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Original article

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ABSTRACT

Background: Patients with a bicuspid aortic valve (BAV) are at risk of developing valve deterioration and aortic dilatation. We aimed to investigate whether blood biomarkers are associated with disease stage in patients with BAV.

Methods: Serum levels of high sensitivity C-reactive protein (hsCRP), high sensitivity troponin T (hsTnT), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and total transforming growth factor-beta 1 (TGF-ß1) were measured in adult BAV patients with valve dysfunction or aortic pathology. Age-matched general population controls were included for TGFß-1 measurements. Correlation analyses and multivariable linear regression were used to determine the association between (2log-transformed) biomarker levels and aortic valve regurgitation, aortic valve stenosis, aortic dilatation, or left ventricular function.

Results: hsCRP and hsTnT were measured in the total group of 183 patients (median age 34 years, 25th–75th percentile 23–46), NT-proBNP in 162 patients, and TGF-ß1 beta in 108 patients. Elevated levels of NT-proBNP were found in 20% of the BAV patients, elevated hsTnT in 6%, and elevated hsCRP in 7%. Higher hsTnT levels were independently associated with aortic regurgitation [odds ratio per doubling (OR_{2log}) 1.34, 95% CI 1.01;1.76] and higher NT-proBNP levels with aortic valve maximal velocity ($ß_{2log}$ 0.17, 95%CI 0.07;0.28) and aortic regurgitation (OR_{2log} 1.41, 95%CI 1.11;1.79). Both BAV patients with (9.9 ± 2.7 ng/mL) and without aortic dilatation (10.4 ± 2.9 ng/mL) showed lower TGF-ß1 levels compared to general population controls (n = 85, 11.8 ± 3.2 ng/mL).

Conclusions: Higher NT-proBNP and hsTNT levels were associated with aortic valve disease in BAV patients. TGF-ß1 levels were lower in BAV patients than in the general population, and not related to aortic dilatation. Longitudinal data are needed to further investigate the prognostic value of biomarkers in these patients.

Introduction

A bicuspid aortic valve (BAV) is an aortic valve with only two cusps instead of three or with three cusps of which two or more are fused with a raphe in between the cusps. It is the most common

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congenital heart malformation with a prevalence of 0.5–2% in the general population [1,2]. BAV is accompanied by aortic valve stenosis in about 65% of cases and by aortic valve regurgitation in about 40%, although numbers vary between studies, depending on the age of the patients [3,4]. Furthermore, approximately 50% of the BAV patients develop aortic dilatation throughout their lifetime [5]. To guide the optimal timing for surgical interventions in patients with BAV, accurate prognostication and monitoring of disease progression of aortic stenosis, aortic regurgitation, or aortic dilatation, is of great importance. Circulating blood biomarkers might provide additional information to determine who is at highest risk for future complications. Currently, there are limited data on the use of biomarkers in patients with BAV and the data that are available mainly focus on aortapathology [6]. It is of great interest to obtain further insight into alterations of growth factors and mediators in aortic valve degeneration, aortic dilatation, or myocardial remodeling. The biomarkers troponin-T and N-terminal pro B type natriuretic peptide (NT-proBNP) may be elevated as a result of pressure overload in aortic stenosis and volume overload in aortic regurgitation, which can help to identify patients with increased left ventricular wall stress who may benefit from earlier treatment. In older patients with degenerative aortic valve stenosis, higher C-reactive protein (CRP) is associated with more severe aortic stenosis [7], but no evidence is available on aortic valve stenosis in patients with a BAV. Finally transforming growth factor-beta 1 (TGF-ß1) is found to be elevated in blood samples of patients with aortic dilatation or syndromes [8–11]. Yet, only small numbers ranging between 9 and 30 patients with a BAV were included in these studies. This cross-sectional study aimed to investigate the association between biomarkers [high sensitivity (hs) CRP, hs TnT, NT-proBNP, and TGF-ß1] and the degree of aortic valve stenosis or regurgitation, left ventricular ejection fraction (LVEF), or aortic diameter in a large cohort of adults with BAV.

Methods

Study design and patient population

For this study we included the data of two cohorts of adults with BAV: the BioCon study, an observational prospective cohort study including consecutive adults with moderate to complex congenital heart disease enrolled between 2011 and 2013 [12], and the BAV study, a multicenter observational cohort study performed between 2014 and 2016 including patients with BAV and/or Turner syndrome [13]. We extracted data from adult patients from both cohort studies with BAV and at least one of the following: 1) aortic valve stenosis (maximal velocity >2.5 m/s), 2) aortic valve regurgitation (at least moderate), 3) aortic dilatation of the sinus of Valsalva or ascending aorta (≥40 mm and/or aortic size index ≥2.1 cm/m^2), 4) aortic coarctation, 5) an aortic valve intervention (balloon dilatation, resection subvalvular stenosis or valve repair), or 6) Turner syndrome. Patients who previously underwent aortic valve or aortic replacement were excluded. All three types of a BAV according to the Sievers classification were included [14]. This classification is based on the number of raphes, which is a fused area between two cusps. BAV with no raphe are called type 0, valves with one raphe type 1, and valves with two raphes type 2. For research purposes, patients underwent physical examination, two-dimensional thoracic echocardiography (TTE), and venous blood sampling on the same day. Hypertension was defined as current use of antihypertensive medication. The study complied with the Declaration of Helsinki and was approved by the medical ethical committee of the Erasmus Medical Center (MEC10-165 and MEC14-225). Written informed consent was provided by all patients.

TGF-ß1 measurements in general population controls

Between 2014 and 2015, 145 healthy volunteers were prospectively recruited through an advertisement for healthy subjects and stratified into five age groups: 20–29, 30–39, 40–49, 50–59, and 60–72 years [15]. TGF-ß1 levels were determined. To create an age-matched reference group, only the participants with an age under 50 years were included for the current study for TGF-ß1 measurements. The inclusion criteria required that subjects had normal results on physical examination, echocardiography, and electrocardiography (ECG). Subjects were excluded when they met any of the following criteria: cardiovascular disease (including aortic dilatation, aortic valve stenosis, aortic valve regurgitation); cardiovascular risk factors consisting of hypertension, diabetes mellitus, hypercholesterolemia; systemic disease or medication known to influence cardiac function; or the finding of cardiac abnormalities during examination. Professional athletes, morbidly obese subjects (body mass index >40 kg/m2), pregnant women, and women with breast implants were also excluded. All participants underwent physical examination, TTE, and venous blood sampling on the same day at the outpatient clinic.

