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Impact of Fetuin-A on progression of calcific aortic valve stenosis - The COFRASA - GENERAC study

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ABSTRACT

Background: Aortic stenosis (AS) is an active disease, but the determinants of AS progression remain largely unknown. Low levels of Fetuin-A, a powerful inhibitor of ectopic calcification, have been linked to ectopic calcium tissue deposition but its role in AS progression has not been clearly evaluated.
Methods: In our ongoing prospective cohort (COFRASA/GENERAC), serum Fetuin-A level was measured at baseline and AS severity was evaluated at baseline and yearly thereafter using echocardiography (mean pressure gradient (MPG)) and computed tomography (degree of aortic valve calcification (AVC)). Annual progression was calculated as [(final measurement-baseline measurement)/follow-up duration] for both MPG and AVC measurements.
Results: We enrolled 296 patients (74 ± 10 years,73% men); mean follow-up duration was 3.0 ± 1.7 years. No correlation was found between baseline serum Fetuin-A (0.55 ± 0.15 g/L) and baseline AS severity ($r = 0.25$, $p = 0.87$ for MPG; $r = 0.06$, $p = 0.36$ for AVC). More importantly, there was no correlation between baseline serum Fetuin-A level and AS progression either assessed using MPG or AVC (both $r = 0.01$, $p = 0.82$). In bivariate analysis, after adjustment for age, gender, baseline AS severity, or valve anatomy, Fetuin-A was not associated with AS progression (all $p > 0.20$). The absence of link with AS progression was further confirmed by the absence of link betwen serum Fetuin-A and the occurrence of AS-related events ($p = 0.17$).
Conclusions: In a large prospective cohort of AS patients, serum Fetuin-A was not associated to hemodynamic or anatomic AS progression. Despite its capacity to inhibit ectopic calcium deposition, Fetuin-A serum level seemed to have minor influence on AS progression.

1. Introduction

Aortic valve stenosis (AS) is the most common valvular heart disease in Western countries [1] and its prevalence is going to dramatically increase with the ageing of the population [2,3]. Hemodynamic consequences of AS are due to progressive calcium deposition within the valve leaflets [1]. There are strong clinical and experimental evidences showing that AS is not a passive degenerative disease, but rather an active process that involves several pathways, including lipid infiltration, chronic inflammation, fibrosis formation, osteoblastic activation, and mineralization within the aortic valve [4]. However, despite important progresses in the understanding of AS pathophysiology, mechanisms governing AS progression remain largely unknown. There is currently no medical therapy that can either prevent or slow down AS progression and aortic valve replacement is the only available therapy. Thus, elucidating the determinants of AS progression is a critical issue with major clinical and therapeutic implications.

Fetuin-A belongs to the cystatin superfamily of cysteine protease inhibitors and is a major component of mineralized bone. Fetuin-A, which is primarily produced in adults by the liver, is a strong inhibiting factor of ectopic calcification [5–7]. Fetuin-A acts by binding clusters of calcium and phosphate to stabilize these ions and prevent uptake by

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cells [8]. Furthermore, Fetuin-A deficiency is associated with soft tissue calcification in experimental models and serum Fetuin-A levels are lower in animal models with aortic valve calcification compared to controls [9–12]. Although several experimental studies have investigated the effect of Fetuin-A on valve calcification, clinical studies evaluating the impact of serum Fetuin-A level on AS progression are scarce [13,14]. Thus, in an ongoing prospective cohort of AS patients, we sought to evaluate the relationship between serum Fetuin-A level and AS progression.

