* = 0.038).

Associations between aortic stenosis severity and serum IL-4, OPG, fetuin-A levels and the aortic valve calcium score The analysis of associations between serum IL-4, OPG and fetuin-A levels and AS severity showed a positive correlation only between IL-4 and EOA in subgroup A1 (*r* = 0.341, *P* =0.04).

In all subjects (group A), there was an inverse correlation between the AVCS and EOA (*r* = –0.463, *P* = 0.001) and AVA (*r* = –0.445, *P* = 0.001) (FIGURES 1 and 2).

There were no significant correlations between the AVCS and serum IL-4, OPG, fetuin-A levels. A regression analysis revealed no significant correlations between serum IL-4, OPG, fetuin-A levels, AVCS and the clinical data. There were no independent factors determining the serum level of the biochemical markers studied.

DISCUSSION Due to increasing prevalence of AS, there has been a growing interest in the pathogenesis of degenerative valvular changes. We did not observe any differences in the serum levels of atherosclerotic (IL-4) and osteogenic (OPG, fetuin-A) markers in relation to AS severity.

The OPG/RANKL/RANK system is involved in pathological vascular and valvular mineralization and in AV degenerative processes as suggested by recent studies.[4-6,13] Moreover, a number of investigators reported a correlation between serum OPG levels and severity of concomitant systemic diseases and higher cardiovascular mortality.[23-27] The potential close links between atherosclerotic lesions and valvular calcification[28] might be associated with abnormal serum OPG and RANKL levels in patients with CAD and AS. According to Kaden et al.,[29] serum OPG levels are significantly decreased and RANKL levels increased in patients with AS. Helske et al.[26] revealed significant correlations between increased serum OPG levels in AS and the presence and severity of CAD. OPG levels did not correlate with the severity of AS, which is in line with our results. At the same time, in all our subjects serum OPG levels were higher than the reference range of 2.0–3.5 pmol/l reported in the literature.[25-27] This could be explained by the presence of hypertension and CAD in the majority of our subjects. Thus, OPG might have a potential role in AV cal-

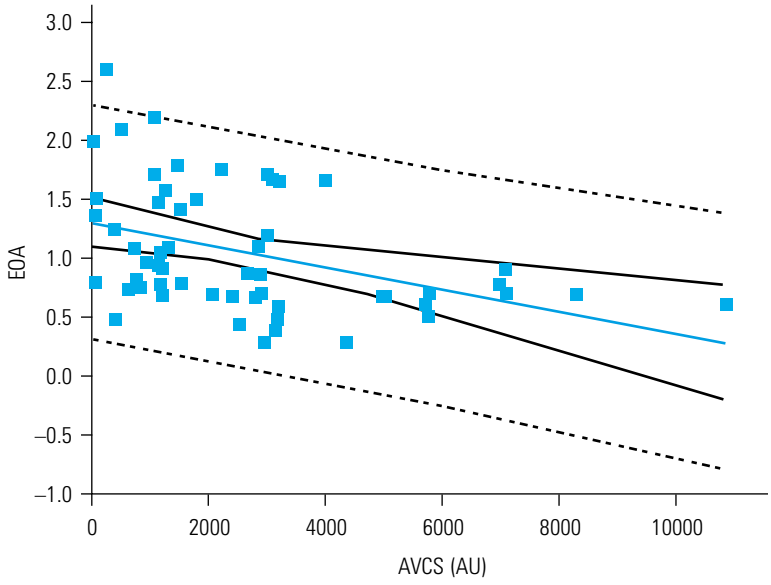

FIGURE 1 Correlation between the aortic valve calcium score (AVCS) and effective orifice area (EAO) in group A