Echocardiography

Standard 2D TTE was performed by an experienced sonographer. All studies were acquired using harmonic imaging on an iE33 or EPIQ7 ultrasound system (Philips Medical Systems, Best, the Netherlands) equipped with a ×5–1 matrix-array transducer (composed of 3040 elements operating at 1–5 MHz). The aorta was measured during diastole with the leading edge-to-leading edge method from either the standard parasternal long-axis view or from a more cranial intercostal window to improve visualization of the ascending aorta [16]. Aortic valve stenosis was defined based on a peak aortic velocity of >2.5 m/s [17] and aortic valve regurgitation was classified as no, mild, moderate, and severe according to the guidelines of the European Association of Echocardiography/American Society of Echocardiography [18]. Aortic dilatation was defined as an aortic diameter ≥40 mm or aortic size index ≥2.1 cm/m^2 at the level of the sinus of Valsalva or ascending aorta. All measurements of LVEF based on strain analyses were performed blinded regarding subject identity using the 2D CPA suite from Tomtec Imaging Systems (Unterschliessheim, Germany).

Laboratory testing

Venous blood sampling was performed for study purposes only. NT-proBNP was measured directly in fresh blood samples with the use of an electrochemiluminesence immunoassay (Roche Diagnostics, Basel, Switzerland) in the clinical chemistry laboratory of the Erasmus MC. The rest of the serum samples were aliquoted and stored at −80 °C within two hours after withdrawal. hsTnT and hsCRP were measured in batches in thawed serum samples using electrochemiluminesence immunoassays (Roche Diagnostics). Lower limit of detection was 3 ng/L for hsTnT, 0.3 mg/L for hsCRP, and 0.6 pmol/L for NT-proBNP. TGF-ß1 measurement was only performed in the patients of the BAV study and healthy controls, and was performed by the laboratory medical immunology of the department of Immunology of the Erasmus MC. Serum concentration of human activated TGF-ß1 was measured by quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions (Duoset® ELISA, R&D Systems Europe, Ltd., Abingdon, UK). Before the assay, the latent TGF-ß1 contained in patients' serum was activated to the immunoreactive form using acid activation and neutralization. The lower limit of detection was 31.25 pg/mL. For the control

subjects, the protocol of TGF-ß1 measurements was exactly the same as for the BAV patients.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD), or median and interquartile range (IQR). Comparison of normally distributed continuous variables was done using the Student's *t* test or, in case of a skewed distribution, the Mann-Whitney test. Biomarker values below the limit of detection (LoD) were substituted with a value that was equal to 50% of this LoD, for analytical purposes. Because of a skewed distribution, all biomarker levels were 2log transformed for further statistical analysis. The upper limit of normal for NT-proBNP was 14 pmol/L (≈125 pg/mL), on the basis of the recommended cut-off for the diagnosis of heart failure in patients presenting with non-acute symptoms [19]. The upper limit of normal was defined as the 99th percentile of the reference distribution, which corresponded with 14 ng/L for hsTnT [20] and with 10 mg/L for hsCRP [21]. First, the Pearson (r_P) or Spearman (r_S) correlation coefficient between biomarkers, as well as between biomarkers and patient characteristics was determined. Second, regression analysis was performed with biomarker levels as independent variable and disease stage as dependent variable. For disease stage the following variables were used: maximum velocity (Vmax) across the aortic valve, aortic valve regurgitation (no, mild, moderate, and severe), aortic diameter, and LVEF. When the dependent variable was continuous, linear regression analysis was used, and in case of an ordinal variable, ordinal logistic regression analysis was used. Significant univariable associations were further analyzed in multivariable analysis. First, we adjusted for age and sex only and secondly also for Vmax, aortic regurgitation, diameter of the sinus of Valsalva and ascending aorta, and ventricular function. Significant associations between biomarker levels and disease stage in multivariable linear regression analysis, were further analyzed using categories of disease stages. Aortic valve stenosis was categorized into no aortic valve stenosis (Vmax <2.5 m/s), mild aortic valve stenosis (Vmax 2.5–3.9 m/s), and severe aortic valve stenosis (Vmax ≥4.0 m/s). Aortic regurgitation was analyzed categorical as no, mild, and moderate/severe aortic valve regurgitation. A sensitivity analysis was performed by excluding Turner patient. Third, we compared biomarkers levels between patients with either aortic valve stenosis or regurgitation and patients without both aortic valve stenosis or regurgitation. Also biomarker levels were compared between BAV patients with different Sievers classifications. Missing data were handled by multiple imputation with five iterations [22]. All tests were two-sided and a *p*-value of <0.05 was considered statistically significant. All statistical analyses were performed using SPSS, version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

The patient selection process is shown in Fig. 1. A total of 183 patients were included with a median age of 34 (IQR 23–46) years of which 82 (45%) were female (Table 1). NT-proBNP measurement was available in 162 patients (89%). In a subset of 108 (58%) patients, total TGF-ß1 levels were determined. Patients in whom TGF-ß1 was measured were significantly older, had larger aortic diameters, and fewer patients had or were previously treated for an aortic coarctation compared to the total group of 188 patients. In the total group, a BAV Sievers type 0 was found in 27 (25%) subjects, Sievers type 1 in 144 (79%) subjects, and Sievers type 2 in 12 (6%) subjects. Moderate or severe aortic valve regurgitation was found in 50 (27%) patients, aortic valve stenosis in 80 (44%) patients, and aortic dilatation in 98 (54%) patients. Both aortic valve regurgitation (at least moderate) and aortic valve stenosis was found in 34 (19%) patients. LVEF of less than the lower limit (5th percentile) of the healthy group, which was 45%, was found in 69 (38%) of the BAV patients.

