European Review for Medical and Pharmacological Sciences 2023; 27: 2358-2376

Endotrophin and matrix metalloproteinase-2 levels in bicuspid aortic valve and hypertension associated aortopathy and their relationship with strain parameters of the ascending aorta

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Abstract. – OBJECTIVE: Bicuspid aortic valve (BAV) is the most common congenital heart defect. Ascending aorta dilatation is related to BAV- and hypertension (HTN)-associated aortopathy. The aim of this study was to investigate aortic elasticity, as well as aortic deformation of the ascending aorta, using strain imaging, and to evaluate the possible relationship of biomarkers, such as endotrophin and matrix metalloproteinase-2 (MMP-2), with ascending aorta dilatation in patients with BAV- or HTN-associated aortopathy.

PATIENTS AND METHODS: This prospective study included patients with ascending aorta dilatation with BAV (n = 33), or normal tricuspid aortic valve with HTN (n = 33), and 20 control subjects. The mean age of the total patients was 42.76 ± 10.4 years (67% male, 33% female). We calculated aortic elasticity parameters using the relevant formula by M-mode echocardiography and determined layer-specific longitudinal and transverse strains of the proximal aorta by speckle-tracking echocardiography. Blood samples of the participants were drawn for the analysis of endotrophin and MMP-2.

RESULTS: Aortic strain and aortic distensibility were significantly decreased, whereas the aortic stiffness index was significantly increased in patient groups with BAV or HTN compared to the control group ($p < 0.001$). Moreover, longitudinal strain of both the anterior and posterior aortic walls of the proximal aorta were significantly impaired in BAV and HTN patients ($p < 0.001$). Serum endotrophin levels were significantly reduced in the patient cohort compared to the controls ($p = 0.001$). Endotrophin was noted to be significantly positively correlated with aortic strain and aortic distensibility ($r = 0.37$, $p = 0.001$; $r = 0.45$, $p < 0.001$, respectively), whereas inversely associated with aortic stiffness index ($r = -0.402$, $p < 0.001$). Furthermore, endotrophin was the single independent predictor of ascending aorta dilatation (OR = 0.986, $p < 0.001$). A cut-off value of endotrophin ≤ 82.38 ng/mL predicted ascending aorta dilatation with a sensitivity of 80.3% and specificity of 78.5% ($p < 0.0001$).

CONCLUSIONS: The present study showed that aortic deformation parameters and elasticity are impaired in BAV and HTN patients, and strain imaging allows for a good analysis of ascending aorta deformation. Endotrophin could be a predictive biomarker of ascending aorta dilatation in BAV and HTN aortopathy.

Key Words:
Endotrophin, Matrix metalloproteinase-2, Bicuspid aortic valve, Aortic dilatation, Strain imaging.

Abbreviations

BAV: Bicuspid aortic valve, HTN: essential hypertension, 2D-STE: two-dimensional speckle-tracking echocardiography, MMP-2: matrix-metalloproteinase-2, TAV: tricuspid aortic valve, BMI: body mass index, TTE: transthoracic echocardiography, LV: left ventricle, LVEF: Left ventricular ejection fraction, LAVI: Left atrial volume index, AoS: systolic aortic diameter, AoD: diastolic aortic diameter, SBP: brachial artery systolic blood pressure, DBP: diastolic blood pressure, PP: Pulse pressure, VVI: velocity vector imaging, LS: longitudinal strain, TS: transverse strain, LD: longitudinal displacement, TD: transverse displacement, LV: longitudinal velocity, CBC: complete blood count, TGF-β: transforming growth factor-β

Introduction

Bicuspid aortic valve (BAV) is the most common congenital heart defect, affecting 1-2% of the general population[1], and BAV causes valvulo-aortopathy. In addition to aortic stenosis or regurgitation, it is associated with ascending aortic dilatation over time and, consequently, an

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increased risk of aortic aneurysm, dissection, and rupture[1,2]. Previous studies[1] have reported that approximately 59% of patients with BAV have ascending aorta dilatation. Several factors play important roles in BAV-associated aortopathy. Accelerated aortic media degeneration and loss of aortic elasticity constitute major risk factors for BAV-associated aortopathy[1,3]. Altered hemodynamic forces on the aortic wall by eccentric flow through the morphologically stenotic valve lead to abnormal turbulent flows in the ascending aorta and increased wall shear stress[3,4]. These alterations in the normal biomechanics of the ascending aorta are major risk factors for degradation in the extracellular matrix with progressive aortic dilatation[1,3,4]. Furthermore, several gene mutations have also been reported to be associated with valve and aortic wall abnormality, in addition to hemodynamic disturbance[5].

Additionally, aortic root or ascending aortic dilatation is a common clinical feature in hypertensive patients. Previous studies[6] have indicated that ascending aorta dilatation is highly prevalent in about 15% of patients affected by essential hypertension (HTN). Dilatation of the ascending aorta was also shown to be associated with arterial stiffness and impaired aortic elasticity[6,7]. Aortic elastic properties determine the intrinsic aortic wall alterations and biomechanics of the ascending aorta. Aortic elasticity can be assessed using different imaging techniques and different plasma biomarkers of extracellular matrix turnover[1,3,8]. Echocardiography is an important imaging tool for evaluating the aortic elasticity of the ascending aorta, such as aortic distensibility, stiffness index, and aortic strain[1,3,8]. However, early alterations in aortic elasticity can be determined by two-dimensional speckle-tracking echocardiography (2D-STE) for the assessment of 2D strain imaging of the ascending aorta[9,10].

Patients with BAV- or HTN-associated aortopathy can develop ascending aorta aneurysms over time and require surgery during their lifetimes. Therefore, early identification of possible determinants of ascending aorta dilatation is crucial, and there is a need for early markers of disease progression. Previous studies[8] have shown that patients suffering from aortic dilatation have an increased level of matrix metalloproteinase-2 (MMP-2) in the plasma. Matrix metalloproteinase-2 is a biomarker of degradation of the extracellular matrix[11]. A newly identified adipokine called endotrophin is a cleavage fragment of the type VI collagen alpha-3 chain secreted from connective tissue, especially adipose tissue[12]. Some recent studies[12,13] have reported that endotrophin plays a significant role in various conditions, such as adipose tissue fibrosis, inflammation, increased insulin resistance, and cancer development. In this study, we aimed to investigate the discriminative role of imaging techniques, and 2D-STE strain imaging in patients having BAV- or HTN-associated aortopathy, and to evaluate whether there is a possible relationship between endotrophin, MMP-2, ascending aorta dilatation, and aortic elastic properties.

Patients and Methods

Study Population and Clinical Data Collection

This prospective observational study enrolled 66 consecutive patients with ascending aorta dilatation with BAV or normal tricuspid aortic valve (TAV) at the Echocardiography Laboratory of Istanbul University School of Medicine between May 2022 and November 2022, and comparisons were made with control subjects. The study population was classified into the following four groups: Group 1 consisted of BAV patients with an aortic aneurysm with a diameter of ascending aorta above 4.5 cm (n = 10). Group 2 included BAV patients with aortic dilatation in the range of 4.0-4.5 cm (n = 23). Group 3 included patients with primary arterial essential HTN with TAV and aortic dilatation (> 4.0 cm) (n = 33), while the control group consisted of 20 volunteers who matched in age, gender, body mass index (BMI), smoking, and alcohol use, but who had no cardiac or ascending aortic disease on the echocardiography in our institution.

