Association of fetuin-A levels with the progression of aortic valve calcification in non-dialyzed patients

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Aims
Fetuin-A has been identified as a potent circulating inhibitor of ectopic calcification. We investigated the relationship between baseline fetuin-A serum levels and the rate of progression of aortic valve calcification (AVC) in non-dialyzed patients with aortic valve disease (AVD).

Methods and results
Seventy-seven patients (mean age 70 ± 8 years) with echocardiographically proven AVD were collected. In all patients, serum fetuin-A levels, creatinine, calcium, lipid parameters, and C-reactive protein were measured at baseline. For quantification of AVC progression, all patients underwent multislice spiral computed tomography examinations at baseline and after a mean follow-up of 12.6 ± 1.4 months (range 7–18 months). In a multifactorial analysis of covariance including fetuin-A levels, baseline AVC score, the covariables sex, age, body mass index, C-reactive protein, glomerular filtration rate, serum lipids, diabetes, smoking status, and hypertension, only serum fetuin-A levels significantly predict the progression of AVC (P, 0.001). Post hoc analysis demonstrated that patients with baseline fetuin-A levels lower than the median of the cohort (0.72 g/L) showed a significantly higher increase of AVC scores (34.6 ± 31.4%) than patients with fetuin-A levels larger than the median (10.0 ± 11.2%, P, 0.001) despite comparable baseline AVC scores. In addition, fetuin-A levels were associated with major adverse clinical events (MACE; P = 0.03).

Conclusion
Serum levels of the calcification inhibitor fetuin-A are associated with the progression of AVC and MACE, independent of the renal function and inflammation.

Keywords
Aortic valve stenosis • Calcification • Multi-slice CT

Introduction
Human fetuin-A (alpha2-Heremans Schmid glycoprotein), a 59 kDa glycoprotein synthesized in the liver, has been identified as a powerful circulating inhibitor of vascular and soft-tissue calcification.1 Most data about the impact of low fetuin-A serum levels upon vascular calcification and increased mortality come from patients with end-stage renal disease (ESRD).2–4 Cohorts of ESRD patients often have low serum concentrations of fetuin-A and their sera showed a reduced capacity to inhibit calcification in vitro.1,5 Fetuin-A can exert its effect by various mechanisms. Fetuin-A represents an integral part of a highly efficient clearing mechanism for small mineral complexes.1,6,7 Intracellular fetuin-A inhibits apoptosis of vascular smooth muscle cells.8 Most notably,
it inhibits the calcification-inducing effects of transforming growth factor-β and bone morphogenetic protein-2, as both have been shown to promote valvular calcification.6,9–13 Valvular calcification is a well-known characteristic feature of fetuin-A knock-out mice, as demonstrated by Westenfeld et al.14 In that study, 80% of fetuin-A null mice, with a relatively calcification-resistant genetic background, exhibited valvular calcifications under certain triggering conditions.

Previous cross-sectional studies in ESRD patients showed a negative correlation between fetuin-A serum levels and coronary as well as valvular calcifications.2,14 Moreover, prospective studies in haemodialysis patients demonstrated concordantly an association between low fetuin-A concentrations and cardiovascular mortality.3,4,14

Degenerative calcific aortic stenosis is the most common valvular heart disease in the elderly, with a prevalence of 2–7% in the population above 65 years of age often leading to heart valve replacement therapy.15 Therefore, predicting its clinical course is of special importance. In contrast to the above-mentioned well-substantiated data derived from ESRD patients, only few data are available on the relationship between serum fetuin-A levels and aortic valve disease (AVD) in patients with normal or only moderately impaired renal function. Two previous studies have investigated the association between low fetuin-A levels and AVD.16,17 These studies support the concept that fetuin-A deficiency is involved in the pathogenesis of calcific aortic stenosis.17 Interestingly, lower serum fetuin-A levels were observed in patients with calcific AVD compared with healthy controls, independent of the renal function.17 However, both studies were cross-sectional in nature and none of these studies assessed the severity of valvular calcification. Therefore, data regarding the association of AVD progression with calcification inhibitor deficiency are as yet unavailable.

