

SERUM RANTES, TRANSFORMING  
GROWTH FACTOR- $\beta$ 1 AND  
INTERLEUKIN-6 LEVELS CORRELATE  
WITH CARDIAC MUSCLE FIBROSIS IN  
PATIENTS WITH AORTIC VALVE STENOSIS

# INTRODUCTION

Progressive aortic valve degeneration leads to severe aortic valve stenosis (AS) in approximately 2 – 7% of the population over 65 years of age . The mechanisms of aortic valve degeneration are multifactorial and not fully understood.

Population-based studies showed a correlation between age and the prevalence of calcific AS . Once calcifications appear, pro-calcific and pro-fibrotic mechanisms are more active, leading to progressive valve degeneration .

Some postulated factors driving AS progression include influence of classic atherosclerotic risk factors.

In AS, not only the aortic valve is affected. In fact, stenosis leads to extravalvular cardiac complications such as left ventricular (LV) remodeling, LV diastolic dysfunction, mitral valve regurgitation, left atrium damage, pulmonary circulation overload, and right ventricular dysfunction.

Myocardial fibrosis results from increased myofibroblast activity and excessive extracellular matrix deposition. Various cells and molecules are thought to be involved in this process, providing targets for potential drug therapies, including transforming growth factor ( $\text{TGF-}\beta$ ), endothelin-1, fibroblast growth factor, matrix metalloproteinases (MMPs), and cytokines such as interleukins (IL-1, IL-6) and tumor necrosis factor ( $\text{TNF-}\alpha$ ). Also the animal model showed an important role of endothelial nitric oxide synthase in hypertrophy remodelling .

# METHODS

- Study population
- Magnetic resonance imaging
- Inflammatory biomarkers
- Echocardiography
- Statistical analysis

# Study population

Forty consecutive patients with moderate (defined as an aortic valve area between 1.0 – 1.5 cm<sup>2</sup> measured using the continuity equation) to severe (AVA < 1.0 cm<sup>2</sup>) AS and without previous history of acute coronary syndromes were included in the study. CAD significance was assessed by coronary angiography or computed tomography angiography. Symptoms of angina were analyzed and estimated based on the Canadian Cardiovascular Classification (CCS) and physical activity limitation based on the New York Heart Association (NYHA) Functional Classification. Additionally, physical activity was measured during the 6-minute walking test (6MWT).

# Magnetic resonance imaging

LV end-diastolic volume and diameters, LV end-systolic volume and diameters, LV ejection fraction (LVEF), and myocardial thickness and mass were determined by magnetic resonance imaging (MRI)

Cine imaging was performed in LV 2-, 3-, and 4-chamber apical views as well as in short-axis views encompassing the LV myocardium using balanced steady-state free precession (SSFP) gradient echo (generalized autocalibrating partially parallel acquisition (GRAPPA))

# Inflammatory biomarkers

Fasting blood was drawn from an antecubital vein without tourniquet and placed in a collection tube. Within 30 minutes of blood collection, plasma was centrifuged for 15 minutes at  $1600 \times g$  at  $4^{\circ}\text{C}$ . Collected serum aliquots were immediately stored at  $\leq -70^{\circ}\text{C}$  until analysis. Biomarker serum levels were determined by ELISA (Human CCL5/RANTES Immunoassay no. DRN00B, Human TGF- $\beta$ 1 Immunoassay no. DB100B, and high sensitivity Human IL-6 Immunoassay no. HS600B, R&D systems, Minneapolis, MN, USA) following the manufacturer's instructions.