Blood biomarkers

The levels of each biomarker are shown by scatterplots in Fig. 2. An elevated NT-proBNP level was found in 37 (20%) patients, an elevated hsTnT level in 10 (6%) patients, and an elevated hsCRP

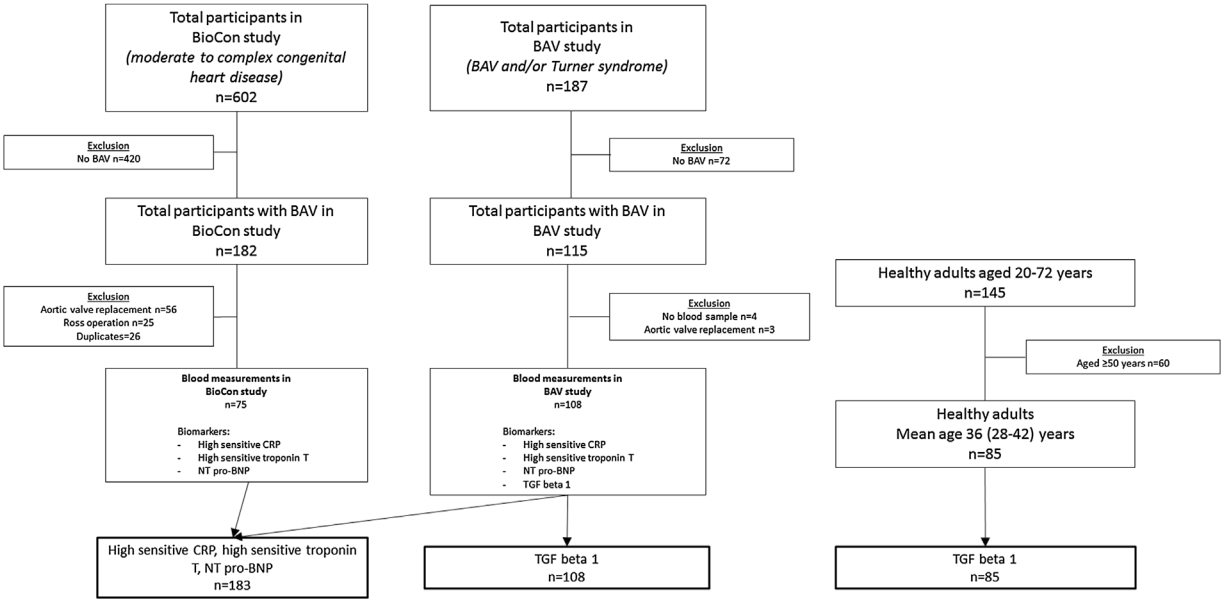

Fig. 1. Flow charts of participants from the BioCon and BAV study in blood marker analysis.
BAV, bicuspid aortic valve; hsCRP, high sensitivity C-reactive protein; hsTnT, high sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TGF beta 1, transforming growth factor beta 1.

Table 1
Baseline characteristics.

	hsCRP and hsTnT (total group) n = 183	NT pro-BNP n = 162	TGFß1 (only measured in BAV study) n = 108	Healthy controls for TGFß1 measurements n = 85
Age (years)	34 (23–46)	31 (23–43)	38 (25–52)[a]	36 (28–42)
Female sex	82 (45%)	72 (44%)	42 (39%)	43 (51%)
Height (cm)	176 (164–183)	176 (165–183)	178 (162–186)	175 (167–182)
Weight (kg)	75 ± 15	75 ± 15	75 ± 15	73 ± 13
Systolic blood pressure (mmHg)	127 ± 17	127 ± 17	126 ± 16	123 ± 12
Diastolic blood pressure (mmHg)	79 ± 11	79 ± 11	78 ± 11	78 ± 8
Aortic diameter, sinus of Valsalva (mm)	35 ± 6	35 ± 6	37 ± 6[a]	31 ± 3[a]
Aortic diameter, ascending aorta (mm)	36 ± 8	36 ± 8	38 ± 7[a]	29 ± 3[a]
Left ventricular ejection fraction (%)	47 ± 8	47 ± 8	46 ± 8	52 ± 4[a]
BAV morphology				
Sievers type 0	27 (15%)	22 (14%)	27 (25%)	–
Sievers type 1	144 (79%)	128 (79%)	70 (65%)	–
Sievers type 2	12 (6%)	12 (7%)	11 (10%)	–
Comorbidities				
Aortic coarctation	59 (32%)	57 (35%)	15 (14%)[a]	0 (0%)
Aortic regurgitation > mild	50 (27%)	46 (28%)	32 (30%)	0 (0%)
Aortic valve stenosis (Vmax≥2.5 m/s)	80 (44%)	70 (43%)	44 (41%)	0 (0%)
Diabetes mellitus	1 (1%)	0 (0%)	1 (1%)	0 (0%)
Hypertension	40 (22%)	33 (20%)	25 (23%)	0 (0%)
Hypercholesterolemia	10 (6%)	6 (4%)	8 (7%)	0 (0%)
Turner syndrome	22 (12%)	14 (9%)	20 (19%)	0 (0%)
Previous aortic valve intervention				
Surgical aortic valve repair	5 (3%)	4 (3%)	1 (1%)	0 (0%)
Percutaneous balloon dilatation	18 (10%)	18 (11%)	12 (11%)	0 (0%)
Resection subvalvular stenosis	7 (4%)	7 (4%)	0 (0%)	0 (0%)

Values are presented as mean (SD) or median (IQR) for continuous variables and N (%) for categorical variables. Data represent non-imputed values. Missing values were present for blood pressure (1% in BAV patients), aortic diameter at the sinus of Valsalva (1% in BAV patients), ascending aortic diameter (2% in BAV patients and 1% in healthy controls), and left ventricular ejection fraction (8% in BAV patients and 14% in healthy controls).
BAV, bicuspid aortic valve; hsCRP, high sensitivity C-reactive protein; hsTnT, high sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TGF beta 1, transforming growth factor beta 1.
[a] Significantly different from the total group, *p*-value <0.05. Values of 0 (0%) could not be tested for significance.

level in 12 (7%) patients. In our reference cohort of 85 volunteers, mean TGF-ß1 levels were 11.8 ± 3.2 ng/mL and not significantly associated with age (r_p = 0.13, p = 0.227) or sex (11.6±2.7 ng/mL in men vs 12.0±3.6 ng/mL in women, p = 0.640) (Supplemental). A significantly lower TGF- ß1 was found in both BAV patients with aortic dilatation (9.9 ± 2.7 ng/mL) and BAV patients without aortic dilatation (10.4 ± 2.9 ng/mL) compared to healthy controls with tricuspid aortic valve (TAV) (11.8 ± 3.2 ng/mL) (Fig. 3), also after correction for age. We found significant correlations between hsCRP and hsTnT (r_s = 0.15, p = 0.042), hsCRP and NT-proBNP (r_s = 0.35, $p < 0.001$), and NT-proBNP and TGF-ß1 (r_s=−0.24, p = 0.025).