Thus, the aim of this prospective study was to investigate the relationship between the serum levels of fetuin-A and the progression of aortic valve calcification (AVC) assessed by multislice spiral computed tomography (MSCT) in non-dialyzed patients with established AVD. The impact of fetuin-A deficiency was assessed in comparison with other risk factors for AVD such as inflammation or traditional cardiovascular risk factors.

**Methods**

**Patient population and study protocol**

Patients with aortic sclerosis as well as patients with mild, moderate, or severe aortic valve stenosis according to echocardiography criteria were recruited. The exclusion criteria were increased serum calcium, chronic kidney disease (CKD) stages 4 or 5 at the time of screening, glomerular filtration rate (GFR) < 30 ml/min or malignant tumours. Ninety-six patients with echocardiographically proven AVC were screened. Patients with increased serum calcium (calcium > 2.6 mmol/L; n = 1), CKD stages 4 or 5 at the time of screening (n = 4), or malignant tumours (n = 2) were excluded from the study. Seven patients eligible for inclusion refused to give their consent.

The final patient cohort included 77 patients [mean age 70 ± 8 years, range 44–84 years, mean body mass index (BMI) 28.2 ± 4.6 kg/m²]. These patients were recruited consecutively at the echocardiography outpatient department or at the inpatient cardiology department of the University Hospital Aachen between May 2004 and July 2006.

The study protocol comprised two non-enhanced MSCT examinations. One was performed within two days after echocardiography shortly after inclusion into the study. After a mean follow-up of 12.6 ± 1.4 months, a second non-enhanced MSCT examination for quantification of AVC progression as well as echocardiography for evaluation of haemodynamic progression was performed.

In all patients, blood samples were taken at study inclusion after an overnight fast of at least 10 h to determine routine laboratory parameters as well as serum fetuin-A and high-sensitivity C-reactive protein levels. Study protocol was approved by the institutional review board. During follow-up, the existence or absence of any major adverse clinical event (MACE) was noted. Informed consent was obtained from each patient prior to the investigation in accordance with the requirements of the Local Ethics Committee.

**Echocardiography**

At study entry, echocardiographic data were obtained in all patients using a commercially available ultrasonographic system (GE Vingmed, Vivid 7, Horten, Norway). Echocardiography was performed by the same experienced echocardiographer (with 7 years experience) in all patients. Aortic valve sclerosis was defined as focal area of increased echogenicity and thickening of the aortic valve leaflets with a transaortic flow velocity < 2.5 m/s on transthoracic echocardiography, using the criteria of Otto et al.18,19 Aortic stenosis was classified as mild in the case of a transaortic flow velocity of ≥ 2.5 m/s and < 3.0 m/s. Patients with a transaortic flow velocity of ≥ 3.0 m/s and < 4.0 m/s were classified as moderate and patients with a transaortic flow velocity ≥ 4.0 m/s were classified as having severe aortic stenosis.19 In patients with left ventricular dysfunction (n = 13, 17%), defined as left ventricular ejection fraction < 55%, aortic valve area was calculated using the continuity equation.

**Coronary angiography**

Coronary angiograms were available in 62 (81%) patients and were assessed retrospectively by visual assessment. The presence of ischaemic heart disease, as defined by a history of myocardial infarction and/or coronary artery stenosis (≥ 50%) on coronary angiography, was evaluated. In addition, the clinical presentations of the patients were stratified according to the Canadian Cardiovascular Society (CCS) and New York Heart Association (NYHA) functional class.

**Multislice spiral computed tomography**

MSCT examinations were performed with a 16-slice MSCT scanner (Sensation 16, Siemens, Forchheim, Germany; collimation 12 x 0.75 mm, tube rotation time 420 ms, table feed 3.4 mm/rotation, tube voltage 120 kV with an effective tube current time product of 150 mAs,eff) using a standardized imaging protocol with retrospective electrocardiography gating. The average heart rate of the patients was 64.6 ± 4.1 b.p.m. Axial images were reconstructed at 60% of the RR interval, as recommended for coronary calcium screening with an effective slice thickness of 3 mm and a reconstruction increment of 2 mm using a dedicated convolution kernel (B35f).20 The field of view was 180 x 180 mm² with a 512² matrix.