# Echocardiography

Comprehensive transthoracic echocardiography was performed in all patients after  $\geq 30$  minutes of rest by 2 independent cardiologists certified in echocardiography. All measurements including the severity of aortic stenosis, dimensions, and LV systolic function were assessed according to European Association of Cardiovascular Imaging (EACVI) guidelines

# Statistical analysis

Table 1. Group I and II baseline characteristics, risk factors, and pharmacotherapy

	<b>Group I</b>	<b>Group II</b>	<b>P-value</b>
Age [years]	65 (9)	67 (8)	0.289*
Female, n (%)	10 (50.0)	10 (50.0)	0.879
Hypertension, n (%)	20 (100.0)	18 (90.0)	0.157
Dyslipidemia, n (%)	20 (100.0)	18 (90.0)	0.157
Diabetes, n(%)	6 (30.0)	5 (25.0)	0.655
Impaired glucose tolerance, n (%)	1 (5.0)	1 (5.0)	0.972
CAD, n (%)	15 (75.0)	17 (85.0)	0.343
Previous PCI, n (%)	6 (30.0)	2 (10.0)	0.098
Stroke, n (%)	1 (5.0)	0 (0.0)	0.300
Smoking history, n (%)	7 (35.0)	1 (5.0)	0.018
BMI [kg/m <sup>2</sup> ]	30.8 (6.0)	30.3 (5.6)	0.904*
Hemoglobin (Hgb) [g/dl]	13.9 (1.7)	13.8 (1.6)	0.862*
Creatinine [umol/l]	85.0 (19.0)	79.0 (18.0)	0.355*
LDL-cholesterol [mmol/l]	3.5 (1.2)	2.8 (1.1)	0.113*
<b>NYHA class, n (%)</b>			< 0.001
I	12.0 (60.0)	2.0 (10.0)	
II	6.0 (30.0)	16.0 (80.0)	
III	0.0 (0.0)	2.0 (10.0)	
<b>CCS class, n (%)</b>			0.029
0	14.0 (70.0)	20.0 (100.0)	
I	3.0 (15.0)	0.0 (0.0)	
II	3.0 (15.0)	0.0 (0.0)	
6-MWT [m]	385 (67)	389 (82)	0.829
<b>Pharmacotherapy</b>			
ACE-I, n (%)	10.0 (55.6)	15.0 (78.9)	0.106
ARB, n (%)	6.0 (33.3)	0.0 (0.0)	0.005
Beta-blocker, n (%)	17.0 (94.4)	17.0 (89.5)	0.612
Diuretic, n (%)	9 (45.0)	9 (45.0)	0.544
Statin, n (%)	18.0 (100.0)	18.0 (94.7)	0.336
Ca-blocker, n (%)	9.0 (50.0%)	3.0 (15.8%)	0.026
Acetylsalicylic acid, n (%)	17.0 (94.4%)	18.0 (94.7%)	0.612

\*Mann-Whitney U test.

Table 2. Echocardiography and MRI measurements.  
 Abbreviations: SV, stroke volume; LVEDD, left ventricle end diastolic diameter; LVEDV, left ventricle end diastolic volume; LA, left atrium; LGE, late gadolinium enhancement.

	<b>Group I</b>	<b>Group II</b>	<b>P-value</b>
<b>Echocardiography</b>			
Ejection fraction, %	71.6 (4.5)	66.4 (10.0)	0.108*
Stroke volume [ml]	103.1 (17.7)	89.0 (17.6)	0.242*
Indexed SV [ml/m <sup>2</sup> ]	47.9 (9.1)	44.4 (10.1)	0.231*
LVEDD [mm]	48 (4.9)	49 (6.5)	0.602*
Indexed LVEDV [ml/ m <sup>2</sup> ]	67.1 (13.4)	69.3 (22.7)	0.779*
LA area [cm <sup>2</sup> ]	25.0 (4.0)	25.1 (5.7)	0.883*
Peak aortic valve gradient [mmHg]	48.9 (9.5)	90.8 (25.6)	< <b>0.001</b> *
Mean aortic valve gradient [mmHg]	28.8 (6.8)	57.9 (14.4)	< <b>0.001</b> *
Aortic valve area [cm <sup>2</sup> ]	1.2 (0.2)	0.7 (0.2)	< <b>0.001</b> *
Peak aortic valve velocity[m/s]	3.5 (3.2:3.8)	4.7 (4.3:5.1)	< <b>0.001</b> *
<b>Magnetic resonance</b>			
Ejection fraction [%]	73.1 (6.1)	70.0 (11.6)	0.947*
Stroke volume [ml]	103.8 (27.9)	95.9 (22.8)	0.369*
Indexed LV mass [g/m <sup>2</sup> ]	74.4 (26.7)	80.8 (24.2)	0.221*
LVEDD [mm]	49.4 (6.8)	48.4 (7.0)	0.632
Indexed LVEDV[ml/m <sup>2</sup> ]	74.3 (17.7)	76.3 (23.8)	0.766
LA area [cm <sup>2</sup> ]	26.4 (6.0)	25.0 (9.5)	0.314*
LGE, n (%)	5.0 (25.0)	8.0 (40.0)	0.311*
LGE mass [g]	5.4 (4.9)	3.7 (2.7)	0.724*

