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The Contribution of Whole Blood Viscosity to the Process of Aortic Valve Sclerosis

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Significance of the Study

- The objective of this study was to assess whole blood viscosity (WBV), a marker of shear stress, as a major risk factor contributing to aortic valve sclerosis (AVS). This study demonstrated that WBV was independently associated with AVS. As an easily measurable laboratory variable, WBV could be a useful indicator of AVS.

Keywords
Aortic valve sclerosis · Whole blood viscosity · Shear stress · Receiver-operating characteristic curve

Abstract
Objective: We aimed to investigate whether increased whole blood viscosity (WBV) could be an important factor for the occurrence of aortic valve sclerosis (AVS). ***Subjects and Methods:*** A total of 209 patients were enrolled in the study. WBV was calculated using the hematocrit and total plasma protein at a low shear rate (LSR) and a high shear rate (HSR). AVS was defined as irregular valve thickening and calcification (without evidence of outflow obstruction) documented by a peak transvalvular velocity <2.5 m/s on echocardiographic examination. The patient group consisted of 109 patients with AVS (77 females, 32 males), and 100 subjects without AVS (65 females, 35 males) were assigned to the control group. ***Results:*** In the AVS group, WBV values were significantly higher for HSR (17.4 ± 0.5 vs. 17.1 ± 0.7 208 s^{-1}, $p < 0.001$) and LSR (65.9 ± 12.5 vs. 59.7 ± 16.7 0.5 s^{-1}, $p = 0.002$). In multivariate logistic regression analysis, WBV at HSR and LSR were independent predictors of AVS (odds ratio, OR: 2.24, 95% confidence interval, CI: 1.38–3.64, $p = 0.001$; OR: 1.026, 95% CI: 1.006–1.046, $p = 0.01$, respectively). Receiver-operating characteristic (ROC) curve analysis indicated that a WBV cutoff value of 65.4 at LSR had a sensitivity of 46.8% and a specificity of 60.0% (area under the ROC curve, AUC: 0.615, 95% CI: 0.535–0.696, $p = 0.004$), and a WBV cutoff value of 17.1 at HSR had a sensitivity of 61.5% and specificity of 53% (AUC: 0.648, 95% CI: 0.571–0.725, $p < 0.001$) for the prediction of AVS. ***Conclusion:*** This study demonstrated that WBV was independently associated with AVS. WBV could be an indicator of inflammation and vessel remodeling without evidence of outflow obstruction.

Introduction

Aortic valve sclerosis (AVS) is a frequent finding at echocardiography in the elderly population [1]. AVS is also an incremental risk factor related to the enhanced deaths ratio from cardiovascular events [1]. The mor-

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phology of atherosclerosis and AVS are similar and associated with similar risk factors [2]. Additionally, it has been claimed that mechanical forces such as blood pressure, membrane tension, and fluid shear stress contribute to the calcification of the aortic valve (AV) [3]. Lesions frequently occur at the aortic side of the leaflets, an area of high turbulent flow and tensile stress with oscillatory shear stress [4]. The center of the valve cusp has the greatest mechanical stress and is more frequently involved than the commissures.

Whole blood viscosity (WBV), a marker of shear stress, may be considered as a major cardiovascular risk factor contributing to AVS. Measurement of WBV may be challenging due to nonstandardized methods in the laboratory, lack of advanced instruments, and insufficient research data [5]. High WBV could disrupt endothelial integrity at the foci of enhanced mechanical stress, such as the aortic surface of AV leaflets in the coronary sinus area of the aorta. Therefore, we aimed to investigate whether increased WBV could be an important factor for the occurrence of AVS in addition to traditional cardiovascular risk factors.

Subjects and Methods

Patient Population

This is a cross-sectional clinical study in which 209 patients were enrolled from August 2014 to November 2016. The patients were divided into 2 groups based on the presence or absence of AVS on echocardiographic examination. The patient group consisted of 109 patients with AVS (77 females, 32 males); 100 subjects without AVS (65 females, 35 males) comprised the control group. AVS was defined as irregular valve thickening and calcification, without evidence of outflow obstruction, as documented by a peak transvalvular velocity <2.5 m/s based on a previous study [6].