None of the biomarkers showed an association with aortic diameter or left ventricular function in multivariable analysis (Table 2). A two-fold higher hsTnT level was independently associated with an increased risk of one higher grade of aortic regurgitation by a factor of 1.34 (95% CI 1.01;1.76). When aortic valve regurgitation was categorized as no, mild, or moderate/severe, hsTnT was only significantly higher in the patients with

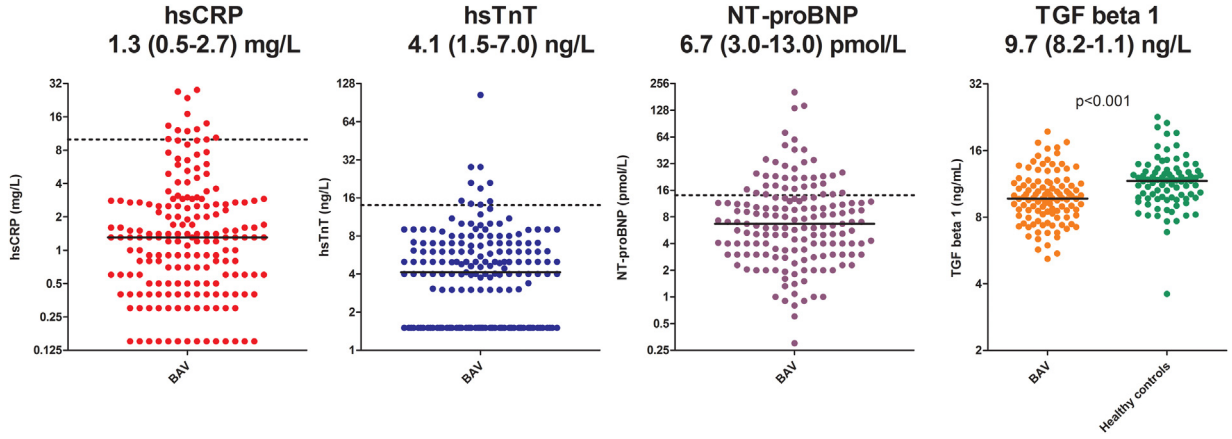

Fig. 2. Biomarker levels in adults with BAV. Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. The dashed line represents the upper limit of normal, defined as the 99th percentile of the reference distribution for hsCRP and hsTnT, corresponding with 10 mg/L and 14 ng/L. The upper limit of normal for NT-proBNP was 14 pmol/L (≈125 pg/mL), on the basis of the recommended cut-off for the diagnosis of heart failure in patients presenting with nonacute symptoms.
BAV, bicuspid aortic valve; hsCRP, high sensitivity C-reactive protein; hsTnT, high sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TGF beta 1, transforming growth factor beta 1.

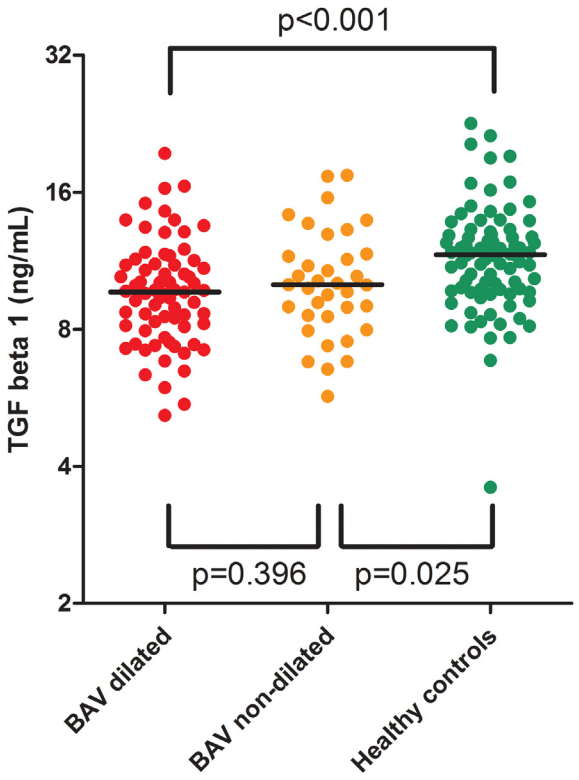

Fig. 3. TGF beta 1 levels in BAV patients with and without aortic dilatation (≥40 mm and/or aortic size index ≥2.1 cm/m2) and healthy controls. Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. BAV, bicuspid aortic valve; TGF beta 1, transforming growth factor beta 1.

moderate/severe aortic valve regurgitation compared to patients with no aortic valve regurgitation (Fig. 4).

A two-fold higher NT-proBNP level was independently associated with a mean 0.17 (95% CI 0.07;0.28) m/s higher Vmax. In addition, a two-fold higher NT-proBNP level was independently associated with an increased risk of one higher level of aortic regurgitation by a factor of 1.41 (95% CI 1.11;1.79). When aortic valve stenosis was categorized into three groups, NT-proBNP levels were only significantly higher in patients with severe aortic valve stenosis (Vmax >4.0 m/s) compared to patients with no aortic valve stenosis (Fig. 4). No significant differences in NT-proBNP levels were found between the three categories of aortic valve regurgitation. The comparison between patients with either aortic stenosis or aortic regurgitation and patients without stenosis and regurgitation can be found in Supplemental. Supplemental shows the comparison in biomarker level between patients with different Sievers classification. When Turner patients were excluded from the analysis, the results remained the same.

Discussion

This cross-sectional study investigated the levels of hsCRP, hsTNT, NTproBNP, and TGF-ß1 in a large cohort of adults with BAV. We can conclude that a substantial number of patients with BAV have elevated levels of NT-proBNP, hsTnT, and hsCRP. TGF-ß1 levels were found to be lower in patients with BAV than in a healthy age-matched control population with TAV. A higher NT-proBNP was significantly associated with a higher maximum velocity across the aortic valve and more severe aortic valve regurgitation. In addition, higher hsTnT levels were associated with more severe aortic valve regurgitation. Specifically, high levels of NT-proBNP were present in patients with severe aortic stenosis (≥4.0 m/s) and high levels of

Table 2
Linear or ordinal logistic regression analysis of biomarkers and continuous variables representing disease progression.