Exclusion criteria were severe valvular heart disease with aortic stenosis or regurgitation, coronary artery disease, patients with cardiovascular disease requiring revascularization or with a history of cardiac surgery, a history of intervention to the aortic valve or aorta, congenital heart disease (i.e., aortic coarctation, etc.), patients with implantable cardiac devices, heart failure with an ejection fraction of < 50%, atrial fibrillation, uncontrolled diabetes mellitus with end-organ damage, chronic kidney disease (estimated glomerular filtration rate < 30 mL/minute/1.73), chronic liver disease, chronic obstructive pulmonary disease, chronic inflammatory diseases, malignancy, thyroid disorder, and poor echogenicity. According to the exclusion

criteria, 14 patients were excluded from the study.

The study protocol was approved by the Local Ethics Committee of Istanbul University, Faculty of Medicine (Approval no: 2022/5/907754), and written informed consent was obtained from all subjects according to the Declaration of Helsinki.

Definitions

The diagnosis of BAV was confirmed by two experienced cardiologists with a clearly defined BAV orifice during systole and two leaflets (with or without raphe) at the short axis on transthoracic echocardiography[11]. Any disagreement in the diagnosis was resolved by transesophageal echocardiography. Moreover, the types of BAV disease were classified as follows: type 0 indicates BAV with no raphe, type 1 indicates a right-left cusps fusion (> 70% of the patients), type 2 indicates a fusion of the right and non-coronary cusps, and type 3 indicates a fusion of the left and non-coronary cusps[14,15]. Patients with primary essential HTN were defined as patients with TAV and a blood pressure ≥ 140/90 mm Hg measured at any time or patients taking any antihypertensive drugs. A diagnosis of secondary HTN was excluded.

2D Transthoracic Echocardiography

All participants underwent a detailed transthoracic echocardiography (TTE) with a Siemens Acuson SC2000 cardiac ultrasound system (Mountain View, CA, USA), using an 4V1C (1.75-4.3 MHz) transducer by an experienced cardiologist blinded to the study groups to minimize the variability of measurements. Conventional 2D echocardiographic images were obtained using the techniques recommended by the American Society of Echocardiography[16]. The blood pressure was measured at the same time at rest for at least 5 minutes.

Aortic diameters were determined at the aortic root, sinus of valsalva, sinotubular junction, and ascending aorta at the parasternal long-axis view, as described earlier[16]. Using M-mode echocardiography, the diameters and wall thicknesses of the left ventricle (LV) were measured. The left ventricular ejection fraction (LVEF) was calculated using biplane Simpson's method[17]. The left atrial volume index (LAVI) and left ventricular mass index were also measured. The LV diastolic function was determined by the ratio of the peak early diastolic filling velocity (E) to the late diastolic filling velocity (A): E/A ratio, and the ratio of transmittal E to the mean of LV septal and lateral early diastolic tissue velocities (mean e'): E/e' ratio. The tricuspid annular plane systolic excursion (TAPSE) was calculated for the right ventricular function. Transaortic systolic peak flow velocity and systolic peak pressure gradient were assessed using continuous-wave Doppler[11]. The presence and degree of aortic regurgitation were evaluated on color-Doppler according to standard criteria[11,16].

The systolic (AoS) and diastolic (AoD) aortic diameters were measured at 3 cm above the aortic valve using a 2D-guided M-mode echocardiography in the parasternal long axis[11]. The AoD was measured at the peak of the R wave by a simultaneously recorded electrocardiogram, while the AoS was measured at the maximal anterior motion of the anterior aortic wall. These aortic diameters were indexed to the body surface area. Additionally, brachial artery systolic (SBP) and diastolic blood pressure (DBP) were measured with a properly sized cuff sphygmomanometer. Pulse pressure (PP) was measured as the difference between SBP and DBP. The following parameters of aortic elasticity were calculated: aortic strain (%) = 100 (AoS - AoD)/AoD; aortic stiffness index = ln(SBP/DBP)/[(AoS - AoD)/AoD]; aortic distensibility (10^{-6} cm^2 dyn^{-1}) = [2(AoS - AoD)/AoD(PP)], as previously described[18].

Strain Imaging of Ascending Aorta Using 2D-STE Analysis

Strain imaging of the proximal aorta was performed by layer-specific 2D-STE in the parasternal long-axis view. For strain analysis, all images were digitally stored on the machine and subsequently analyzed offline using a velocity vector imaging (VVI) program. Since the tracking software program does not include aortic wall strain, a two-chamber analysis of LV was used to evaluate aortic deformation, similar to previous studies[9]. In the standard apical two-chamber view, six segments were decreased to four by dividing the aortic wall into two anterior and two posterior segments and excluding apical segments.

We then calculated the peak longitudinal strain (LS) and transverse strain (TS) of the aortic wall for each layer (endocardial, myocardial, and epicardial); the longitudinal (LD) and transverse (TD) displacements, defined as a change in the position of the body; and the longitudinal velocity (LV), displacement of an object per time unit for each layer of the ascending aorta.

Laboratory Tests

Blood samples of the patients were collected in appropriate tubes for complete blood count (CBC), coagulation tests, and routine biochemical tests. All tests were performed within three hours following collection. Some of the serum samples were aliquoted for ELISA tests and stored at -80°C until used.

The CBC analyses were performed by COULTER® LH780 Hematology Analyzer (Beckman Coulter Inc., Brea, CA, USA). Routine biochemical tests were performed using Cobas 6000 (Roche Diagnostics, Mannheim, Germany) analyzers. The MMP-2 and endotrophin concentrations of the serum samples were measured using commercial kits based on the ELISA method (Elabscience Biotechnology Inc., Catalog No: E-EL-H1445 and Sunred Biological Technology, Catalogue No: 201-12-9305, respectively).

Statistical Analysis

The Kolmogorov-Smirnov test was used to analyze the normality of the data. Parametric continuous data are expressed as mean ± standard deviation (SD), and non-parametric continuous data, median (minimum-maximum), and categorical data are expressed as percentages. A Chi-squared test or Fisher's exact test was used to assess the differences in categorical variables between the groups, where necessary. A Student's *t*-test or the Mann-Whitney U test was used to compare unpaired samples as needed. The relationships among the parameters were assessed using Pearson's or Spearman's correlation analyses according to the normality of the data. The primary analysis used ANOVA to compare all reported data for parametric variables, whereas the Kruskal-Wallis test was used for comparison among non-parametric variables between groups. Logistic regression analysis was used to determine the independent predictors of ascending aorta dilatation. Multiple linear regression analyses of the longitudinal average strain and transverse strain were performed. Standardized partial regression coefficients (β) were used to compare the effect on the dependent variable, and 95% confidence intervals (CI) were determined. The receiver operating characteristic (ROC) curves were obtained to determine the best cut-off values for LS, LV, LD, TS, TD, aortic strain, aortic stiffness index, aortic distensibility, and laboratory parameters in the prediction of ascending aorta dilatation. Significance was assumed at a two-sided $p < 0.05$. All statistical tests, except for the ROC curve analysis, were conducted using the Statistical Package for the Social Sciences 26.0 for Windows (IBM Corp., Armonk, NY, USA). The ROC curve analyses were performed with MedCalc® Statistical Software version 20.015 (MedCalc Software Ltd, Ostend, Belgium).

Results

Patient Characteristics

A total of 86 consecutive patients were enrolled in the present study (Figure 1). The mean age of the total study population was 42.76 ± 10.4 years (67% male, 33% female). The baseline clinical characteristics and echocardiographic and laboratory findings of the study groups are presented in Table I. No statistically significant differences were observed with respect to age, gender, BMI, or current smoking or alcohol status between the groups ($p > 0.05$). The LV diastolic function parameters, such as E/A ratio, E/e' ratio, LAVI, LV mass index, cardiac output, stroke volume, and the presence of aortic regurgitation, were significantly different between the groups (Table I). The most common type of BAV was type 1, accounting for about 60% of the BAV patients in our study.