Medical history was obtained from patients and their hospital records, and physical examination was performed on all patients and controls. Demographic, clinical, and laboratory data of the subjects were collected from medical records. Exclusion criteria were prior coronary artery bypass surgery or percutaneous coronary intervention, left ventricular ejection fraction <50%, a history of heart valve surgery, bicuspid AV, rheumatic heart disease, renal failure, acute or chronic liver disease, chronic pulmonary disease, acute and chronic infections or inflammatory diseases, malignancies, hematological disorders (including anemia), or oral warfarin therapy. Anemia was defined as a reduction in either the percentage of red blood cells (hematocrit, Hct) or a reduction in the concentration of hemoglobin in a sample of venous blood compared with reference values.

Hypertension was defined as a systolic blood pressure of 140 mm Hg or more or a diastolic blood pressure of 90 mm Hg or more, or taking antihypertensive medication. Diabetes mellitus was defined as the use of antidiabetic drugs and a fasting blood glucose >126 mg/dL. Hyperlipidemia was identified in patients with total cholesterol >200 mg/dL, low-density lipoprotein (LDL) >130 mg/dL, triglyceride levels >150 mg/dL, and in patients treated with lipid-lowering drugs. Body mass index was defined as weight in kilograms divided by the square of the height in meters. The study was approved by the Institutional Ethics Committee, and written informed consent was obtained from all the patients.

Transthoracic Echocardiography

Each patient underwent a complete transthoracic echocardiography using the recommendations from the American Society of Echocardiography's Guidelines and Standards Committee [7]. Echocardiograms were performed using a Vivid S5 (General Electronic, Waukesha, WI, USA) with a 2.5- to 3.5-MHz transducer, placed on the IIIrd–IVth left intercostal space along the parasternal line, with patients being supine in left lateral decubitus position with the head of the bed kept at 30°. All examinations were performed by an experienced cardiologist blinded to the patient's clinical information.

AVS was defined as the presence of irregularly increased echogenicity and thickening of the leaflets, no restriction of leaflet motion, with peak instantaneous transaortic jet velocity <2.5 m/s. Patients with poor echogenicity were excluded from the study. AVS was defined as restricted systolic opening of the valve leaflets; patients with a mean transvalvular pressure gradient of at least 10 mm Hg and/or with peak instantaneous transaortic jet velocity >2.5 m/s were also excluded from the study. Mitral annular calcification (MAC) was defined echocardiographically as an echodense, irregular shelf-like structure involving the mitral valve annulus with associated acoustic shadowing.

Laboratory Analysis

Blood samples were drawn through venipuncture from all subjects following 12 h of fasting into standardized tubes containing dipotassium ethylenediaminetetraacetic acid (EDTA). All hematological measurements were performed using the XT-2000i analyzer (Sysmex Corporation of America, Long Grove, IL, USA). Biochemical measurements were made using a molecular analyzer (Roche Diagnostics, Manheim, Germany).

Extrapolation of WBV

The calculation of WBV was done with a formula from Hct and total plasma protein (TP) for wall shear stress [8]. WBV was calculated for both LSR (0.5 s^{-1}) and HSR (208 s^{-1}) from Hct and TP protein concentration using a validated formula [8].

HSR: WBV (208 s^{-1}) = (0.12 × Hct) + 0.17 (TP – 2.07).
LSR: WBV (0.5 s^{-1}) = (1.89 × Hct) + 3.76 (TP – 78.42).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) for Windows was used for all statistical calculations. Categorical variables were expressed as numbers and proportions while continuous variables were expressed as means ± SD. The Shapiro-Wilk test was used to evaluate whether the distribution of continuous variables was normal. Continuous variables were compared with the Student *t* test (while comparing normally distributed variables) or Mann-Whitney U test (while comparing nonnormally distributed variables). The χ^2 test was used to compare groups regarding categorical variables. Variables with $p < 0.10$ in univariate analysis were identified as potential risk markers and included in the full multivariate logistic regression model as covariates. The receiver-operating characteristic (ROC) curve was used to demonstrate the sensitivity and specificity of WBV at HSR and LSR and their cutoff values for predicting AVS. A value of $p < 0.05$ was considered as significant.