MSCT images were assessed in a consensus reading by an experienced radiologist and an experienced cardiologist. Both readers were blinded to all patient data. Image analysis was performed on a separate computer workstation (Leonardo, Siemens, Forchheim, Germany) equipped with a dedicated software tool for calcium
scoring (Calcium Scoring CT, Siemens, Forchheim, Germany). For quantitative assessment of AVC, the Agatston AVC score was calculated with a detection threshold of 130 Hounsfield units. In addition, we calculated the Agatston score with the same detection threshold for assessment of mitral annular calcification. A detailed description of valvular calcification assessment has been published previously.

**Blood samples**

Venous blood was collected in the morning after an overnight fast of at least 10 h. Serum was collected after centrifugation at 2500 × g for 20 min and stored at −25 °C until further analyses. We routinely measured concentrations of total cholesterol, serum calcium, HDL and LDL cholesterol, triglycerides, and creatinine at hospital admission with standard autoanalyser methods. The GFR was assessed by the modified modification of diet in renal disease formula. Serum analysis for high-sensitivity C-reactive protein was performed by particle-enhanced immunonephelometry using a standard ‘CardioPhase hs-CRP’ (Dade Behring Holding GmbH, Liederbach, Germany), as described previously. Interday precision controls revealed coefficients of variation below 6%.

Fetuin-A serum measurements were performed by the nephelometry method using a polyclonal rabbit anti-human antibody against fetuin-A as previously described. The within-run precision obtained from a 20-fold measurement of identical samples revealed a variation coefficient of 7.75%. The day-to-day precision obtained from repetitive measurements of control serum was determined as a variation coefficient of 8.11%.

**Cardiovascular risk factors**

Cardiovascular risk factors were assessed from the patient interview and chart review. Risk factors recorded were nicotine abuse, hypertension (use of antihypertensive medication or blood pressure at rest > 140/90 mmHg), diabetes mellitus (use of insulin or oral antidiabetic agents or fasting serum glucose > 130 mg/dL), hypercholesterolaemia (total fasting serum cholesterol > 200 mg/dL or use of cholesterol-lowering medication), and obesity [BMI (body weight/body length²) > 30 kg/m²].

**Statistical analysis**

Continuous variables were expressed as mean values ± corresponding standard deviation and were compared using the Student’s t-test. Categorical data were summarized by absolute and relative frequencies. Pearson’s correlation coefficients were calculated between fetuin-A and the percental increase of mitral annular calcification scores.

Multifactorial analysis of covariance was used to explore the effects of fetuin-A levels, baseline AVC score, and the covariables sex, age, BMI, C-reactive protein, GFR, cholesterol, diabetes, hypercholesterolaemia, smoking status, and hypertension, on the progression of AVC scores. Because of the comparatively large numbers of independent factors, univariate analysis, using either simple linear regression (in the case of the continuous covariables AVC score, age, BMI, and GFR) or one-factorial analysis of variance (ANOVA; in the case of binary factors), were conducted first in order to select relevant factors possibly influencing the progression of AVC (factors with a P-value of ≤0.2); only these factors and GFR, as a parameter known to influence calcification, were examined in the final multivariate analysis. To evaluate a possible dependence of fetuin-A from diabetes status, we incorporated the fetuin × diabetes interaction term into our final multivariate model.