Aortic Diameters and Aortic Elastic Properties

Table II shows a comparison of the aortic elastic parameters and biochemical markers among the groups. Patients with BAV or HTN had significantly higher indexed diastolic or systolic aortic diameters than the control group ($p < 0.001$). Moreover, the aortic stiffness index was statistically significantly higher in the BAV and HTN patient groups than in the controls ($p < 0.001$), whereas aortic strain and aortic distensibility were significantly reduced in patient groups compared to the control group ($p < 0.001$). Additionally, peak aortic velocity and aortic gradients were significantly different among the groups ($p < 0.001$).

2D-STE Analysis of Ascending Aorta

The aortic deformation parameters of the proximal aorta by 2D-STE analysis in the study groups are presented in Table III. The layer-specific strain imaging of the proximal aorta showed that the LS of the anterior and posterior aortic walls and the average LS in all three layers were significantly reduced in the BAV and HTN patient groups

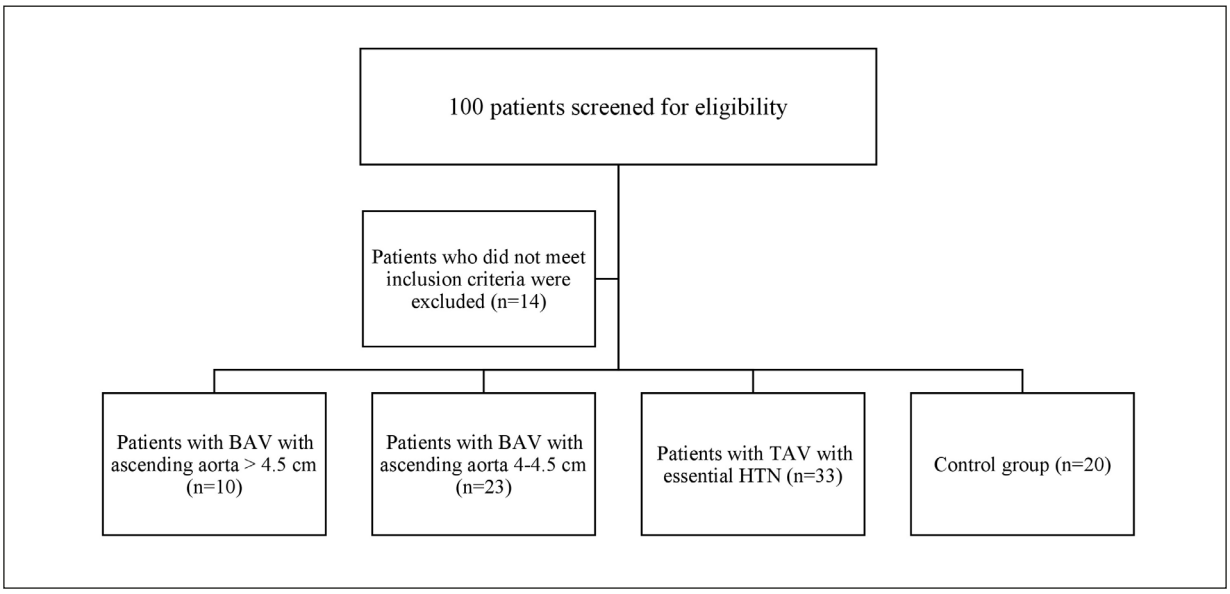

Figure 1. Flow diagram of the study design.

Table I. Baseline clinical characteristics, laboratory findings, treatment and conventional echocardiographic parameters of the study groups.

	BAV with ascending aorta >4.5 cm (n=10)	BAV with ascending aorta 4-4.5 cm (n=23)	HTN (n=33)	Control group (n=20)	*p*-value
Clinical characteristics					
Age, (years)	45.86 ± 7.6[b]	40.22 ± 14[d]	49.25 ± 3.5	41.35 ± 8.3	0.158
Gender Male, n (%) Female, n (%)	 6 (7%) 4 (4.7%)	 16 (18.6%) 7 (8.1%)	 26 (30.2%) 7 (8.1%)	 10 (11.6%) 10 (11.6%)	0.171
BMI (kg/m^2)	30.68 ± 3.1	28.9 ± 2	29.84 ± 3.1	27.19 ± 1.2	0.063
BSA (m^2)	1.99 ± 0.1[c]	1.9 ± 0.1	1.97 ± 0.1[f]	1.82 ± 0.2[c,f]	0.001
Heart rate (bpm)	69.5 (63-84)[c]	76 (57-103)[d]	68 (60-90)[d,f]	76 (65-92)[c,f]	0.014*
Systolic blood pressure, (mmHg)	110 (90-160)	120 (80-190)	120 (90-150)	100 (90-172)	0.065
Diastolic blood pressure, (mmHg)	70 (65-90)	80 (45-100)[e]	80 (60-90)[f]	70 (60-80)[e,f]	0.033*
Pulse pressure, (mmHg)	40 (20-80)	40 (20-90)	40 (20-60)	40 (30-93)	0.878
Disease duration, (years)	0.5 (0-5)[b]	0 (0-25)[d,e]	8 (0-30)[b,d,f]	0[e,f]	<0.001*
Comorbidities					
HTN, n (%)	6 (7%)[c]	7 (8.1%)[d,e]	29 (33.7%)[d,f]	0 (0%)	<0,001*
DM, n (%)	1 (1.2%)[b]	3 (3.5%)[d]	12 (14%)[b,d,f]	1 (1.2%)[f]	0.021*
Smoking, n (%)	3 (3.5%)	5 (5.8%)	9 (10.5%)	6 (7%)	0.927
Alcohol use, n (%)	0 (0%)	0 (0%)	1 (1.2%)	1 (1.2%)	0.685
Laboratory findings					
Glucose, (mg/dL)	97.5 (76-188.9)	97 (73-177)	96.5 (73-144)	86.5 (72-146)	0.356

Table continued

Table I (*Continued*). Baseline clinical characteristics, laboratory findings, treatment and conventional echocardiographic parameters of the study groups.