	Correlation analysis		Univariable regression analysis		Multivariable regression analysis[a]	
	Correlation coefficient	*p*-value	ß or OR (95% CI)[b]	*p*-value	ß or OR (95% CI)[b]	*p*-value
Log2 hsCRP						
Aortic valve stenosis (Vmax in m/s)	0.00	0.981	0.02 (-0.07;0.11)	0.300		
Aortic valve regurgitation (no, mild, moderate, severe)	−0.02	0.827	0.99 (0.85;1.15)	0.716		
Aortic diameter at the level of sinus of Valsalva (mm)	0.00	0.996	0.02 (-0.51;0.56)	0.938		
Aortic diameter at the level of ascending aorta (mm)	0.01	0.855	0.01 (-0.64;0.67)	0.972		
Left ventricular ejection fraction (%)	0.01	0.191	0.01 (-0.57;0.83)	0.725		
Log2 hsTnT						
Aortic valve stenosis (Vmax in m/s)	0.11	0.132	0.09 (-0.04;0.22)	0.164		
Aortic valve regurgitation (no, mild, moderate, severe)	0.16	0.035	1.29 (1.03;1.57)	0.030	**1.34 (1.01;1.76)**	**0.041**
Aortic diameter at the level of sinus of Valsalva (mm)	0.29	<0.001	1.56 (1.17;1.95)	<0.001	0.07 (-0.61;0.76)	0.838[c]
Aortic diameter at the level of ascending aorta (mm)	0.19	0.011	1.39 (0.44;2.33)	0.004	−0.40 (-1.40;0.60)	0.431[c]
Left ventricular ejection fraction (%)	0.10	0.260	0.49 (-0.63;1.61)	0.384		
Log2 NT-proBNP						
Aortic valve stenosis (Vmax in m/s)	0.19	0.016	0.16 (0.05;0.26)	0.003	**0.17 (0.07;0.28)**	**0.002**
Aortic valve regurgitation (no, mild, moderate, severe)	0.14	0.070	1.22 (1.01;1.47)	0.035	**1.41 (1.11;1.79)**	**0.005**
Aortic diameter at the level of sinus of Valsalva (mm)	−0.04	0.593	−0.18 (-0.81;0.46)	0.592		
Aortic diameter at the level of ascending aorta (mm)	0.03	0.694	0.16 (-0.64;0.95)	0.693		
Left ventricular ejection fraction (%)	0.17	0.035	0.94 (0.08;0.09)	0.033	0.36 (-0.73;1.44)	0.518[c]
Log2 TGFß1						
Aortic valve stenosis (Vmax in m/s)	−0.11	0.277	−0.38 (-0.88;0.12)	0.137		
Aortic valve regurgitation (no, mild, moderate, severe)	−0.01	0.944	0.92 (0.37;2.30)	0.866		
Aortic diameter at the level of sinus of Valsalva (mm)	0.19	0.045	3.01 (0.10;5.92)	0.043	1.32 (-1.06;3.70)	0.278[c]
Aortic diameter at the level of ascending aorta (mm)	−0.03	0.758	−0.60 (-4.37;3.18)	0.757		
Left ventricular ejection fraction (%)	−0.13	0.205	−2.60 (-6.59;1.38)	0.200		

Bold = significant in multivariable analysis.
hsCRP, high sensitivity C-reactive protein; hsTnT, high sensitivity troponin T; NT-proBNP, N-terminal pro-B-type natriuretic peptide; TGF beta 1, transforming growth factor beta 1.
[a] Adjusted for age, sex, Vmax, aortic valve regurgitation, aortic diameters, and left ventricular ejection fraction.
[b] Beta coefficient was given for linear regression analysis performed with Vmax, aortic diameter, or left ventricular ejection fraction as dependent variables. Odd ratio was given for ordinal logistic regression analysis performed with aortic regurgitation (no, mild, moderate, severe) as dependent variable.
[c] Not significant after adjustment for only age and sex.

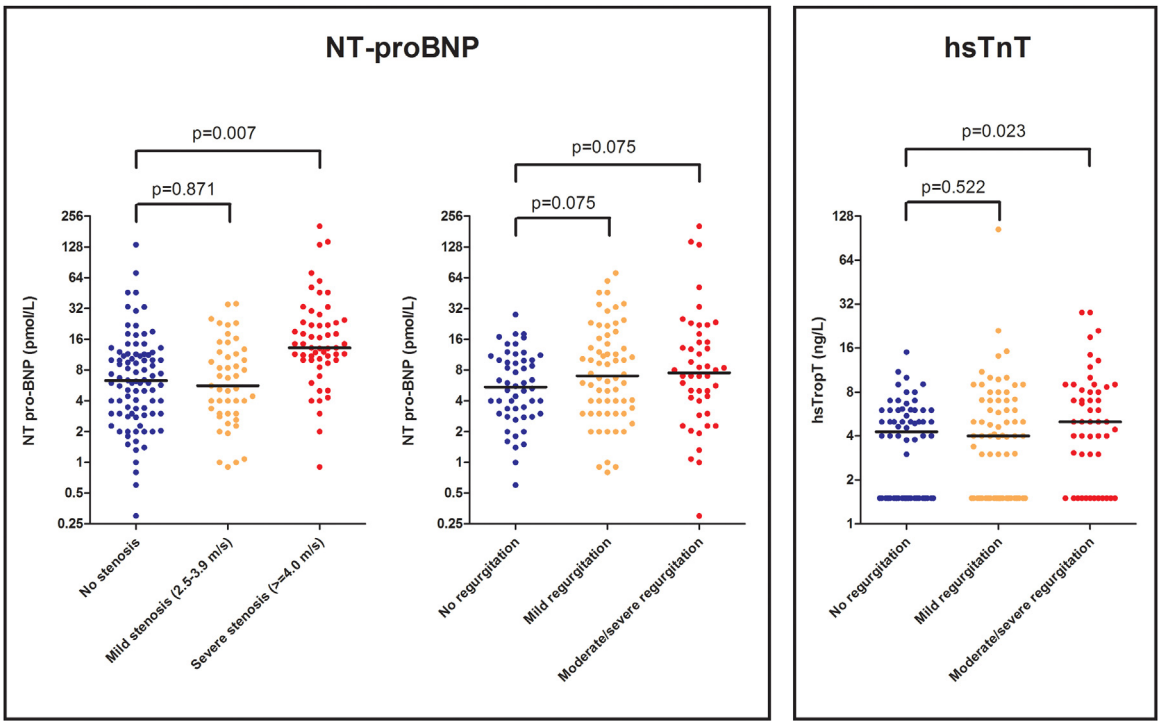

Fig. 4. NT-proBNP and hsTnT levels in patients with different stages of aortic valve stenosis and aortic valve regurgitation. Biomarker levels are presented at the Y-axis on the 2log-scale. The continuous line represents the median. NT-proBNP, pro-B-type; hsTnT, high-sensitivity troponin T.