After fitting the final multivariate linear model, we checked the assumption that the error terms of this model have to be normally distributed by drawing a QQ plot of the error terms (i.e. comparing the observed quantiles of the error terms with according quantiles of a normal distribution). The inspection of the QQ plot of the error terms of this multivariable model indicated clear deviations from the normality assumption. Because of the right-skewed distribution of AVC scores, we decided to re-fit the model to log-transformed AVC scores instead of the obtained raw AVC scores. Checking the normal distribution of the error terms of this model again, the created QQ plot now suggested that the normality assumption of the error terms of this model appears to be fulfilled. Consequently, we used this model for drawing our conclusions regarding statistical significance.

In addition, we performed a multiple linear regression analysis to explore the effects of fetuin-A, peak transvalvular flow velocity, and baseline AVC score (independent variables) on MACE (dependent variable). Statistical significance of the independent factors used in these models was assessed by global F-tests. To illustrate the importance of fetuin-A levels, we compared patients with fetuin-A levels below and above the median, respectively, with respect to AVC progression in a descriptive post hoc analysis. In addition, we stratified the patients according to the development of the MSCT calcification score into two groups: ‘progressors’, with >10% increase in MSCT AVC score, and ‘non-progressors’, with <10% increase, as this cut-off value is clearly above the median interscan reproducibility of approximately 8% for MSCT data of patients with AVC. In addition, interobserver variabilities of AVC scores were calculated as the standard deviation of the absolute difference between two measurements divided by the mean of both the measurements, and expressed as a percentage. Interobserver correlations were evaluated using the Pearson’s correlation coefficient.

The global significance level of α = 0.05 was chosen for all statistical tests conducted. A two-sided P-value of ≤0.05 can be interpreted as a statistically significant test result. Statistical analysis was performed using SAS statistical analysis software package (Version 9.1, SAS Institute, Cary, NC, USA).

**Results**

**Baseline characteristics**

The clinical and laboratory baseline characteristics of the patient population are given in Table 1. The clinical presentation of the patients as well as coronary angiography data are shown in Table 2. There were no significant differences in CCS and NYHA functional class between patients with low fetuin-A levels (CCS class 1 ± 0.2; NYHA class 1.9 ± 0.9) and patients with high fetuin-A levels (CCS class 0.8 ± 1.1, P = 0.14; NYHA class 2.0 ± 0.7, P = 0.63). Fifty patients (65%) had coronary artery disease.

The mean calculated GFR was 64 ± 18 mL/min (range 30–132 mL/min) without significant differences in GFR between patients with low- and high-fetuin-A levels, respectively (Table 1). Thirty-two (42%) patients had CKD stage III (calculated GFR 30–59 mL/min), 39 (51%) patients showed CKD stage II (calculated GFR 60–89 mL/min), and six (8%) patients had a calculated GFR > 90 mL/min.

At baseline echocardiography, 32 patients (42%) demonstrated aortic sclerosis, 28 patients (36%) presented with mild aortic valve stenosis, 16 patients (21%) revealed moderate, and one
levels and patients with high fetuin-A levels were observed.

At study inclusion, the mean Agatston AVC score for the entire group was 1041.0 ± 1243 (range 0.4–7496). Patients with fetuin-A levels below the median (0.72 g/L) showed comparable baseline AVC scores compared with patients with fetuin-A levels above the median (1077 ± 1047 vs. 1003 ± 1413, P = 0.79). The mean increase of AVC score at follow-up was 22 ± 27% (median 17%, range –10% to 157%) for the whole study population.

Interobserver variability for the AVC score was 8.03%. In addition, interobserver correlation was r = 0.99.

**Table 1** Clinical and laboratory characteristics of the study cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall group (n = 77)</th>
<th>Low fetuin-A* (n = 39)</th>
<th>High fetuin-A* (n = 38)</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>70 ± 8</td>
<td>71 ± 8</td>
<td>69 ± 7</td>
<td>0.21</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.2 ± 4.6</td>
<td>28.6 ± 5.3</td>
<td>27.8 ± 3.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Hypertension</td>
<td>61 (79%)</td>
<td>28 (72%)</td>
<td>33 (87%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>58 (75%)</td>
<td>30 (77%)</td>
<td>28 (74%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Smoking</td>
<td>30 (39%)</td>
<td>15 (38%)</td>
<td>15 (39%)</td>
<td>0.93</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus</td>
<td>14 (18%)</td>
<td>6 (15%)</td>
<td>8 (21%)</td>
<td>0.52</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>50 (65%)</td>
<td>29 (74%)</td>
<td>21 (55%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Previous MI</td>
<td>20 (26%)</td>
<td>12 (31%)</td>
<td>8 (21%)</td>
<td>0.33</td>
</tr>
</tbody>
</table>