	BAV with ascending aorta >4.5 cm (n=10)	BAV with ascending aorta 4-4.5 cm (n=23)	HTN (n=33)	Control group (n=20)	*p*-value
HbA1C, (%)	5.5 (5-8.5)	5.5 (4.7-9.9)	6.1 (5-9.5)	5.3 (4.2-7.7)	0.098
BUN, (mg/dL)	11.6 (10.1-22.6)	13.6 (9.5-18.5)[d]	15.2 (11-35.3)[d,f]	11.6 (6.9-19.5)[f]	0.006*
Creatinine (mg/dL)	0.9 (0.6-1.5)[c]	0.8 (0.6-1.1)	0.9 (0.7-1.4)[e,f]	0.8 (0.5-1)[f]	0.023*
GFR, (mL/min/1.73 m²)	100.26 ± 29.4	109.43 ± 20.1[d]	86.5 ± 12.8[d,f]	109.89 ± 9.7[f]	<0.001*
AST (U/I)	17.41 ± 3.7	20.2 ± 8.7	18.01 ± 3.8	18.56 ± 5.9	0.543
ALT (U/I)	24.04 ± 10.8	23.91 ± 13.2	18.15 ± 7.7	18.04 ± 9	0.106
Sodium (mmol/L)	141 ± 2.4	139.94 ± 2.1	141 ± 2.3	139.89 ± 2.4	0.247
Potassium (mmol/L)	4.43 ± 0.3	4.51 ± 0.4	4.54 ± 0.5	4.27 ± 0.3	0.133
Uric acid, (mg/dL)	5.76 ± 1.2	4.93 ± 0.8	5.4 ± 1	4.86 ± 1.3	0.108
CRP, (mg/L)	2.3 (0.7-8.7)[c]	2.4 (0.3-63.8)[e]	3.1 (0.4-11.4)[f]	0.9 (0.4-4.8)[c,e,f]	0.013*
Hgb (gr/dL)	14.6 (10.2-16.3)	13.9 (10.3-16.9)	14.4 (11.2-15.7)	14.6 (12.4-16)	0.425
Hematocrit (%)	42.7 (31.5-48.1)	41.3 (30.5-49)	43.6 (34.4-47.4)	43.1 (37.2-47.4)	0.612
WBC (10^3/µl)	7 (4.9-9)	6.8 (4.4-14.1)	7.4 (5-13.3)	6.4 (4.6-10.4)	0.186
Neutrophile (10^3/µl)	3.8 (2.3-6)	4 (1.6-9.8)	4.9 (2.6-9)	4 (2.4-7.1)	0.397
Lymphocyte (10^3/µl)	2.2 (1.4-3.9)	1.8 (1.1-3.3)	2.2 (1.2-4.2)	1.8 (1.1-3.2)	0.089
Platelet (10^3/µl)	243 (133-365)	237 (87-377)	268 (95-586)	247 (177-370)	0.513
RDW (%)	13.5 (12.4-16.7)[b]	13.8 (12.4-17.6)	14 (13-18.3)[b,f]	13.4 (12.3-14.4)[f]	0.007*
MPV (fL)	8.6 (7.4-10.4)	9.2 (7-10.9)	8.5 (6.3-12.6)	8.5 (6.9-9.9)	0.650
PDW (fL)	16.6 (16.1-17.4)	16.8 (16-17.7)	16.5 (15.9-18.8)	16.5 (16.1-17.4)	0.722
MCV (fL)	86.7 (78.9-95.7)	88.2 (69.2-93.7)	86.8 (63.8-99.6)	84.8 (80.1-92.6)	0.724
Total cholesterol, (mg/dL)	191.92 ± 39.6	187.33 ± 34	200.47 ± 36.1	203.23 ± 35.6	0.580
HDL-C, (mg/dL)	46.4 (37-69.7)	45.3 (33-77.8)	43.6 (26.7-76.2)	46.3 (30.1-76.6)	0.415
LDL-C, (mg/dL)	114.06 ± 33.2	114.72 ± 22.9	124.93 ± 29.8	131.11 ± 26.9	0.258
Triglyceride, (mg/dL)	153.6 (84.7-239.7)[c]	104.9 (53.8-192.5)[d]	130.7 (60-516.6)[d,f]	102.2 (53.2-02.1)[e,f]	0.007*
Treatment					
Beta blocker, n (%)	5 (5.8%)[c]	8 (9.3%)[e]	14 (16.%)[f]	0 (0%)[c,e,f]	0.005*
CCB, n (%)	1 (1.2%)	3 (3.5%)	15 (3.5%)[f]	0 (0%)[f]	<0.001*
Diuretic, n (%)	2 (2.3%)	1 (1.2%)	11(12.8%) [f]	0 (0%)[f]	0.004*
Statin, n (%)	3 (3.5%)	4 (4.7%)	4 (4.7%)	0 (0%)	0.110
ACE inhibitor, n (%)	2 (2.3%)	3 (3.5%)	12 (14%)[f]	0 (0%)[f]	0.010*
ARB, n (%)	3 (3.5%)	1 (1.2%)	10 (11.6%)[f]	0 (0%)[f]	0.006*
Transthoracic echocardiographic findings					
LVEDD (cm)	4.91 ± 0.5	4.99 ± 0.6	4.86 ± 0.4	4.62 ± 0.4	0.064
LVESD (cm)	3.2 (2.6-3.9)[c]	3.2 (2.7-4.5)[e]	3.1 (2.4-3.9)[f]	2.9 (2.4-3.6)[c,e,f]	0.024*
IVSd (cm)	1.2 (1-1.9)[c]	1.2 (0.7-1.4)[e]	1.2 (1.1-1.5)[f]	0.8 (1.1-0.9)[c,e,f]	<0.001*
LVPWd, (cm)	1.1 (1-1.5)[c]	1 (0.7-1.2)[e]	1.1 (0.9-1.3)[f]	0.9 (0.6-1.1)[c,e,f]	<0.001*
LVEF, (%)	63.6 ± 4.9	63.18 ± 5.1	64.66 ± 5.2	66.45 ± 6.1	0.240
RV, (mm)	2.77 ± 0.2	2.79 ± 0.2	2.92 ± 0.2[f]	2.62 ± 0.2[f]	<0.001*
LA, (mm)	3.63 ± 0.3[b]	3.72 ± 0.5[d,e]	4.06 ± 0.3[b,d,f]	3.42 ± 0.2[e,f]	<0.001*

Table continued

Table I (*Continued*). Baseline clinical characteristics, laboratory findings, treatment and conventional echocardiographic parameters of the study groups.

	BAV with ascending aorta >4.5 cm (n=10)	BAV with ascending aorta 4-4.5 cm (n=23)	HTN (n=33)	Control group (n=20)	*p*-value
RA, (mm)	3.38 ± 0.3	3.23 ± 0.3	3.47 ± 0.2	3.18 ± 0.2	0.003
E/A ratio	1 (0.4-1.4)[b]	0.9 (0.5-2.1)[d]	0.7 (0.5-1.5)[b,d,f]	1.2 (0.7-1.5)[f]	<0.001*
E/e' ratio	8.4 (5.4-13)	7.8 (4-15)[e]	8.2 (3.2-19.6)[f]	6.6 (4.8-9.4)[e,f]	0.021*
LAVI (ml/m²)	23 (14.5-37)	21.8 (10-39)[d]	29 (19-109)[d,f]	20 (14-32)[f]	0.001*
LV mass index (gr/m²)	106.5 (72-272.4)[c]	111 (50-279.8)[e]	110.5 (72-297)[f]	75 (58-96)[c,e,f]	<0.001*
TAPSE (mm)	2.26 ± 0.3	2.21 ± 0.3	2.12 ± 0.3	2.07 ± 0.3	0.320
Cardiac output, (L/min)	5.7 (4-7.2)	5.9 (4.3-9.4)[e]	6.1 (4.4-34.8)[f]	4.9 (3-7.4)[e,f]	0.003*
Stroke volume, (mL)	87.2 (43-117)[c]	81.7 (59-124.1)[e]	91.5 (56.3-199)[f]	63.8 (38-94.1)[c,e,f]	<0.001*
Mild-moderate mitral regurgitation, n (%)	10 (11.6%)	22 (25.6%)	28 (32.6%)	15 (17.4%)	0.123
Mild-moderate aortic regurgitation, n (%)	9 (10.5%)[c]	20 (23.3%)[e]	21 (24.4%)[f]	3 (3.5%)[c,e,f]	<0.001*
Bicuspid aortic valve type					
Type 0, n (%)	1 (1.2%)	3 (3.5%)	0 (0%)	0 (0%)	0.076
Type 1, n (%)	7 (8.1%)[b,c]	13 (15.1%)[d,e]	0 (0%)[b,d]	0 (0%)[c,e]	<0.001*
Type 2, n (%)	2 (2.3%)[b,c]	2 (2.3%)[d,e]	0 (0%)[b,d]	0 (0%)[c,e]	0.033*
Type 3, n (%)	0 (0%)	1 (1.2%)	0 (0%)	0 (0%)	0.428

BVA: Bicuspid aortic valve, BMI: body mass index, BSA: body surface area, HTN: essential hypertension, DM: diabetes mellitus, HbA1C: hemoglobin A1C, BUN: blood urea nitrogen, GFR: glomerular filtration rate, AST: aspartate aminotransferase, ALT: alanine aminotransferase, CRP: C-reactive protein, Hgb: hemoglobin, WBC: white blood cell, RDW: red cell distribution width, MPV: mean platelet volume, PDW: platelet distribution width, MCV: mean corpuscular volume, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, CCB: calcium channel blocker, ARB: angiotensin receptor blocker, ACE: angiotensin converting enzyme, LVEDD: left ventricle end diastolic diameter, LVESD: left ventricle end systolic diameter, IVSd: interventricular septum diameter, LVPWd: left ventricule posterior wall diameter, LVEF: left ventricle ejection fraction, RV: Right ventricle, LA: left atrium, RA: right atrium, LAVI: left atrial volume index, LV:left ventricle, TAPSE: tricuspid annular plane of systolic excursion. [a] There is a statistically significant difference between the groups BAV with ascending aorta>4.5cm and BAV with ascending aorta 4-4.5 cm. [b] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and HTN. [c] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and the control. [d] There is a statistically significant difference between the groups BAV with ascending aorta 4-4.5 cm and HTN. [e] There is a statistically significant difference between the groups BAV with ascending aorta 4-4.5 cm and the control. [f] There is a statistically significant difference between the groups HTN and the control; *$p<0.05$.

compared to the controls (all p-values < 0.001) (Figures 2-3). However, the TS of the anterior and posterior aortic walls and the average TS were similar within the study population. For TD and LV, there was no significant difference between the groups.