2. Methods

2.1. Study design

The population comprised asymptomatic AS patients enrolled between November 2006 and March 2015, in an ongoing prospective cohort COFRASA/GENERAC (clinical Trial.gov number NCT 00338676 and clinical Trial.gov number NCT00647088) aiming at evaluating the determinants of AS progression with at least 1 year of follow-up. Inclusion criteria were pure, at least mild (defined by a mean pressure gradient (MPG) ≥10 mmHg and aortic valve structural changes (thickening/calcification)), isolated, degenerative asymptomatic AS (patients had to be free of dyspnea, angina and chest pain). Exclusion criteria were rheumatic disease or radiotherapy, prior infective endocarditis, more than mild coexisting aortic regurgitation (defined by a vena contracta width ≥ 3 mm or a regurgitant volume ≥ 30 ml) or associated valvular disease and severe renal insufficiency (creatinine clearance ≤30 ml/min). All patients underwent a comprehensive clinical evaluation, a transthoracic echocardiography (TTE) and a multislice computed tomography (MSCT) at enrollment and yearly thereafter. Measurement of fetuin-A was performed at baseline. Echocardiographic, MSCT and fetuin-A measurements were performed blinded one of each other. Patients were contacted every 6 months and seen at our research center every year. Occurrence of AS related events (sudden death, congestive heart failure, or new onset of symptoms (dyspnea, angina or syncope)) was prospectively recorded. Our regional ethic committee approved the study and all patients gave a written informed consent.

2.2. Echocardiography

All echocardiographies were performed at baseline and on yearly basis by the same experienced echocardiographer (last author) using commercially ultrasonographic systems (Vivid 7 ® General Electric or iE33/Epic ® Phillips). Severity of AS was evaluated based on peak velocity (PV), mean pressure gradient (MPG) and the aortic valve area (AVA) calculated using the continuity equation as written by current guidelines [15]. AVA was calculated as an absolute value and indexed (AVAi) to body surface area (BSA). Mild AS was defined by MPG <20 mmHg (or AVA between 1.5 and 2 cm^2, PV between 2 and 3 m/s), moderate AS defined as MPG between 20 and 40 mmHg (or AVA between 1 and 1.5 cm^2, PV between 3 and 4 m/s) and severe AS as a MPG > 40 mmHg (or AVA <1 cm^2, PV > 4 m/s). Aortic valvular anatomy (bicuspid or tricuspid aortic valve) was determined using TTE at inclusion in a parasternal short axis view. LV mass was calculated using Devereux's formula. LV ejection fraction (LVEF) was assessed using the biplane Simpson method or visually and considered abnormal if <50%.

2.3. Multislice computed tomography

MSCT examinations was carried out same day than TTE using a Philips scanner (MX 8000 IDT 16, Phillips Medical Systems, Andover, MA, USA) or a General Electric scanner (Light speed VCTTM, General Electric Company, Fairfield, Connecticut, USA) [16–19]. A scan run consisted of a prospective acquisition of 43-mm thick contiguous transverse slices. Acquisition time was 0.5 s/slice ECG triggered at 75% of the RR interval. No contrast enhancement was needed nor was a beta-blocker administered for the purpose of the examination. Measurements were performed using dedicated semi-automatic software (Heart Beat Calcium Scoring, Philips Medical Systems or SmartScore, General Electric Medical Systems). Calcification was defined as four adjacent pixels with density >130 Hounsfield units. The degree of AVC was quantitatively assessed according to the Agatston method (calcium score) expressed in arbitrary units (AU). AVC was defined as calcification within the valve leaflets, aortic annulus, or aortic wall immediately adjacent to leaflet or annular calcification. Two MSCT runs were performed sequentially with 1 or 2 mm initial interval. Each run was independently scored and the two scores were averaged. Radiation exposure was typically between 2 and 3 mSV. MSCT images were evaluated by experienced radiologists. Readers were blinded of clinical, echocardiographic and biological data.

2.4. Blood samples

All blood samples were collected from a peripheral vein at inclusion and processed under identical conditions, at 08:00 after 12-h fasting the same day than the TTE. Concentrations of total cholesterol, serum calcium, phosphate high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, triglycerides, creatinine were routinely measured using standard methods. The remaining serum was separated within 30–60 min of collection and aliquots were frozen to −80 °C and kept on site without freeze–thaw cycles. All fetuin-A dosages were performed at 1 core laboratory blinded of any clinical or imaging information by an ELISA sandwich method (Epitope Diagnostics Inc. Human Fetuin A ELISA, Immunodiagnostic Systems,Ltd, Tyne and Wear, UK) using 2 selected polyclonal goat antibodies against different epitopes of human Fetuin-A. The within-run and between run precision were respectively <5% and < 7%. The 95% percentile normal range was 0.35–0.95 g/L (mean 0.57 g/L).

