Valvular expression of factor XI correlates with valve calcification and aortic stenosis severity

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INTRODUCTION

Aortic stenosis (AS) is a progressive disease with a pathogenesis similar to atherosclerosis [1]. AS progression is associated with aortic valve orifice and leaflet mobility reduction, and there is no available pharmacological treatment to prevent or at least retard disease progression [1]. Currently, the only therapeutic options for AS are surgical aortic valve replacement or transcatheter aortic valve implantation [2, 3]. Associated activation of both coagulation and inflammation leading to valvular calcification has been shown in AS [4, 5]. Growing evidence indicates a contribution of factor XI (FXI) to thrombosis [6] and atherogenesis [7]. However, the role of the intrinsic pathway of coagulation, especially FXI expression, in AS progression has not been studied. FXI plays an important role in blood coagulation and its activation to FXIa is mediated by activated FXII (FXIIa), through the feedback activation by tissue factor (TF)/thrombin, or *via* autoactivation [8]. FXIa converts FIX to its active form, but its activation is also catalyzed by the FVIIa-TF complex [8]. Kossmann et al. [9] demonstrated that FXI inhibition in mice, beyond antithrombotic effects, protects *also* against vascular inflammation, namely reactive oxygen species formation, leukocyte infiltration, and fibrotic remodeling. Importantly, FXIa inhibitors and antibodies to FXI reduced atherogenesis and inflammation in mice [10]. Here we investigated whether FXI is present within stenotic leaflets in severe AS patients and if its expression correlates with disease severity.

METHODS

We enrolled 20 patients between April 2022 and June 2023 with symptomatic severe AS. All patients underwent first-time elective surgical aortic valve replacement at the Department of Cardiovascular Surgery and Transplantology, John Paul II Hospital, Kraków, Poland. Data on demographics, medical history, and current treatment were collected using a standardized questionnaire. Severe AS was defined as mean transvalvular pressure gradient (PG_{mean}) ≥40 mm Hg, peak transvalvular velocity (V_{max}) ≥4.0 m/s, and aortic valve area (AVA) ≤1 cm^2 on transthoracic echocardiography [11]. Arterial hypertension, hypercholesterolemia, and atherosclerosis were diagnosed as previously described [12]. The exclusion criteria for AS patients included atherosclerotic vascular disease requiring revascularization, acute infection including infective endocarditis, rheumatic AS, diabetes mellitus, advanced chronic kidney disease, need for concomitant valvular surgery (e.g., mitral valve repair), percutaneous coronary intervention, recent (<3 months) acute coronary syndrome or cerebrovascular episode, diagnosed malignancy, and pregnancy. Angiographically documented coronary artery stenosis greater than 20% of the diameter was the exclusion criterion to avoid any influence of nonobstructive atherosclerosis [12, 13].

The ethics committee approved the study (8/KBL/OIL/2019 and 53/KBL/OIL/2022), and all participants provided their written informed consent in accordance with the Declaration of Helsinki.

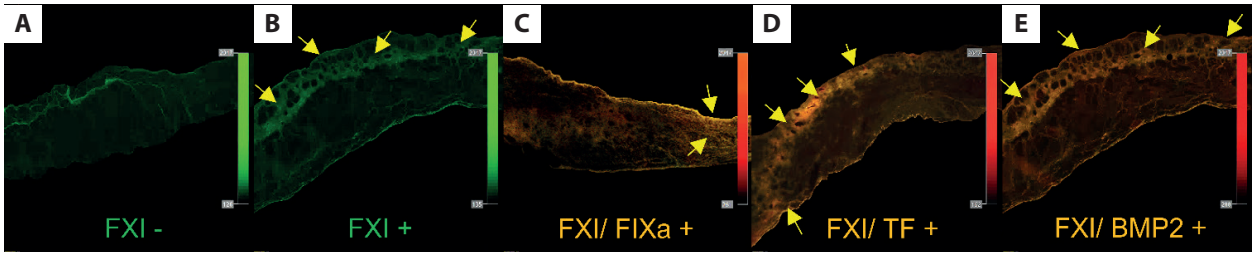

Figure 1. Valvular factor (F)XI expression together with active FIX(a), tissue factor (TF), and bone morphogenetic protein 2 (BMP2). Representative microphotographs of **A**. Valvular expression of (FXI) in control leaflets and **B**. Stenotic leaflets. **C**–**E**. Colocalization (orange) of FXI (green) and active FIX(a) (FIXa), TF, or bone morphogenetic protein 2 (BMP2) (red) within stenotic leaflets. Red arrowheads indicate the aortic side of the leaflet; yellow arrowheads indicate the immunopositive areas. Scale bar 200 µm, original magnification 4×

Fasting venous blood was drawn before aortic valve replacement. Citrated blood (9:1 of 0.106 M sodium citrate) was centrifuged at 2500 g for 20 minutes at 20°C, while blood drawn into serum tubes was centrifuged at 1600 g for 10 minutes at 4°C. Routine laboratory assays were used to determine glucose, creatinine, lipid profile, C-reactive protein, and fibrinogen.