hsTnT were present in patients with moderate to severe aortic valve regurgitation.

In contrast to imaging biomarkers, blood biomarkers can be obtained in all patients and at relatively low cost, making them very suitable as diagnostic and useful in prediction models. This study already showed that patients with BAV have higher levels of NT-proBNP, hsTnT, and hsCRP and lower levels of TGF- ß1, which can help us to understand the pathophysiology of complications in patients with BAV. In addition, this is the first study that evaluated associations between biomarkers and disease stage in this specific group of patients with BAV and demonstrated that particularly NT-proBNP and hsTNT may have potential prognostic value in adults with a BAV. Whether biomarker levels can predict deterioration of aortic valve pathology and can be used for clinical decision-making in BAV patients should be further investigated in longitudinal studies.

NT-proBNP in BAV patients

NT-proBNP is associated with ventricular dysfunction in patients with congenital aortic valve stenosis [23] and is elevated in older individuals with calcified aortic valve stenosis [24] or aortic valve regurgitation [25]. In older patients with aortic stenosis, the prognostic value of NT-proBNP has also been proven [26,27]. However, BAV patients differs genetically, histopathologically, and in age from the patient group of aortic valve degeneration represented in literature. BAV patients are prone to develop aortic valve stenosis, aortic valve regurgitation, or aortic dilatation at a relatively young age [28]. We showed that NT-proBNP levels are associated with aortic valve stenosis and regurgitation in BAV patients. NT-proBNP expression is induced by diastolic and systolic myocardial wall stretch [29]. This can be a result of both aortic valve stenosis and regurgitation due to increased left ventricular pressure afterload or volume overload respectively. Two studies [29,30] that included relatively old patients with aortic valve stenosis, showed that NT-proBNP and BNP are already elevated in patients with normal LV end-diastolic pressure or left atrial pressure, compared to controls. This might suggest that NT-proBNP is already elevated before patients with aortic valve stenosis or regurgitation develop increased pressures and compensated hypertrophy. However, to answer the question whether NT-proBNP could help to identify BAV patients with future LVEF deterioration, longitudinal studies are required.

hsTnT in BAV patients

Troponin T, a marker of myocardial injury, is not only elevated in acute coronary syndrome, but it can also be elevated in chronic heart failure [31] as a result of multiple suspected contributing mechanisms including cardiomyocyte damage from inflammatory cytokines or oxidative stress, apoptosis, or a stretch-related mechanism [32]. Troponin T levels also have a predictive value of long-term outcome in patients with non-ischemic heart failure [33]. Again these studies are performed in older patients compared to the BAV patients included in our study and therefore probably represent another disease etiology. Although we did not find an association between hsTnT levels and LVEF, we did find that hsTnT levels were associated with more severe aortic valve regurgitation in BAV patients. One hypothetical explanation might be the reduced coronary blood flow during diastole found in patients with aortic regurgitation [34,35], which can result in cardiomyocyte damage and therefore increased hsTnT levels. A reduced coronary blood flow is also found in patients with aortic stenosis [36], while aortic stenosis was not associated with increased hsTnT levels in our cohort.

hsCRP in BAV patients

In older patients with aortic stenosis, Galante et al. found that inflammatory markers, such as CRP, were elevated [37]. Statins

have been tested and suggested as a new treatment to reduce the progression of aortic valve stenosis, because they reduce vascular inflammatory processes besides their already known cholesterol-lowering effect [38]. Nevertheless, large randomized controlled trials did not find an effect of statin therapy on cardiovascular events, mortality, or valve dynamics in patients with aortic valve stenosis [39]. In our study, CRP levels did not show an association with the severity of aortic valve stenosis in BAV patients, suggesting that inflammation is less likely to be involved as the underlying process of aortic valve stenosis development in BAV patients. This finding confirms that indeed there is no clear role for statin therapy. A theory of dilatation of the aorta is presumed to be a process of inflammation, in this study we did not find an association between aorta diameter and the level of hsCRP. The reason for this could be that this process only plays a role in late stage of dilatation and the number of patients with a large aortic diameter might have been too small.

TGF-beta 1 in BAV patients

There has been attention for biomarkers in BAV-associated aortic pathology, focusing on matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinase (TIMP), and TGF-ß [6,40]. Although TGF-ß also seems to be important in thoracic aortic aneurysm development in BAV [41], we did not find an association between serum TGF-ß1 levels and aortic diameter in BAV patients. Our results did show, however, that TGF-ß1 levels are decreased in patients with BAV, both with and without aortic dilatation. This is in line with a study investigating aortic tissue, since they found that a higher proportion of TGF-ß sequestered in the extracellular matrix found in BAV compared to a TAV, probably leads to less free TGF-ß being available to activate the TGFß-pathway [42]. The development of aortic dilatation in BAV patients does not seem to be associated with an increased TGF-ß signaling, such as in Marfan and Loeys-Dietz syndrome. This is confirmed by another study, which also presented a group of non-dilated BAV patients in whom TGF-ß was less expressed in aortic tissue [43]. This different activation of the TGF-ß pathway in aortic dilatation between BAV patients and patients with Marfan or Loeys-Dietz syndrome needs further attention. Contradictory, other studies presented results with higher TGF-ß1 in BAV patients [10,11] or no difference in TGF-ß1 between BAV and TAV participants [9], but these studies contained only 30, 24, and 12 BAV patients. Also it is important to mention that platelets are a major source of TGF-ß1 in the circulation. It has been shown that patients with BAV tend to have a higher mean platelet volume [44], indicating increased platelet activation. If this increased platelet activation had affected the TGF-ß1 measurements, BAV patients would have had higher TGF-ß1 levels. However, we found lower levels of TGF-ß1 in BAV patients, but no information on platelet levels was available to substantiate this assumption.