2.5. Statistical analysis

Continuous variables were expressed as mean values ± standard deviation or median (IQR), or number of patients (per cent). As MPG and AVC were not normally distributed a log transformation was used. Annualized progression was calculated as (final measurement - baseline measurement)/follow-up duration for hemodynamic and anatomic measurements. Comparisons between patients groups with Fetuin-A above or below the median range were performed using Student's *t*-test, χ^2 test, Mann-Whitney-Wilcoxon test, and Kruskal-Wallis test as appropriate. Univariate and bivariate regression model were conducted to assess impact of Fetuin-A level on hemodynamic and anatomic AS progression after adjustment for age, sex, baseline AS severity or valve anatomy. Event-free survival (composite endpoint of AS-related events defined by sudden death, congestive heart failure, or new onset of symptoms (dyspnea, angina or syncope)) was assessed using the Kaplan-Meier analysis. Comparison of event-free survival according to Fetuin-A median level was performed using log-rank test. Cox proportional hazard model was used to evaluate the predictive value of Fetuin-A level for event-free survival. A p value <0.05 was considered statistically significant. Statistical analyses were performed using JMP 10 software (SAS institute, Cary, NC, USA).

3. Results

3.1. Baseline characteristics

A total of 296 patients constituted our population. Patient characteristics are summarized in Table 1. Briefly, 73% (217/296) were men, mean age was 74 ± 10 years and 22% (66/296) had diabetes. History of coronay artery disease (CAD) was present in 31% (93/296). By design no patients had severe renal insufficiency and mean creatinine clearance was 77 ± 21 ml/min/m^2. Mean left ventricle efection fraction (LVEF) was 64 ± 5%. The aortic valve was tricuspid in 76% (225/296) and bicuspid in 24% (71/296). Mean MPG at enrollement was 25 ± 16 mmHg and 44% (130/296) had mild AS, 43% (127/296) moderate AS and 13% (39/296) severe AS. Mean AVC was 1339 ± 1217 AU.

3.2. Overall progression

Mean follow-up duration was 3.0 ± 1.7 years. Hemodynamic and anatomic AS progression are presented in Table 1. MPG increased from 25 ± 16 mmHg to 34 ± 19 mmHg, (+4 ± 4 mmHg/year (median + 2 mmHg, [1–6])) and mean AVC score increased from 1339 ± 1217 AU to 1934 ± 1640 AU (+223 ± 301 AU/year (median + 132, [47–283])).

3.3. Determinants of Fetuin-A

Mean Fetuin-A was 0.55 ± 0.15 g/L (0.53 g/L [0.44–0.61]). As shown in the right part of Table 1, patients with Fetuin-A level above the median value were younger (71 ± 10 vs 74 ± 10 years, p = 0.001), more frequently smoker (56% vs 44%, p = 0.04), presented with higher LDL-cholesterol and Triglycelides levels (2.9 ± 0.9 vs 2.5 ± 0.9 mmol/l, p = 0.0007 and 1.4 ± 0.7 vs 1.2 ± 0.7 mmol/l, p = 0.02 respectively) than those with Fetuin-A level below the median value. There were no other difference regarding clinical and biological characteristics. No correlation was found between Fetuin-A level and baseline parameters of AS severity (r = 0.25, p = 0.87 with MPG (mmHg) and r = 0.06, p = 0.36 with AVC (AU)) (Fig. 1A, B). Fetuin-A level was aslo not different between patients with mild, moderate and severe AS (p = 0.14) (Fig. 1D). There was a significant although modest inverse correlation between age and Fetuin-A level (r = −0.21, p = 0.03) (Fig. 1C) but none with creatinine clearance (r = 0.0044, p = 0.45).