Table 1. Baseline characteristics and laboratory findings of the patients with aortic value sclerosis (AVS) and controls

Parameters	Controls (n = 100)	AVS (n = 109)	p values
Age, years	66.0±6.4	65.0±7.4	0.296
Gender			
Females	65	77	0.458
Males	35	32	
Body mass index	30.3±3.8	31.2±5.0	0.168
Hyperlipidemia	51 (51.0%)	65 (59.6%)	0.214
Diabetes mellitus	34 (34%)	45 (41.3%)	0.318
Hypertension	51 (51.0%)	63 (57.8%)	0.334
Smoking	30 (30%)	24 (22%)	0.208
Systolic blood pressure, mm Hg	124.53±19.4	127.94±18.4	0.194
Diastolic blood pressure, mm Hg	74.7±15.9	74.3±10.3	0.829
Heart rate, bpm	68.6±12.7	67±11.7	0.332
Echocardiographic measurements			
Ejection fraction, %	60.8±3.2	59.8±5.0	0.121
Left atrial diameter, cm	3.75±0.3	3.78±0.4	0.661
Ascending aorta diameter, cm	3.49±0.30	3.49±0.26	0.998
Aortic valve jet velocity, m/s	1.33±0.18	1.97±0.13	<0.001
Mitral annular calcification	14 (14%)	28 (25.7%)	0.039
Medications			
β-Blockers	35 (35.0%)	35 (32.4%)	0.769
ACE inhibitors/ARB	41 (41.0%)	48 (44.0%)	0.677
ASA	37 (37.0%)	42 (38.5%)	0.887
Statins	26 (26.0%)	25 (22.9%)	0.632
Laboratory parameters			
WBC, $\times 10^3/\mu L$	7.7±2.3	7.3±1.9	0.173
Hemoglobin, g/dL	13.7±1.2	13.8±1.0	0.465
Hct, %	40.9±5.4	41.6±4.6	0.266
Platelets, $\times 10^3/\mu L$	256.8±73.0	244.0±57.7	0.159
Glucose, mg/dL	115.8±41.4	119.0±49.1	0.612
Creatinine, mg/dL	1.0±0.2	0.9±0.2	0.112
hs-CRP, mg/dL	2.5±2.4	3.4±3.3	0.044
Total cholesterol, mg/dL	195.2±41.3	205.8±44.5	0.079
LDL cholesterol, mg/dL	119.0±36.3	127.9±38.0	0.084
HDL cholesterol, mg/dL	45.3±10.2	47.3±10.1	0.162
Triglycerides, mg/dL	154.8±68.6	152.7±74.6	0.833
Albumin, mg/dL	4.2±0.8	4.4±0.8	0.424
Total protein, g/L	73.8±6.3	75.0±4.9	0.104
WBV at HSR, 208 s^{-1}	17.1±0.7	17.4±0.5	<0.001
WBV at LSR, 0.5 s^{-1}	59.7±16.7	65.9±12.5	0.002

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; Hct, hematocrit, HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; HSR, high shear rate; LDL, low-density lipoprotein; LSR, low shear rate; WBC, white blood cell count; WBV, whole blood viscosity.