In addition, we provided reference values for TGF-ß1, based on 85 healthy volunteers without aortic dilatation with an age range from 20 to 50 years. The mean TGF-ß1 levels that we found (11.8 ± 3.2 ng/mL) were both lower [45] or higher [8,9] than in previously published studies. This could possibly be due to differences in affinity of the antibodies in the immunoassays or inclusion of different reference groups (patient without BAV versus local population). Since the current literature presents a large variation in reference levels of circulating TGF-ß1, reference levels should be measured with the same technique, preferably measured in the same laboratory.

Limitations

Some limitations of our study need to be addressed. The lack of follow-up data prevented us to evaluate the longitudinal prognostic value of these biomarkers. In addition, patients who previously underwent aortic valve or aortic replacement were excluded and therefore BAV patients with the most severe disease stage were less likely to be included in this study. Finally, measurements of LVEF were based on strain analyses in both BAV patients and healthy controls, while the biplane method of disks is the currently recommended 2D method to assess LVEF [46]. A substantial variation between techniques has to be taken into account, with the values found in our study being lower compared to the values referred to as abnormal by the guidelines: LVEF of $<$52% for men and $<$54% for women are suggestive of abnormal LV systolic function [16].

Conclusion

A substantial number of patients with BAV have elevated levels of NT-proBNP, hsTnT, and hsCRP and they show lower levels of TGF-ß1 compared to healthy controls with TAV. In BAV patients, higher NT-proBNP was associated with more severe aortic valve stenosis and regurgitation, while higher hsTnT levels were associated with more severe aortic valve regurgitation. No independent association between hsCRP and TGF-ß1 with the degree of aortic valve stenosis or regurgitation, LVEF or aortic diameter was found. This is the first step toward the identification of a biomarker that can be used in prognostic staging and risk prediction.

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Disclosures

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jjcc.2020.02.023.

References

[1] Basso C, Boschello M, Perrone C, Mecenero A, Cera A, Bicego D, et al. An echocardiographic survey of primary school children for bicuspid aortic valve. Am J Cardiol 2004;93:661–3.
[2] Hoffman JI, Kaplan S. The incidence of congenital heart disease. J Am Coll Cardiol 2002;39:1890–900.
[3] Mordi I, Tzemos N. Bicuspid aortic valve disease: a comprehensive review. Cardiol Res Pract 2012;2012196037.
[4] Roberts WC, Vowels TJ, Ko JM. Natural history of adults with congenitally malformed aortic valves (unicuspid or bicuspid). Medicine (Baltimore). 2012;91:287–308.
[5] Verma S, Siu SC. Aortic dilatation in patients with bicuspid aortic valve. N Engl J Med 2014;370:1920–9.
[6] Naito S, Hillebrand M, Bernhardt AMJ, Jagodzinski A, Conradi L, Detter C, et al. The value of circulating biomarkers in bicuspid aortic valve-associated aortopathy. Thorac Cardiovasc Surg 2018;66:278–86.
[7] Imai K, Okura H, Kume T, Yamada R, Miyamoto Y, Kawamoto T, et al. C-Reactive protein predicts severity, progression, and prognosis of asymptomatic aortic valve stenosis. Am Heart J 2008;156:713–8.
[8] Sepetiene R, Patamsyte V, Zukovas G, Jariene G, Stanioniene Z, Benetis R, et al. Blood plasma TGF- beta1 concentration in sporadic dilatative pathology of ascending aorta: more questions than answers. PLoS One 2015;10e0129353.
[9] Rueda-Martinez C, Lamas O, Carrasco-Chinchilla F, Robledo-Carmona J, Porras C, Sanchez-Espin G, et al. Increased blood levels of transforming growth factor