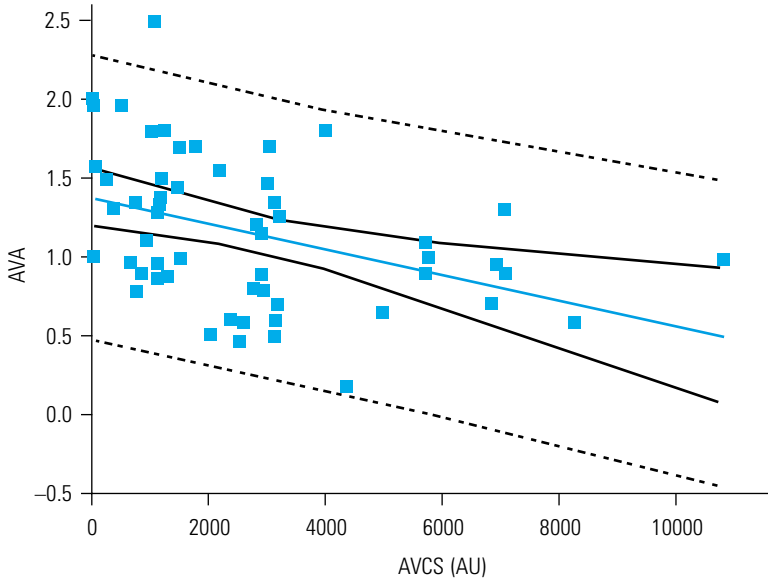

FIGURE 2 Correlation between the aortic valve calcium score (AVCS) and aortic valve area (AVA) in group A

cification. However, the analysis had to take into account concomitant diseases.

The subgroup analysis confirms the above hypothesis. It revealed that OPG levels in subjects without CAD were higher in severe AS than in moderate AS. The analysis of the subgroup with moderate AS showed significantly higher OPG levels in patients with AS and CAD. The results confirm that CAD affects serum marker levels only in the early stages of the disease. Atherosclerotic process possibly accompanies pathological aortic valve calcification, especially in the early stages. It seems that serum OPG levels are not specific for one of the above mechanisms but are dependent on both mechanisms. Previous studies on CAD patients showed a positive correlation between serum OPG levels and CAD severity and coronary artery narrowing.[25,30] Increased OPG levels after myocardial infarction or after coronary angioplasty suggest that OPG is associated with proinflammatory activation.[27] Single studies suggested a major role of OPG in heart failure in severe AS. Helske et al.[26] reported strong correlations between severity of heart failure in the course of AS and CAD and serum OPG levels. At the same time, they did not show any correlations between OPG and LVEF and AVA. Serum OPG levels decreased significantly after aortic valve replacement. Other reports showed that OPG levels are not associated with AS severity but closely correlate with the presence and severity of CAD.[13,26,27,30]

The role of IL-4 and fetuin-A as potential pathogenic factors in patients with AS remains unclear. We observed a positive correlation between serum IL-4 levels and EOA only in patients with severe AS – increased IL-4 levels corresponded to less severe AS. Potential inhibition of AS progression by IL-4 as an anti-inflammatory cytokine may explain this finding. On the other hand, IL-4 increases calcification and bone formation by promoting osteoblast differentiation. A potential autoregulatory mechanism in severe AS might be related to a decrease in IL-4 levels.[9,16] Our analysis revealed that IL-4 levels were significantly higher in patients with AS and CAD. There were no significant correlations between AS severity, CAD, and serum fetuin-A levels.

Severity of AV mineralization processes may be measured using the AVCS. In our study, the AVCS differed significantly between study subjects and the control group and correlated with the severity of AS. Associations between the AVCS and AS severity and cardiovascular risk factors have been confirmed in many studies.[18-20] A number of investigators reported that the presence of aortic valve calcifications correlates positively with the severity of CAD and the risk of changes in coronary arteries is 3-fold higher in patients with aortic valve calcification compared with those without aortic valve calcification.[18] In the present paper, the AVCS in severe AS was high irrespective of CAD. In patients with moderate AS, higher AVCS was observed in those with coronary artery lesions. We observed the analogy in the results of biochemical parameters, which confirms that early AV calcification and atherosclerosis have common pathomechanisms. At the same time, correlations in severe AS were not evident.

Finally, our results suggest that atherosclerotic and calcification processes are associated only with the early stages of cardiovascular remodeling. Of note, in severe AS, no significant correlations between calcification processes and atherosclerosis can be observed.

The hypothesis that there is a mechanism that leads to AV degeneration and is independent of atherosclerosis is becoming increasingly popular. The results of clinical trials on aggressive hypolipemic therapy in patients with mild and

moderate AS (SEAS) did not demonstrate regression of AS severity despite regular drug administration (statins and ezetimibe).[31] Otto[32] emphasized the differences in the pathophysiology and morphology of AV and coronary artery lesions. AV calcifications are much more extensive and advanced compared with coronary artery atherosclerotic plaque distribution in patients with AS.