Plasma MMP-2 and Endotrophin Levels

The plasma endotrophin levels were significantly decreased in the BAV and HTN patient groups compared to the controls (p = 0.001) (Table II) (Figure 4). However, we found no significant difference in plasma MMP-2 levels among the groups (p = 0.106).

Additionally, a multivariable logistic regression analysis was performed to determine the independent predictors of ascending aorta dilatation. Furthermore, multiple linear regression analyses of the average LS and TS were performed. In our study, endotrophin was found to be the single independent predictor of ascending aorta dilatation (OR = 0.986, 95% CI 0.978-0.994, p < 0.001, Table IV). Linear regression analysis revealed that

ascending aorta diameter was the single independent predictor of the average LS of different layers of the proximal aorta.

In correlation analysis, a significant positive correlation was found between endotrophin, aortic strain, and aortic distensibility whereas a significant inverse correlation was observed between endotrophin, aortic stiffness index, indexed diastolic, and systolic aortic diameter (Table V, Figure 5). For the 2D-STE strain analysis, there was a significant positive correlation between endotrophin, LV, and the LS of different layers of the proximal aorta (Table VI, Figure 6).

In the ROC curve analysis, a cut-off value of 82.38 ng/mL was identified as the predictive endotrophin value for determining ascending aorta dilatation with a sensitivity of 80.3% and a specificity of 78.5% (AUC = 0.808, 95% CI 0.705-0.888, $p < 0.0001$) (**Supplementary Table I**, Figure 7). The plasma MMP-2 levels predicted ascending aorta dilatation with 77.2%

Table II. Comparison of patients with bicuspid aortic valve, essential hypertension, and control subjects in aortic elastic parameters and biochemical markers.

Aortic diameter at (cm)	BAV with ascending aorta >4.5 cm (n=10)	BAV with ascending aorta 4-4.5 cm (n=23)	HTN (n=33)	Control group (n=20)	*p*-value
Aortic root	2.7 (2.3-3.4)	2.6 (2.2-3.8)	2.7 (2.1-3.6)	2.5 (2.2-3.5)	0.308
Sinus of Valsalva	4 (3.2-4.6)[c]	3.7 (3-35.1)[d,e]	4.2 (3.3-5)[d,f]	3.1 (2.7-3.9)[c,e,f]	<0.001*
Sinotubular junction	3.8 (3.3-4.5)[a,c]	3.3 (2.8-29)[a,d,e]	3.7 (3.1-4.4)[d,f]	2.7 (2.3-3.3)[c,e,f]	<0.001*
Ascending aorta	4.8 (4-5.1)[a,c]	4.1 (3.9-4.7)[a,d,e]	4.5 (4-5.1)[d,f]	3.1 (2.8-3.8)[c,e,f]	<0.001*
Peak aortic velocity (cm/s)	189.5 (87-429)[b,c]	207 (122-361)[d,e]	133 (87-206)[b,d]	132 (107-176)[c,e]	<0.001*
Mean aortic gradient (mmHg)	7 (2-48)	11.5 (4-32)[d,e]	3.7 (1-90)[d]	4.2 (2.9-5.5)[e]	<0.001*
Peak aortic gradient (mmHg)	15.5 (3-73)[b,c]	22.5 (6-52)[d,e]	7.6 (3-17)[b,d]	6.9 (4.6-9.1)[c,e]	<0.001*
Indexed diastolic aortic diameter (mm/m^2)	23.1 (20.1-24.7)[a,c]	19.8 (16.3-23.6)[a,e]	21.8 (18.2-25.6)[f]	14.2 (11.4-17.9)[c,e,f]	<0.001*
Indexed systolic aortic diameter (mm/m^2)	24.2 (21.5-25.3)[a,c]	21.5 (19.3-25.1)[a,e]	23.1 (19.4-26.7)[f]	18.1 (14.0-20.6)[c,e,f]	<0.001*
Aortic strain (%)	4.4 (0.1-7.1)[a,c]	7.6 (0.1-18.2)[a,e]	5.8 (2-11.1)[f]	21.6 (10-34.6)[c,e,f]	<0.001*
Aortic stiffness index	10.1 (5.8-28.8)[a,c]	6.2 (2-33.1)[a,e]	7.4 (2.9-20.3)[f]	1.9 (1.2-5.6)[c,e,f]	<0.001*
Aortic distensibility ($cm^2dyn^{-1}10^{-6}$)	1.7 (0.1-2.7)[c]	2.3 (0.1-9.3)[e]	2.1 (0.8-5.2)[f]	8.3 (2.2-17.3)[c,e,f]	<0.001*
Matrix metalloproteinase-2 (ng/mL)	146.6 (104.2-840)	163 (116.8-753)	177.9 (83.9-797)	137.4 (97.5-267.5)	0.106
Endotrophin (ng/mL)	44 (14.3-67.4)[c]	58.8 (33.6-278.1)[e]	56.7 (31.5-357.6)[f]	210.4 (38.5-430.8)[c,e,f]	0.001*

BAV: bicuspid aortic valve, HTN: essential hypertension. [a] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and BAV with ascending aorta 4-4.5 cm. [b] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and HTN. [c] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and the control. [d] There is a statistically significant difference between the groups BAV with ascending aorta 4-4.5 cm and HTN. [e] There is a statistically significant difference between the groups BAV with ascending aorta 4-4.5 cm and the control. [f] There is a statistically significant difference between the groups HTN and the control; *p<0.05.

Table III. Comparison of deformation parameters of proximal aorta between the study groups.