beta in patients with aortic dilatation. Interact Cardiovasc Thorac Surg 2017;25:571–4.
[10] Hillebrand M, Millot N, Sheikhzadeh S, Rybczynski M, Gerth S, Kolbel T, et al. Total serum transforming growth factor-beta1 is elevated in the entire spectrum of genetic aortic syndromes. Clin Cardiol 2014;37:672–9.
[11] Gallo A, Agnese V, Coronnello C, Raffa GM, Bellavia D, Conaldi PG, et al. On the prospect of serum exosomal miRNA profiling and protein biomarkers for the diagnosis of ascending aortic dilatation in patients with bicuspid and tricuspid aortic valve. Int J Cardiol 2018;273:230–6.
[12] Eindhoven JA, Roos-Hesselink JW, van den Bosch AE, Kardys I, Cheng JM, Veenis JF, et al. High-sensitive troponin-T in adult congenital heart disease. Int J Cardiol 2015;184:405–11.
[13] Bons LR, Duijnhouwer AL, Boccalini S, van den Hoven AT, van der Vlugt MJ, Chelu RG, et al. Intermodality variation of aortic dimensions: how, where and when to measure the ascending aorta. Int J Cardiol 2019;276:230–5.
[14] Sievers HH, Schmidtke C. A classification system for the bicuspid aortic valve from 304 surgical specimens. J Thorac Cardiovasc Surg 2007;133:1226–33.
[15] Menting ME, McGhie JS, Koopman LP, Vletter WB, Helbing WA, van den Bosch AE, et al. Normal myocardial strain values using 2D speckle tracking echocardiography in healthy adults aged 20 to 72 years. Echocardiography 2016;33:1665–75.
[16] Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr 2015;28:1–39. e14.
[17] Baumgartner H, Hung J, Bermejo J, Chambers JB, Edvardsen T, Goldstein S, et al. Recommendations on the echocardiographic assessment of aortic valve stenosis: a focused update from the european association of cardiovascular imaging and the american society of echocardiography. J Am Soc Echocardiogr 2017;30:372–92.
[18] Lancellotti P, Tribouilloy C, Hagendorff A, Popescu BA, Edvardsen T, Pierard LA, et al. Recommendations for the echocardiographic assessment of native valvular regurgitation: an executive summary from the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2013;14:611–44.
[19] McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur Heart J 2012;33:1787–847.
[20] Giannitsis E, Kurz K, Hallermayer K, Jarausch J, Jaffe AS, Katus HA. Analytical validation of a high-sensitivity cardiac troponin T assay. Clin Chem 2010;56:254–61.
[21] Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. Clin Chim Acta 1981;117:13–23.
[22] Hayati Rezvan P, Lee KJ, Simpson JA. The rise of multiple imputation: a review of the reporting and implementation of the method in medical research. BMC Med Res Methodol 2015;15:30.
[23] Eindhoven JA, van den Bosch AE, Ruys TP, Opic P, Cuypers JA, McGhie JS, et al. N-terminal pro-B-type natriuretic peptide and its relationship with cardiac function in adults with congenital heart disease. J Am Coll Cardiol 2013;62:1203–12.
[24] Ferrari G, Sainger R, Beckmann E, Keller G, Yu PJ, Monti MC, et al. Validation of plasma biomarkers in degenerative calcific aortic stenosis. J Surg Res 2010;163:12–7.
[25] Song BG, Park YH, Kang GH, Chun WJ, Oh JH, Choi JO, et al. Preoperative, postoperative and one-year follow-up of N-terminal pro-B-type natriuretic peptide levels in volume overload of aortic regurgitation: comparison with pressure overload of aortic stenosis. Cardiology 2010;116:286–91.
[26] Clavel MA, Malouf J, Michelena HI, Suri RM, Jaffe AS, Mahoney DW, et al. B-type natriuretic peptide clinical activation in aortic stenosis: impact on long-term survival. J Am Coll Cardiol 2014;63:2016–25.
[27] Goodman A, Kusunose K, Popovic ZB, Parikh R, Barr T, Sabik JF, et al. Synergistic utility of brain natriuretic peptide and left ventricular strain in patients with significant aortic stenosis. J Am Heart Assoc 2016;5.
[28] Michelena HI, Prakash SK, Della Corte A, Bissell MM, Anavekar N, Mathieu P, et al. Bicuspid aortic valve: identifying knowledge gaps and rising to the challenge from the International Bicuspid Aortic Valve Consortium (BAVCon). Circulation 2014;129:2691–704.
[29] Vanderheyden M, Goethals M, Verstreken S, De Bruyne B, Muller K, Van Schuerbeeck E, et al. Wall stress modulates brain natriuretic peptide production in pressure overload cardiomyopathy. J Am Coll Cardiol 2004;44:2349–54.
[30] Qi W, Mathisen P, Kjekshus J, Simonsen S, Bjornerheim R, Endresen K, et al. Natriuretic peptides in patients with aortic stenosis. Am Heart J 2001;142:725–32.
[31] Korff S, Katus HA, Giannitsis E. Differential diagnosis of elevated troponins. Heart 2006;92:987–93.
[32] Kociol RD, Pang PS, Gheorghiade M, Fonarow GC, O'Connor CM, Felker GM. Troponin elevation in heart failure prevalence, mechanisms, and clinical implications. J Am Coll Cardiol 2010;56:1071–8.
[33] Sato Y, Nishi K, Taniguchi R, Miyamoto T, Fukuhara R, Yamane K, et al. In patients with heart failure and non-ischemic heart disease, cardiac troponin T is a reliable predictor of long-term echocardiographic changes and adverse cardiac events. J Cardiol 2009;54:221–30.
[34] Rabkin SW. Differences in coronary blood flow in aortic regurgitation and systemic arterial hypertension have implications for diastolic blood pressure targets: a systematic review and meta-analysis. Clin Cardiol 2013;36:728–36.
[35] Icli A, Mutlu H, Karabag T, Kahraman H. Decreased coronary blood flow velocity in patients with aortic insufficiency but normal coronary arteries: the use of TIMI frame count in aortic insufficiency cases. Int J Clin Exp Med 2015;8:16358–63.
[36] Banovic MD, Vujisic-Tesic BD, Kujacic VG, Callahan MJ, Nedeljkovic IP, Trifunovic DD, et al. Coronary flow reserve in patients with aortic stenosis and nonobstructed coronary arteries. Acta Cardiol 2011;66:743–9.
[37] Galante A, Pietroiusti A, Vellini M, Piccolo P, Possati G, De Bonis M, et al. C-reactive protein is increased in patients with degenerative aortic valvular stenosis. J Am Coll Cardiol 2001;38:1078–82.
[38] Takemoto M, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. Arterioscler Thromb Vasc Biol 2001;21:1712–9.
[39] Loomba RS, Arora R. Statin therapy and aortic stenosis: a systematic review of the effects of statin therapy on aortic stenosis. Am J Ther 2010;17:e110–4.
[40] Maredia AK, Greenway SC, Verma S, Fedak PWM. Bicuspid aortic valve-associated aortopathy: update on biomarkers. Curr Opin Cardiol 2018;33:134–9.
[41] van de Pol V, Kurakula K, DeRuiter MC, Goumans MJ. Thoracic aortic aneurysm development in patients with bicuspid aortic valve: what is the role of endothelial cells? Front Physiol 2017;8:938.
[42] Paloschi V, Gadin JR, Khan S, Bjorck HM, Du L, Maleki S, et al. Aneurysm development in patients with a bicuspid aortic valve is not associated with transforming growth factor-beta activation. Arterioscler Thromb Vasc Biol 2015;35:973–80.
[43] Grewal N, Gittenberger-de Groot AC, DeRuiter MC, Klautz RJ, Poelmann RE, Duim S, et al. Bicuspid aortic valve: phosphorylation of c-Kit and downstream targets are prognostic for future aortopathy. Eur J Cardiothorac Surg 2014;46:831–9.
[44] Bilen E, Tanboga IH, Kurt M, Kocak U, Ayhan H, Durmaz T, et al. Mean platelet volume is increased in patients with bicuspid aortic valve. Clin Appl Thromb Hemost 2012;18:351–5.
[45] Young DG, Skibinski G, Mason JI, James K. The influence of age and gender on serum dehydroepiandrosterone sulphate (DHEA-S), IL-6, IL-6 soluble receptor (IL-6 sR) and transforming growth factor beta 1 (TGF-beta1) levels in normal healthy blood donors. Clin Exp Immunol 1999;117:476–81.
[46] Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015;16:233–70.