**Table 2** Clinical presentation and coronary angiography data of the study cohort

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall group (n = 77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical presentation</td>
<td></td>
</tr>
<tr>
<td>NYHA class</td>
<td>4 (5)/14(18)/37(47)/22(28)/0</td>
</tr>
<tr>
<td>CCS class</td>
<td>39 (51)/13 (17)/16 (21)/8 (10)/1 (1)</td>
</tr>
<tr>
<td>Coronary angiography data</td>
<td></td>
</tr>
<tr>
<td>No cath and no previous MI</td>
<td>15 (20)</td>
</tr>
<tr>
<td>Exclusion CAD by cath, n%</td>
<td>12 (15)</td>
</tr>
<tr>
<td>Single-vessel disease, n%</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Two-vessel disease, n%</td>
<td>14 (18)</td>
</tr>
<tr>
<td>Three-vessel disease, n%</td>
<td>27 (35)</td>
</tr>
</tbody>
</table>

Baseline aortic valve calcification score

At study inclusion, the mean Agatston AVC score for the entire group was 1041 ± 1243 (range 0.4–7496). Patients with fetuin-A levels below the median (0.72 g/L) showed comparable baseline AVC scores compared with patients with fetuin-A levels above the median (1077 ± 1047 vs. 1003 ± 1413, P = 0.79). The mean increase of AVC score at follow-up was 22 ± 27% (median 17%, range –10% to 157%) for the whole study population.

Interobserver variability for the AVC score was 8.03%. In addition, interobserver correlation was r = 0.99.

**Determinants of AVC progression**

Univariate analysis comprising clinical patient characteristics, cardiovascular risk factors, laboratory data, baseline AVC score, and fetuin-A revealed that only fetuin-A at baseline was significantly associated with the progression of AVC (P = 0.0002; Table 4). Figure 1 shows the significant linear correlation between systemic fetuin-A levels and the progression of AVC (r = −0.61, P < 0.001). In addition, there was also a significant association between fetuin-A levels and the progression of AVC in the subgroup of patients with aortic valve stenosis (P = 0.01).

A multivariate analysis comprising fetuin-A and GFR confirmed that only fetuin-A was associated with the progression of AVC (P = 0.0002). As shown in Figure 2, no significant relationship between the stage of CKD and the increase of AVC scores in patients with normal to moderately impaired renal function were observed. A post hoc analysis was conducted for patients with fetuin-A levels below and above the median, respectively. There were no significant differences in the baseline characteristics of patients with low fetuin-A levels vs. patients with high fetuin-A levels (Table 1). Patients with fetuin-A levels below the median showed a higher increase of AVC scores (34.6 ± 31.4%) than patients with fetuin-A levels above the median (10.0 ± 11.2%, P < 0.001, Figure 3). In addition, patients with a relevant increase of AVC scores > 10% (progressors; n = 48), clearly above the
median interscan reproducibility of 7.9% for MSCT data of patients with AVC (26), revealed significantly lower fetuin-A levels (0.66 ± 0.13) compared with non-progressors (n = 29) with a minimal or absence in the increase of AVC scores (0.82 ± 0.11, P < 0.001).

Haemodynamic course of aortic valve disease

There was no significant change in ejection fraction assessed by echocardiography between baseline and the follow-up examination after a mean of 12.6 months. As shown in Table 3, there was a mean increase of peak transvalvular aortic flow velocity of 9% from baseline to follow-up. However, no significant differences in the increase of aortic valve stenosis severity between patients with low fetuin-A levels and patients with high fetuin-A levels were observed (Table 3).