Results

The mean age of the participants was 65.5 ± 6.9 years. The baseline characteristics, laboratory values, and echocardiographic measurements of both study groups are presented in Table 1. There were no significant differences between the groups with respect to mean age, gender, body mass index, diabetes mellitus, hypertension, hyperlipidemia, and smoking. In echocardiographic measurements, mean AV jet velocity was 1.97 ± 0.13 m/s in the patient group and 1,33 ± 0.18 m/s in the control group ($p < 0.001$). Mean left ventricular ejection fraction, left atrial size, and ascending aorta diameter were similar in both groups. The presence of MAC was significantly higher in the AVS group than in the control group (28 [25.7%] vs. 14 [14%]; $p = 0.039$).

Table 2. Model 1: independent predictors of aortic valve sclerosis

Parameters	OR	95% CI for OR		*p* values
		lower	upper	
MAC	2.015	0.961	4.227	0.064
hs-CRP	1.087	0.982	1.205	0.109
LDL cholesterol	1.008	1.000	1.016	0.063
WBV at HSR, 208 s^{-1}	2.245	1.381	3.648	0.001

CI, confidence interval; hs-CRP, high-sensitivity C-reactive protein; HSR, high shear rate; LDL, low-density lipoprotein; MAC, mitral annular calcification; OR, odds ratio; WBV, whole blood viscosity.

Table 3. Model 2: independent predictors of aortic valve sclerosis

Parameters	OR	95% CI for OR		*p* values
		lower	upper	
MAC	2.073	0.995	4.319	0.052
hs-CRP	1.088	0.983	1.205	0.103
LDL cholesterol	1.007	0.999	1.015	0.078
WBV at LSR, 0.5 s^{-1}	1.026	1.006	1.046	0.010

LSR, low shear rate (see also Table 2 for abbreviations).

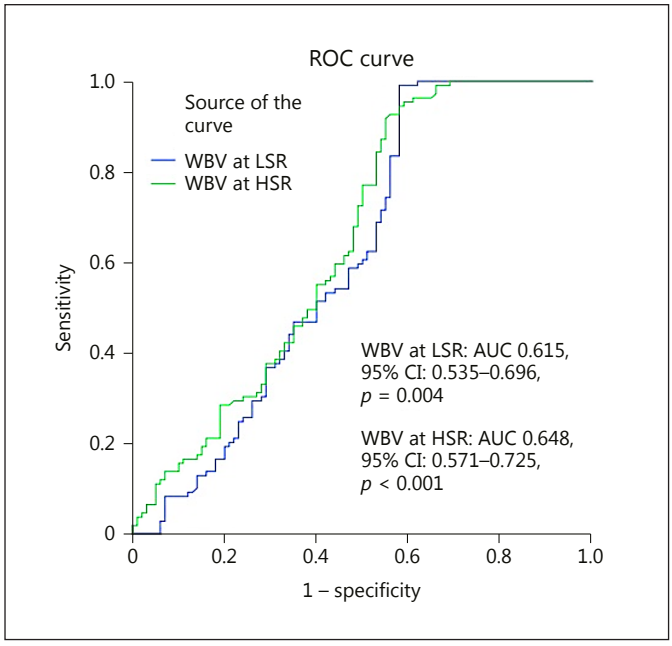

Fig. 1. Comparison of receiver-operating characteristic (ROC) curves for whole blood viscosity (WBV) at low (LSR) and high shear rate (HSR). 95% confidence intervals (CIs) for ROC are also displayed. AUC, area under the ROC curve.

Both groups were similar in regard to laboratory parameters except for higher high-sensitivity C-reactive protein levels ($p = 0.044$) in the AVS group. Total and LDL cholesterol levels were higher in the AVS group, but the differences were not statistically significant (p values: 0.079 and 0.084, respectively). In the AVS group, WBV values were significantly higher for HSR than in the control group (17.4 ± 0.5 vs. 17.1 ± 0.7 208 s^{-1}, $p < 0.001$) and LSR (65.9 ± 12.5 vs. 59.7 ± 16.7 0.5 s^{-1}, $p = 0.002$).