Aortic deformation parameters (Speckle-tracking imaging)		BAV with ascending aorta >4.5 cm (n=10)	BAV with ascending aorta 4-4.5 cm (n=23)	HTN (n=33)	Control group (n=20)	*p*-value
Longitudinal strain (%)						
Anterior wall	Endo	9.1 (-17-15.7)[c]	15.6 (-28.7-40.7)[e]	13 (-16.3-39.2)[f]	48.3 (21.7-107.1)[c,e,f]	<0.001*
	Myo	9.7 (-14.9-16.7)[c]	15.3 (-20.4-34.7)[e]	12.5 (-13.4-38.3)[f]	52.2 (25.3-89.9)[c,e,f]	<0.001*
	Epi	9.8 (-13.5-18.4)[c]	15.3 (-5.5-29.3)[e]	10.3 (-11.9-41.8)[f]	42 (24.6-90.4)[c,e,f]	<0.001*
Posterior wall	Endo	19.2 (6.9-27.5)[c]	19.2 (-16.4-56)[e]	8.7 (-24.1-28.1)[f]	49.3 (22.1-118.2)[c,e,f]	<0.001*
	Myo	14.8 (2.6-19.6)[c]	14.9 (-25.9-60)[e]	8.7 (-23.6-32.5)[f]	31.9 (13.4-72.1)[c,e,f]	<0.001*
	Epi	9.5 (-1.7-19)	14.7 (-26.4-62.3)	6.9 (-23.6-33.1)[f]	27.7 (-21.9-43)[f]	<0.001*
Average	Endo	11.8 (-4.8-21.6)[c]	16.6 (-21.5-43.2)[e]	9.9 (-17.5-31.7)[f]	54.6 (21.9-107)[c,e,f]	<0.001*
	Myo	10.4 (-3.9-16.8)[c]	15.3 (-23.2-34.9)[e]	8.5 (-18.2-28.1)[f]	42 (25.1-71)[c,e,f]	<0.001*
	Epi	9.8 (-2.1-12.8)[c]	11.9 (-15.9-28.8)[e]	9.4 (-17.5-29.2)[f]	31.1 (11.8-56.4)[c,e,f]	<0.001*
Longitudinal velocity (cm/s)						
Anterior wall	Endo	2.05 ± 2	2.99 ± 1.9	2.15 ± 1.5	3.82 ± 2.9	0.090
	Myo	1.76 ± 1.4	2.48 ± 1.6	1.86 ± 1.4	3.35 ± 2.8	0.108
	Epi	1.72 ± 0.9	2.2 ± 1.4	1.78 ± 1.4	3.24 ± 3	0.132
Posterior wall	Endo	2.69 ± 2.3	4.21 ± 2.6	3.81 ± 2.5	5.04 ± 4.1	0.470
	Myo	2.75 ± 1.9	3.93 ± 2.3	3.45 ± 2.4	4.59 ± 3.6	0.529
	Epi	3.07 ± 1.4	3.88 ± 2.2	3.44 ± 2.4	4.16 ± 3.3	0.787
Average	Endo	2.11 ± 1.6	3.52 ± 2.2	2.57 ± 1.7	4.43 ± 3.4	0.161
	Myo	2.04 ± 1.4	3.14 ± 1.9	2.28 ± 1.5	3.97 ± 3.2	0.175
	Epi	2.24 ± 1.1	2.97 ± 1.7	2.27 ± 1.5	3.7 ± 3	0.279
Longitudinal displacement (mm)						
Anterior wall	Endo	0.7 (0.4-5.3)	0.5 (0-6)	0.7 (0-4.6)	0.2 (0-0.8)	0.143
	Myo	0.9 (0.5-4.2)	0.2 (0-4.2)	0.6 (0-3.6)	0.1 (0-1.1)	0.098
	Epi	1.1 (0.6-3.3)	0.4 (0-3.4)	0.6 (0-3.1)	0.2 (0-1.3)	0.118
Posterior wall	Endo	0.9 (0.1-5.9)	1.3 (0-5)	0.8 (0.1-6.9)	0.6 (0.1-1.5)	0.142
	Myo	1.4 (0.1-5.4)	1.1 (0.1-4.7)[e]	1.2 (0.1-4.9)[f]	0.5 (0-1.4)[e,f]	0.030*
	Epi	2.2 (0.1-4.8)[c]	1.4 (0.3-4.6)[e]	1.4 (0-4.2)[f]	0.5 (0-1.3)[c,e,f]	0.009*
Average	Endo	0.8 (0.2-4.5)	0.7 (0-5)	0.9 (0.2-2.5)	0.4 (0.1-1.1)	0.120
	Myo	1.1 (0.3-4)	0.5 (0.2-3.8)	0.9 (0.1-2.2)	0.3 (0-1.2)	0.074
	Epi	1.6 (0.3-3.4)[c]	0.7 (0.2-3.3)[e]	1 (0-2.2)[f]	0.3 (0-1.3)[c,e,f]	0.031*
Transverse strain (%)						
Anterior wall		33.4 (8.6-51.2)	13.7 (-41.8-77)	25.7 (-43.7-97.8)	1.5 (-40.2-73.3)	0.300
Posterior wall		-9.7 (-30.4--3)	-10.2 (-97.7-63.5)	13.9 (-46.8-88.7)	-11.8 (-67.8-19.7)	0.076
Average		6.6 (1.8-25.7)	-5.9 (-38-56.3)	18.7 (-35.5-70.4)	-8.6 (-39.7-30.7)	0.090
Transverse displacement (mm)						
Anterior wall	Endo	0.3 (0-0.9)	0.3 (0-1)	0.4 (0-6.7)	0.3 (0-1.2)	0.391
	Myo	0.2 (0-0.4)	0.3 (0-1.2)	0.5 (0-7.1)	0.3 (0.1-1.3)	0.285

Table continued

Table III (*continued*). Comparison of deformation parameters of proximal aorta between the study groups.

Aortic deformation parameters (Speckle-tracking imaging)		BAV with ascending aorta >4.5 cm (n=10)	BAV with ascending aorta 4-4.5 cm (n=23)	HTN (n=33)	Control group (n=20)	*p*-value
Posterior wall	Epi	0.1 (0-0.3)	0.3 (0-1.2)	0.5 (0-7.5)	0.2 (0-1.3)	0.241
	Endo	5.4 (2-6.2)	5.6 (3.4-7.9)	5.9 (0.1-10.8)	6.7 (2.6-8.9)	0.610
	Myo	5.4 (2.1-7.1)	5.9 (3.7-8.2)	6.7 (0.1-10)	7.2 (3.3-9.7)	0.253
Average	Epi	5.4 (2.1-8.2)	6.5 (3.7-10.2)	6.4 (0.1-11.4)	8.3 (3.6-11.5)	0.062
	Endo	2.7 (1.2-3.1)	2.8 (1.9-4.2)	3.6 (0.9-4.8)	3.6 (1.5-4.8)	0.246
	Myo	3.2 (1.2-4)	3 (2.4-4.3)	3.5 (1.2-5.2)	3.7 (0.7-4.9)	0.774
	Epi	3.2 (1.2-4.1)	3.4 (2.1-5.1)	3.6 (1.5-5.8)	4.3 (0.7-5.8)	0.610

BAV: bicuspid aortic valve, HTN: essential hypertension, Endo: endocardium, Myo; myocardium, Epi: epicardium. [a] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and BAV with ascending aorta 4-4.5 cm. [b] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and HTN. [c] There is a statistically significant difference between the groups BAV with ascending aorta>4.5 cm and the control. [d] There is a statistically significant difference between the groups BAV with ascending aorta 4-4.5 cm and HTN. [e] There is a statistically significant difference between the groups BAV with ascending aorta 4-4.5 cm and the control. [f] There is a statistically significant difference between the groups HTN and the control; *$p<0.05$.

sensitivity and 56.2% specificity, with a cut-off of 137.82 ng/mL (AUC = 0.670, 95% CI 0.558-0.770, $p = 0.028$) (**Supplementary Table I**, Figure 7). In the ROC curve analysis of deformation parameters, the prediction values of LS of the proximal aorta in the different layers for aortic dilatation are presented in **Supplementary Table II** ($p < 0.0001$).

Discussion

In the present study, we compared aortic elastic properties and aortic deformation parameters in all three layers of the proximal aorta by 2D-STE strain imaging between BAV- and HTN-associated aortopathy. Furthermore, we evaluated the relationship between endotrophin and the aortic elasticity and the deformation of the ascending aorta in patients with BAV- or HTN-associated aortopathy. We showed that endotrophin is associated with aortic elasticity and ascending aorta dilatation. Additionally, we found that endotrophin is an independent predictor of ascending aorta dilatation. For the first time, we have shown that endotrophin molecules can be a predictive marker for ascending aortic dilatation.