Mitral annular calcification

At study inclusion, in 13 patients mitral annular calcification was present (mean Agatston score for the entire group was 655 ± 899 (range 34–3191). The mean increase of mitral annular Agatston score at follow-up was 21 ± 28% (median 17%, range – 2% to 25%).

**Table 3** Echocardiographic data at baseline and 12-months follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall group (n = 77)</th>
<th>Low fetuin-A (n = 39)</th>
<th>High fetuin-A (n = 38)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (mm)</td>
<td>50 ± 7.8</td>
<td>50 ± 8.0</td>
<td>50 ± 7.7</td>
<td>0.97</td>
</tr>
<tr>
<td>EF (%)</td>
<td>57 ± 7.3</td>
<td>58 ± 8.2</td>
<td>57 ± 6.4</td>
<td>0.81</td>
</tr>
<tr>
<td>P&lt;sub&gt;max&lt;/sub&gt; (mmHg)</td>
<td>31 ± 16</td>
<td>31 ± 17</td>
<td>34 ± 15</td>
<td>0.48</td>
</tr>
<tr>
<td>P&lt;sub&gt;mean&lt;/sub&gt; (mmHg)</td>
<td>16 ± 9</td>
<td>16 ± 10</td>
<td>18 ± 9</td>
<td>0.46</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; (m/s)</td>
<td>2.57 ± 0.6</td>
<td>2.57 ± 0.7</td>
<td>2.58 ± 0.6</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Echocardiography at 12 months follow-up

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall group (n = 77)</th>
<th>Low fetuin-A (n = 39)</th>
<th>High fetuin-A (n = 38)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEDD (mm)</td>
<td>50 ± 6.9</td>
<td>49 ± 6.2</td>
<td>51 ± 7.8</td>
<td>0.15</td>
</tr>
<tr>
<td>EF (%)</td>
<td>56 ± 7.0</td>
<td>56 ± 7.8</td>
<td>55 ± 6.3</td>
<td>0.71</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt; (m/s)</td>
<td>2.76 ± 0.9</td>
<td>2.75 ± 0.9</td>
<td>2.77 ± 0.8</td>
<td>0.90</td>
</tr>
<tr>
<td>Increase P&lt;sub&gt;max&lt;/sub&gt; (%)</td>
<td>12.1 ± 30.0</td>
<td>11.5 ± 29.9</td>
<td>12.8 ± 30.6</td>
<td>0.86</td>
</tr>
<tr>
<td>Increase P&lt;sub&gt;mean&lt;/sub&gt; (%)</td>
<td>20.0 ± 33.0</td>
<td>24.1 ± 37.2</td>
<td>15.7 ± 28</td>
<td>0.27</td>
</tr>
<tr>
<td>Increase V&lt;sub&gt;max&lt;/sub&gt; (%)</td>
<td>9.02 ± 15.1</td>
<td>9.38 ± 15.4</td>
<td>8.65 ± 15.2</td>
<td>0.84</td>
</tr>
</tbody>
</table>

*P-values are for comparisons between low-fetuin-A and high fetuin-A group. P<sub>max</sub>, peak transvalvular aortic gradient; P<sub>mean</sub>, mean transvalvular aortic gradient; V<sub>max</sub>, peak transvalvular aortic flow velocity; EF, ejection fraction; LVEDD, left ventricular end-diastolic diameter.