In order to find predictors of AVS, 2 multivariate logistic regression models were considered separately by WBV at HSR and LSR values, which contain MAC, high-sensitivity C-reactive protein, and LDL cholesterol (Tables 2, 3). The WBV values at HSR and LSR were independent predictors of AVS (WBV at HSR: odds ratio, OR: 2.24, 95% confidence interval, CI: 1.38–3.64, $p = 0.001$; WBV at LSR: OR: 1.026, 95% CI: 1.006–1.046, $p = 0.01$; Tables 2, 3). In the ROC curve analysis, a WBV cutoff value of 65.4 at LSR had a sensitivity of 46.8% and a specificity of 60.0% for the prediction of AVS (area under the ROC curve, AUC: 0.615, 95% CI: 0.535–0.696, $p = 0.004$), and a WBV cutoff value of 17.1 at HSR had a sensitivity of 61.5% and specificity of 53% for the prediction of AVS (AUC: 0.648, 95% CI: 0.571–0.725, $p < 0.001$; Fig. 1).

Discussion

The present study demonstrated that WBV values were higher in the AVS group than in the controls. Furthermore, WBV values at HSR and LSR were independently associated with AVS. WBV is a measure of the resistance of blood to flow and contributes to endothelial shear stress [9]. This biophysical property makes it a critical determinant of friction against the vessel walls. Shear stress modulates the orientation of endothelial cells in the direction of flow and the "waviness" of the luminal surface of the vessel [10]. Balachandran et al. [11] reported that the exposure of pulsatile shear stress on the aortic surface causes an increase in inflammatory mediator cells. Turbulent flow and resulting oscillatory shear stress on the aortic surface of AV leaflets and in the coronary sinus area lead to increased permeability of endothelial

cells and proatherogenic phenotypic transformation, including augmented matrix calcification [12]. As a common pathomechanism of AVS and atherosclerosis, sclerosis and subsequent calcification of AV leaflets is promoted by the same traditional risk factors that lead to endothelial dysfunction, as well as hemodynamic factors involving formation of secondary flow, where the vessel wall is exposed to oscillatory shear stress [12]. Like AVS, coronary atherosclerotic lesions more commonly occur at sites with the highest oscillatory shear stress, such as coronary vessel bifurcations [13].

Some published studies reported that WBV estimated by the de Simone formula was found to be associated with occurrence of MAC, and coronary collateral circulation in patients with chronic total occlusion, ST-elevation myocardial infarction, and coronary slow-flow phenomenon [14–17]. Moreover, increased Hct levels, as a component of WBV, inversely affect cerebral hemodynamics and result in an increased risk of neurological deficits [8, 18–20]. The strong relationship between high blood pressure and WBV and the components of WBV have also been shown in primary hypertension [21–23]. Coronary artery disease and AV disease may not generally coexist, and associations are complex. Even in the absence of the atherosclerotic process, altered blood flow patterns may be seen in patients with severe aortic stenosis [24–27]. The fluctuations in WBV are an indicator of wall shear stress, and enhanced WBV has been claimed to cause inflammation and vessel remodeling [28].

In a previous study, the rate of AVS was found to be higher in patients who had myocardial infarction without previous coronary risk factors, suggesting that undetectable atherosclerotic processes may have been higher in these patients than expected [1]. Therefore, traditional cardiovascular risk factors such as diabetes, hypertension, smoking, and hyperlipidemia are insufficient to explain the occurrence and development of AVS.

Higher WBV may be an indicator of AVS due to increased AV resistance. Although the WBV is the major component of the Virchow triad, related studies are limited because of the various materials needed for its evaluation. In this study, our findings have also demonstrated that WBV may be an important factor contributing to the development of AVS.

The limitations of this study include the fact that it is a case-control study with a relatively small number of patients; all the data were based on a single measurement, and direct measurements of blood viscosity with a viscometer were not done.

Conclusions

In this study, WBV at HSR and LSR were independently associated with AVS. Enhanced WBV could be an indicator of inflammation and vessel remodeling without evidence of outflow obstruction.

Disclosure Statement

The authors have no conflict of interest to report.

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