Although BAV is the most common congenital cardiac defect with an estimated prevalence of 0.5-2% in the general population, the aortic prognosis in patients with BAV has not been established well[3]. It has been reported that BAV represents 9% of all aortic dissections among young people[3]. In this regard, BAV does not solely cause valvulopathy but is also associated with aortopathy, resulting in the dilatation of different sections of the thoracic ascending aorta and increased risk of aortic dissection[19]. In addition, the chance of an aneurysm forming has been reported to be eight times higher with BAV[14]. An ascending aortic diameter and a sinus of valsalva exceeding 40 mm were regarded as aortic dilatation[20]. Previous studies[20] have reported that surgical intervention was required at 55 mm in the absence of coarctation, high blood pressure, or suspected family forms of aortic dissection. The exact mechanism of BAV-associated aortopathy remains unknown. The hemodynamic alterations on the aortic wall by these mechanisms and increased wall shear stress cause aortic wall cystic medial necrosis, elastic fiber fragmentation, loss

Table IV. Multivariate regression analysis for ascending aortic dilatation.

	OR	95% CI	*p*-value
Endotrophin	0.986	0.978-0.994	<0.001*
E/e' ratio	1.519	0.992-2.326	0.054
LVEDD	4.043	0.665-24.560	0.129

OR: odds ratio, CI: confidence interval, LVEDD: left ventricular end diastolic diameter; *$p<0.05$.

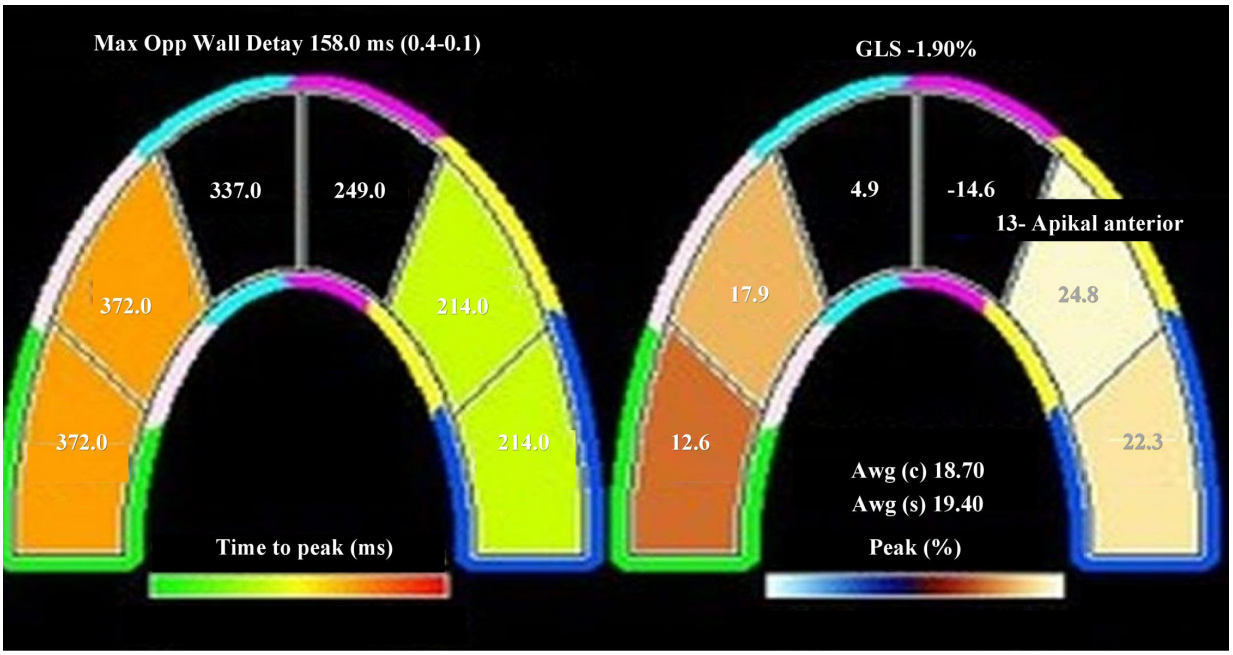

Figure 2. Strain imaging of ascending aortic wall using vector velocity imaging in patient group.

of smooth muscle cells, and augmentation of collagen fibers[1,21,22]. Consequently, these structural alterations result in reduced aortic wall elasticity and increased aortic stiffness[3,5,21].

Systemic essential HTN, referred to as high blood pressure in the systemic arteries, constitutes a major cause of cardiovascular morbidity and mortality in the adult population[23]. Ascending aorta dilatation is associated with HTN-related organ damage and is a surrogate indicator of HTN-associated aortopathy[24]. Recent studies[25-28] have shown that increased aortic stiffness and decreased aortic strain and distensibility are also associated with HTN-related ascending aorta dilatation. Likewise, Song et al[29] found that aortic elastic properties were significantly impaired in patients with essential HTN compared to healthy subjects. Previously, M-mode echocardiography was shown to determine abnormal aortic elasticity and increased stiffness in BAV and HTN patients[15,30]. In the present study, we confirmed reduced aortic elasticity and increased aortic stiffness in patients with BAV and HTN aortopathy using M-mode measurements. In addition, BAV patients with an ascending aorta above 4.5 cm had more reduced aortic strain and increased aortic stiffness than BAV patients with a diameter in the range of 4.0-4.5 cm. However, we could not show significant differences between the BAV and HTN groups.

Furthermore, aortic wall longitudinal strain measurement by 2D-STE imaging may be a promising new technique[1,14,15,30]. Aortic STE imaging analysis was developed to obtain the longitudinal and circumferential deformation of the aortic wall[1,14,15,30]. Several studies[1,9,10,14] have used STE imaging to determine the deformation of the proximal aorta. Longobardo et al[1] found a significant reduction in the LS of the proximal aorta by 2D-STE in BAV patients compared to controls. A stiffer aorta shows a lower deformation capacity and therefore it has a higher risk of dissection or rupture. Moreover, previous studies[31] have revealed that the ascending aorta in the BAV patients is stiffer and has reduced distensibility compared to the healthy population, even with a normal aortic diameter. In one study[14], aortic LS was suggested to be a good deformation parameter for evaluating aortic distensibility. On the other hand, Ozkaramanli Gur et al[9] found ascending aortic deformation in transverse strain to be impaired in ankylosing spondylitis. In our study, we assessed and compared the aortic deformation of the proximal ascending aorta in the BAV and HTN patients by this new method using VVI. We also assessed LS and TS in all three layers of the proximal aorta. This STE imaging provided a reproducible analysis for the evaluation of the aortic deformation. In our study, the LS of the anterior and posterior walls of the ascending