**Table 4** Univariate analysis of clinical patient characteristics with respect to progression of aortic valve calcification (AVC)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>df</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetuin-A</td>
<td>1</td>
<td>15.06</td>
<td>0.0002</td>
</tr>
<tr>
<td>Age</td>
<td>1</td>
<td>0.36</td>
<td>0.5510</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.00</td>
<td>0.9627</td>
</tr>
<tr>
<td>Body mass index</td>
<td>1</td>
<td>0.37</td>
<td>0.5461</td>
</tr>
<tr>
<td>Baseline AVC score</td>
<td>1</td>
<td>0.08</td>
<td>0.7726</td>
</tr>
<tr>
<td>High-sensitivity C-reactive protein</td>
<td>1</td>
<td>0.33</td>
<td>0.5654</td>
</tr>
<tr>
<td>eGFR</td>
<td>1</td>
<td>0.26</td>
<td>0.7960</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1</td>
<td>0.88</td>
<td>0.3511</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1</td>
<td>0.55</td>
<td>0.4590</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>1</td>
<td>0.74</td>
<td>0.3926</td>
</tr>
<tr>
<td>Smoking</td>
<td>1</td>
<td>0.04</td>
<td>0.8431</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>1</td>
<td>0.04</td>
<td>0.8464</td>
</tr>
</tbody>
</table>

df, degrees of freedom; degrees of freedom for error 110; F-value, value of test statistic of global F-test; eGFR, estimated glomerular filtration rate.

![Figure 1](https://academic.oup.com/eurheartj/article-abstract/30/16/2054/630709) Correlation between serum fetuin-A levels and the progression of aortic valve calcification (r = –0.61; P = 0.0002 for fetuin-A). Regression equation: AVC (%) = 75.59 – 73.69 × Fetuin.
to 95%) for the whole study population. There was a moderate but significant correlation between serum fetuin-A levels and the per-
cental increase of mitral annular calcification ($P = 0.04$, $r = -0.58$).

**Major adverse clinical events**

During the mean follow-up of 12.6 months, six of 77 patients (8%) developed a MACE. Of these six patients, three underwent aortic valve replacement. Three patients suffered from onset of symptoms related to haemodynamic progression of AVD. Multiple linear regression analysis showed fetuin-A levels ($P = 0.034$) and peak transvalvular flow velocity ($P = 0.038$) as risk factors for MACE.

**Discussion**

This is the first longitudinal, prospective study investigating an association of serum fetuin-A levels with the progression of AVC. The main results of this study suggest that: (i) low levels of the systemic calcification inhibitor serum fetuin-A are associated with an accelerated progression of AVC in patients with established calcific AVD as well as with MACE; (ii) the AVC progression was already detectable while the haemodynamic profile of aortic valve stenosis or change in NYHA functional class within 12 months follow-up remain constant; (iii) the impact upon AVC pro-
gression was independent of the renal function, inflammation, baseline calcification level, as well as ‘traditional’ risk factors such as hypercholesterolaemia in non-dialyzed patients.

MSCT is a well-established and quantitative method for the assessment of valvular calcification with a low median interscan reproducibility of approximately 8%. Thus, MSCT enables to reliably study and monitor the progression of AVC over time. This is of special importance as AVC load is associated with the aortic valve stenosis haemodynamic severity with a strong diagnos-
tic value in predicting the presence of severe aortic valve steno-
sis. In addition, AVC load provides an independent outcome determinant in aortic valve stenosis. The present data corroborate the rapidly progressive nature of calcific AVD reflected by the mean annual AVC score increase of 22%. In addition, the data suggest that the progression of AVC precede the haemodynamic progression of aortic valve stenosis assessed by echocardiography. Thus, identification of possible determinants of accelerated AVC progression other than the previously mentioned association with certain cardiac risk factors are of particular interest.

In this context, calcification inhibitor deficiency may be of importance. An inverse association of low fetuin-A serum levels with valvular calcification and cardiovascular mortality has been reported in ESRD patients. Two previous cross-sectional studies showed an association of low fetuin-A levels with calcific AVD in patients with normal or moderately impaired renal func-
tion.16,17 The larger of these two studies stratified patients into ter-
tiles of fetuin-A serum levels. In non-diabetic patients, there was a significantly higher prevalence of aortic stenosis in the lowest fetuin-A tertile compared with the highest tertile (12% vs. 4%).16 In a recent study reported by Kaden et al., lower serum fetuin-A levels were demonstrated in patients with calcific AVD compared with healthy controls, independent of the renal function. In contrast to the latter study, which exclusively included patients with severe aortic valve stenosis, the present study is longitudinal and mainly included patients with mild and moderate aortic valve stenosis.