Aortic valves were collected during open heart surgery, embedded in Cryomatrix (Thermo Scientific, Kalamazoo, MN, US), and sectioned into 5 µm slices with a Leica CM1520 cryostat. Five control valves were obtained at autopsy from apparently healthy individuals of similar age.

Activation of FXI was assessed indirectly by double staining of FXI with active FIX (FIXa) or TF. Immunostaining was conducted according to the previously described protocol [12] using primary antibodies against FXI (Santa Cruz Biotechnology, Dallas, TX, US), FIXa (Antibodies-online, Aachen, Germany), TF (Abcam, Cambridge, UK), and bone morphogenetic protein 2 (BMP2; Abcam). The corresponding secondary antibodies conjugated with AlexaFluor 488 or 594 (Abcam) were applied. A negative IgG isotype control was performed routinely. Olympus BX43 microscope (Tokyo, Japan) was used to analyze the images. The percentage of immunopositive areas was calculated as previously described [12, 13], and 15 serial step sections were analyzed per valve by two independent observers blinded for the sample origin.

Statistical analysis

All statistics were performed using STATISTICA software (Version 13.3, TIBCO Software, Palo Alto, CA, US). Categorical variables were presented as numbers and percentages, while continuous variables were expressed as means and standard deviations (SD) or medians and quartiles Q1–Q3. Normality was analyzed by the Shapiro-Wilk test. Associations between variables were calculated using Pearson or Spearman correlation coefficients, as appropriate. A *P*-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Baseline characteristics of AS patients are shown in Table S1 (Supplementary material, *Table S1*). *In loco* analysis revealed valvular expression of FXI within all studied stenotic valves, but not within control ones (Figure 1A and B). The mean (SD) FXI-immunopositive valve area constituted 21.5 (1.4)% of the total leaflet area, while means (SD) for FIXa and TF were 17.2 (2.5)% and 26.5 (5.1)%, respectively. The mean (SD) BMP2-positive area was 23.9 (4.1)%. The expression of studied proteins was observed at the aortic side of the stenotic leaflets and presented a condensed pattern of fluorescence. Interestingly, the expression of FXI co-expressed with FIXa in 66%, suggesting local activation of the intrinsic pathway as well as in 71% with TF, the major component of the extrinsic coagulation pathway (Figure 1C and D). Moreover, FXI co-expressed with BMP2 in 83% (Figure 1E), which supports our previous finding that coagulation activation is involved in leaflet calcification [4, 5, 12, 14]. Importantly, valvular amounts of FXI correlated with disease severity reflected by V_{max} (r = 0.54; *P* = 0.01), both transvalvular pressure gradients (PG_{mean} r = 0.49; *P* = 0.03; PG_{max} r = 0.53; *P* = 0.02), and AVA (r = –0.53; *P* = 0.02) (Supplementary material, *Figure S1*). Valvular expression of FIXa correlated with AVA (r = –0.49; *P* = 0.03) and V_{max} (r = 0.44; *P* = 0.049) but not transvalvular pressure gradients (both *P* >0.05).

To the best of our knowledge, this report provides the first evidence that the intrinsic coagulation pathway is activated within stenotic aortic valves. A strong co-expression of FXI with FIXa, TF, and BMP2 highlights the involvement of both coagulation pathways in valve calcification. Coagulation activation within stenotic valves is implicated in both valvular inflammation and calcification *via* the nuclear transcription factor kappa B (NF-κB) pathway [12]. Since the current study showed that abundant FXI valvular expression was associated with disease severity, it is tempting to speculate that FXIa inhibitors, such as asundexian or milvexian [15] might not only attenuate coagulation activation but also inflammatory response and thus retard AS progression. Importantly, phase II clinical trials showed that FXIa inhibitors prevent stroke and systemic embolism in patients with atrial fibrillation without increasing bleeding risk compared to non-vitamin K antagonist oral anticoagulants [15]. Therefore, FXIa inhibition might offer a novel strategy for preventing AS development and/or progression, at least in patients with an indication for anticoagulant therapy. Moreover, taking into account FXI

contribution to inflammation in atherosclerosis, targeting FXI might influence the cross-talk between coagulation and inflammation, resulting in retardation of aortic valve leaflets calcification. Clinical relevance of our findings requires further studies.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/kardiologia_polska.

Article information

Conflict of interest: None declared.

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