	<b>RANTES</b>	<b>IL-6</b>	<b>TGF-<math>\beta</math>1</b>
<b>Echocardiography</b>			
Ejection fraction	0.152 (0.349)	0.025 (0.88)	<b>0.328 (0.043)</b>
Peak aortic valve gradient	-0.216 (0.182)	-0.075 (0.646)	-0.227 (0.158)
Mean aortic valve gradient	-0.220 (0.173)	-0.083 (0.609)	-0.212 (0.189)
Aortic valve area	0.209 (0.195)	0.074 (0.651)	0.229 (0.154)
<b>Magnetic resonance</b>			
Ejection fraction	0.216 (0.181)	-0.002 (0.991)	0.238 (0.139)
LV mass	<b>-0.336 (0.034)</b>	<b>0.328 (0.039)</b>	-0.225 (0.163)
LGE mass	-0.058 (0.850)	<b>0.680 (0.010)</b>	-0.113 (0.713)

Table 4. Correlations between parameters of aortic stenosis, measured in echocardiography and MRI, and inflammatory biomarker serum levels in the overall study population r and (P) value.

	<b>Group I</b>	<b>Group II</b>	<b>P-value</b>
TGF- $\beta$ 1 [ng/ml]	33.6 (9.4)	31.7(10.9)	0.563
RANTES [ng/ml] (Q1:Q3)	35.2 (26.4 – 8.2)	32(18.9 – 49.9)	0.289
IL-6 [pg/ml] (Q1:Q3)	2.2(1.3 – 5.2)	2.2(1.8 – 2.9)	0.904

Table 3. Serum levels of transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), RANTES and interleukin 6 (IL-6) and severity of aortic stenosis

# RESULTS

Group I included twenty patients with moderate AS while group II included twenty patients with severe AS (Table 1). The prevalence of cardiovascular risk factors was similar in both groups except for active smoking, which was more prevalent in group I (Table 1).

Subjects from group II reported more frequent intensification of symptoms in the NYHA scale (NYHA  $\geq$  II), although Group I presented with more frequent symptoms in the CCS scale. The analyzed patient population was asymptomatic or low-symptomatic: 16 NYHA 0-I patients, 22 NYHA II patients, and only 2 patients with NYHA III symptoms

In previous studies, significant differences in serum levels of TGF- $\beta$ 1 were found in severe AS patients and in asymptomatic moderate to severe AS patients (29-31). Additionally, a positive correlation between TGF- $\beta$ 1 and mean AS gradient was demonstrated (30). In our study, we evaluated TGF- $\beta$ 1 levels and did not find differences between both groups or a significant relationship between TGF- $\beta$ 1 serum level and parameters of stenosis severity. Interestingly, a positive correlation between TGF- $\beta$ 1 levels and ejection fraction, as measured by echocardiography, was found.

# Conclusions

Although there is an increasing interest in the immunopathogenesis of AS, relatively little is known about the relationship of inflammatory factors with severity of the disease and its clinical implications. The relationship between selected inflammatory biomarkers, LV ejection fraction, LV mass, and LV muscle mass with LGE appeared to be independent of valvular pathobiologic process severity, as we did not observe differences in IL-6, RANTES, or TGF- $\beta$ 1 levels between the two groups differing in severity. In contrast, these markers appear to be linked with myocardial function and remodeling, which may provide valuable insights into the pathobiology of AS and contribute to the development of future detection strategies

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