Prior cross-sectional studies showed an association between fetuin-A levels and the presence of AVC in ESRD patients at baseline.14,16 In contrast to these studies, we quantitated the amount of valvular calcification by MSCT in a patient cohort comprising mainly patients with moderately impaired renal function and we
were not able to show an association between fetuin-A levels and the severity of valve calcification at baseline. Thus, fetuin-A may not always reflect a given amount of pre-existing calcification but rather reflects ongoing calcification. This may partially result from the fact that fetuin-A exerts a decisive role as an inhibitor of pathological calcification at multiple stages of disease progression. The importance of fetuin-A calcification inhibitor deficiency for the development of valvar calcifications was recently demonstrated using a Ahsg (fetuin)−/−/apolipoprotein E (ApoE−/−) double-deficient murine model.32 In this study, Westenfeld et al.32 were able to show in an established murine model of atherosclerosis that fetuin-A deficiency enhanced aortic calcification and AVC in ApoE-deficient mice. These experimental data support our findings and the relevance of fetuin-A for the progression of AVC.

The longitudinal data of our study are the first to suggest that low fetuin-A levels are associated with an accelerated progression of AVC. No previous study, neither in dialysis nor in non-dialysis patients, used two MSCT examinations to exactly quantify the development of AVC over time. It is noteworthy that both univariate and multivariate analysis in our study point towards a close association between C-reactive protein levels and the progression of AVC, which has been reported recently demonstrating no association between C-reactive protein levels and the progression of AVC.33 Moreover, progressors (increase of AVC score) showed significantly lower serum fetuin-A levels than non-progressors.

In addition, low fetuin-A levels were significantly associated with MACES demonstrating the possible impact on patient management, which has to be evaluated in future studies. Although the baseline AVC score failed to reach statistical significance as a risk factor for MACES in our study, presumably because of one patient with minor valve calcification in our small subgroup with MACE, the importance of AVC score for adverse clinical events has been demonstrated in previous studies.28–30

Interestingly, we did not observe an association between C-reactive protein levels and the progression of AVC in our study, although the overall importance of inflammation for the development of cardiovascular disease and calcification has been elucidated in the last few years.34 This is in line with a similar observation, which has been reported recently demonstrating no association between C-reactive protein levels and the progression of AVD.35

Limitations

Some limitations of the study should be acknowledged. The findings of the study are based on a relatively small patient population with known AVC from a single-centre as well as a short follow-up interval. Therefore, larger multi-centre studies with longer follow-up intervals are needed to confirm these findings. However, a longer follow-up with repeated annual MSCT examination for evaluation of AVC progression may be considered unethical because of the radiation exposure. Thus, data on the relationship between morphology, i.e. valvar calcification and functional echocardiographic data in the long-term follow-up is still missing.

Clinical implications

Taking the possible role of low fetuin-A levels in the progression of AVC into account, an important question arises regarding the therapeutic influence of serum fetuin-A levels in patients with AVC. There is only limited data in patients with severely impaired renal function available indicating that certain oral phosphate-binders elevate serum fetuin-A levels.36 A therapeutic approach has not yet been investigated in patients with mildly impaired or normal renal function.

Larger future studies are needed to evaluate if fetuin-A might serve as a biomarker to identify those at risk for accelerated AVC progression as well as for MACE not only in calcific AVD but also for the calcific valvar deposition in patients with aortic valve bioprostheses. Possibly, an accelerated calcification of bioprostheses may decrease the durability of aortic valve bioprostheses.

Conclusions

In summary, the present data support an association of the humoral factor fetuin-A with the progression of AVC in non-dialyzed patients and show an association between fetuin-A and MACE. However, a yet unmet issue is the question whether the association of low serum fetuin-A and a rapid progression of AVC is directly causal or whether a yet unidentified trigger causes both low fetuin-A levels and progressive AVC.

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References