Limitations A limitation of the study was a relatively small number of patients. Limitations of potential echocardiographic mistakes were minimized by assigning the examinations to only one experienced sonographer. Only subjects with preserved EF were enrolled into the study; therefore, a possible effect of low cardiac output on cytokine activation was not addressed. In all groups, blood pressure and pulse pressure values were comparable; therefore, the limitation of potential differences in parameters during examinations affecting AS severity might be ignored. Hypertension and CAD were observed in the majority of patients and controls.

Conclusions Potential biochemical markers of cardiovascular system remodeling – IL-4, OPG, and fetuin-A – do not differentiate patients with and without degenerative AS. The presence of CAD in patients with moderate AS is related to higher AVCS values and elevated serum OPG levels. There is a possible association between atherosclerotic and degenerative processes in the early stages of cardiovascular system remodeling. In patients with severe AS, no significant correlations between calcification processes and atherosclerosis have been observed.

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ARTYKUŁ ORYGINALNY

Markery wapnienia i miażdżycy u chorych z degeneracyjną stenozą aortalną a współwystępowanie choroby wieńcowej

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SŁOWA KLUCZOWE

fetuina A, interleukina 4, miażdżyca, osteoprotegryna, stenoza aortalna

STRZESZCZENIE

WPROWADZENIE Procesy degeneracyjne zastawki aortalnej są nadal przedmiotem dyskusji. Potencjalnie w różnicowaniu ich etiologii mogą być przydatne niektóre markery wapnienia i zmian miażdżycowych.

CELE Celem pracy była ocena biochemicznych markerów wapnienia i zmian miażdżycowych u chorych z degeneracyjną stenozą aortalną (*aortic stenosis* – AS) w zależności od wartości wskaźnika uwapnienia zastawki aortalnej (*aortic valve calcium score* – AVCS) oraz towarzyszącej choroby wieńcowej (*coronary artery disease* – CAD).

PACJENCI I METODY Do badania włączono 88 chorych: 68 z degeneracyjną AS (grupa A), w tym 44 z ciężką AS (A1; 25 chorych z CAD) i 24 z umiarkowaną AS (A2; 13 chorych z CAD), oraz 20 chorych dobranych pod względem wieku i płci stanowiących grupę kontrolną (18 chorych z CAD). U wszystkich chorych zebrano dane kliniczne i przeprowadzono badania laboratoryjne (w tym analizę stężenia interleukiny 4 [IL-4], osteoprotegryny [OPG] i fetuiny A w surowicy), wykonano koronarografię oraz określono wartość AVCS.

WYNIKI W badanych grupach i podgrupach stwierdzono porównywalne stężenia IL-4, OPG i fetuiny A w surowicy. Wykazano istotne różnice w wartościach AVCS u chorych z ciężką AS, umiarkowaną AS i w grupie kontrolnej (odpowiednio 3605 ±2542 jednostek Agatstona [*Agatston* units – AU], 1390 ±1143 AU, 100 ±194 AU; p <0,001). Nie wykazano istotnych zależności pomiędzy AVCS a stężeniami IL-4, OPG i fetuiny A w surowicy. W przypadku chorych z umiarkowaną AS stężenie OPG było wyższe u osób z towarzyszącą CAD: 5,84 ±1,4 *vs* 4,03 ±1,3 pmol/l (p = 0,036). W przypadku chorych z ciężką AS średnie wartości AVCS były podobne u osób z towarzyszącą CAD i bez niej. Większe wartości AVCS obserwowano jedynie u chorych z umiarkowaną AS i towarzyszącą CAD w porównaniu z chorymi bez CAD (1644 ±1285 *vs* 902 ±789 AU, p = 0,038).

WNIOSKI Między chorymi z degeneracyjną AS i bez niej nie występują istotne różnice w stężeniach wybranych markerów biochemicznych. Współwystępowanie CAD w umiarkowanej AS wiąże się ze zwiększonymi wartościami AVCS i zwiększonym stężeniem OPG w surowicy, co może sugerować wpływ procesów miażdżycowych na wczesne etapy wapnienia zastawki. U osób z ciężką AS nie wykazano zależności pomiędzy markerami wapnienia a markerami zmian miażdżycowych.

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