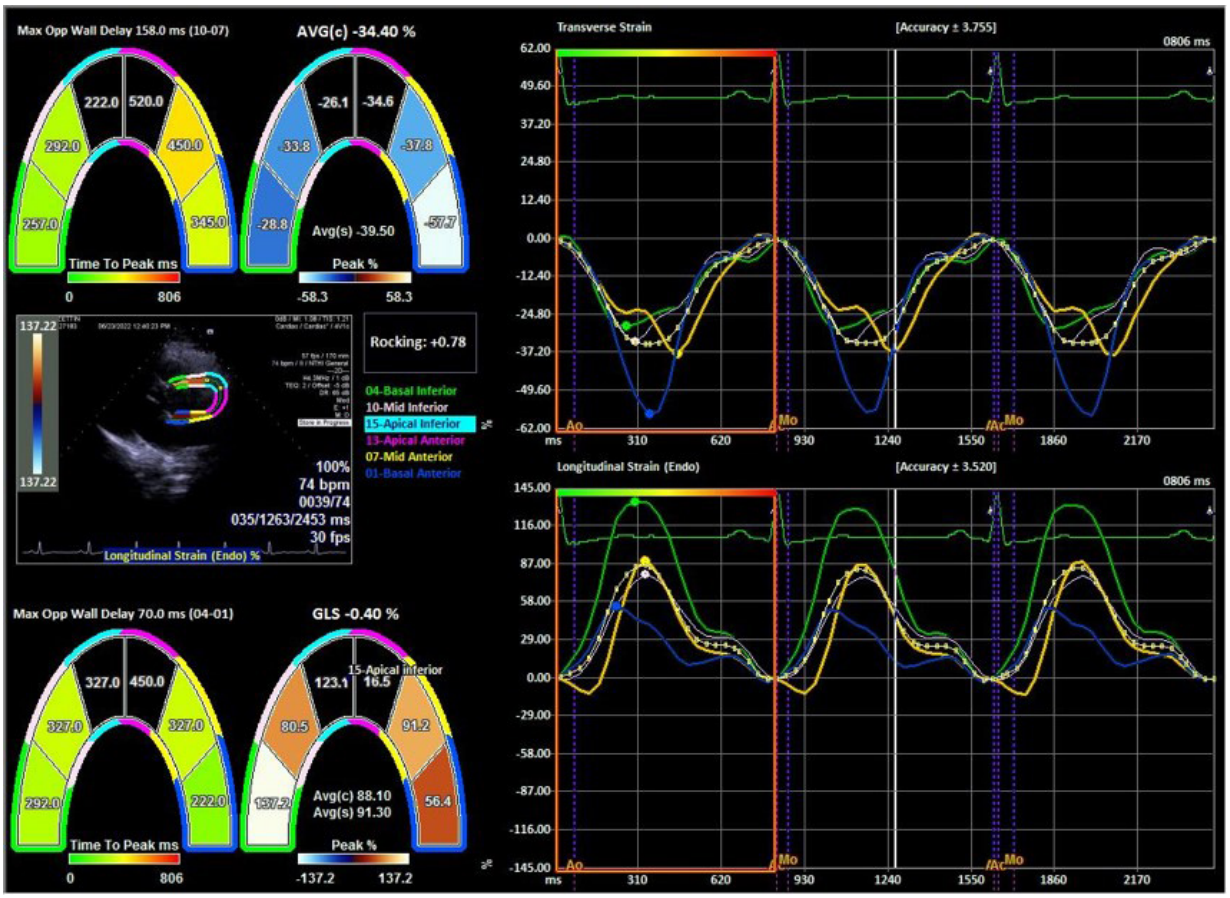

Figure 3. Strain imaging of ascending aortic wall in control group.

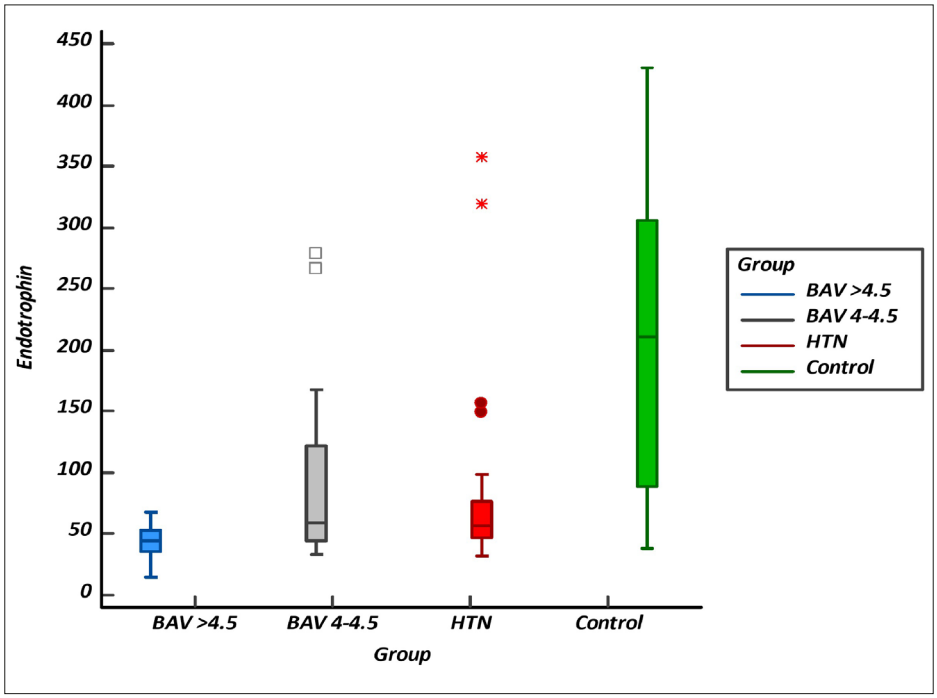

Figure 4. Serum endotrophin levels in study and control groups. The *shows high values.

Table V. Correlation of endotrophin and matrix metalloproteinase-2 with aortic elastic and conventional echocardiographic parameters

	Matrix metalloproteinase-2		Endotrophin	
	r	*p*-value	r	*p*-value
Indexed diastolic aortic diameter	0.279	0.017*	-0.438	<0.001*
Indexed systolic aortic diameter	0.269	0.021*	-0.417	<0.001*
Aortic strain	-0.192	0.086	0.37	0.001*
Aortic stiffness index	0.167	0.137	-0.402	<0.001*
Aortic distensibility	-0.167	0.143	0.454	<0.001*
LVEDD	-0.013	0.906	0.025	0.829
LVESD	0.095	0.403	-0.024	0.832
IVSd	0.106	0.348	-0.272*	0.016*
LVPWd	0.010	0.933	-0.232*	0.041*
LVEF	-0.195	0.083	0.117	0.308
RV	0.137	0.233	-0.027	0.816
LA	0.122	0.282	-0.052	0.651
RA	0.052	0.689	0.039	0.767
E/A ratio	-0.215	0.057	0.050	0.666
E/e' ratio	0.123	0.279	-0.121	0.294
LAVI	0.066	0.567	-0.188	0.106
LV mass index	0.036	0.770	-0.010	0.935
TAPSE	-0.166	0.177	-0.119	0.341
Cardiac output	0.069	0.574	-0.103	0.411
Stroke volume	0.121	0.326	0.053	0.674
CRP	0.187	0.133	-0.211	0.091
Uric Acid	-0.122	0.311	0.024	0.847
Disease duration	0.193	0.095	-0.311	0.007*
HTN	0.135	0.226	-0.446	<0.001*
DM	0.180	0.106	-0.063	0.580

LVEDD: left ventricle end diastolic diameter, LVESD: left ventricular end systolic diameter, IVSd: interventricular septum diameter, LVPWd: left ventricle posterior wall diameter, LVEF: left ventricle ejection fraction, RV: right ventricle, LA: left atrium, RA: right atrium, LAVI : left atrium volume index, LV: left ventricle, TAPSE: tricuspid valve plane systolic excursion, CRP: C reactive protein, HTN: essential hypertension, DM: diabetes mellitus; *$p<0.05$.

aorta was significantly impaired in the BAV and HTN cohorts compared to the controls. However, we did not find a significant difference in the LS analysis between the BAV and HTN groups. In addition, the TS of the anterior and posterior aortic walls, TD, and LV did not differ among the groups. Therefore, we suggest that BAV and HTN impair aortic deformation in the longitudinal axis. According to our findings, we showed that values below 20.88% of LS for the anterior aortic wall, 26.17% for the posterior aortic wall, and 21.55% for the average LS significantly predicted ascending aortic dilatation. Again, values above 0.77 mm of LD for the anterior aortic wall, 0.78 mm for the posterior aortic wall, and 0.57 mm for the average LD significantly predicted ascending aortic dilatation.

An important mechanism of the pathogenesis of the structural alterations of the BAV-associated aortopathy is the extracellular matrix degradation due to the increased activity of matrix metalloproteinases, the most important family of proteases. Thus, in BAV-associated aortopathy, the presence of matrix degeneration is augmented

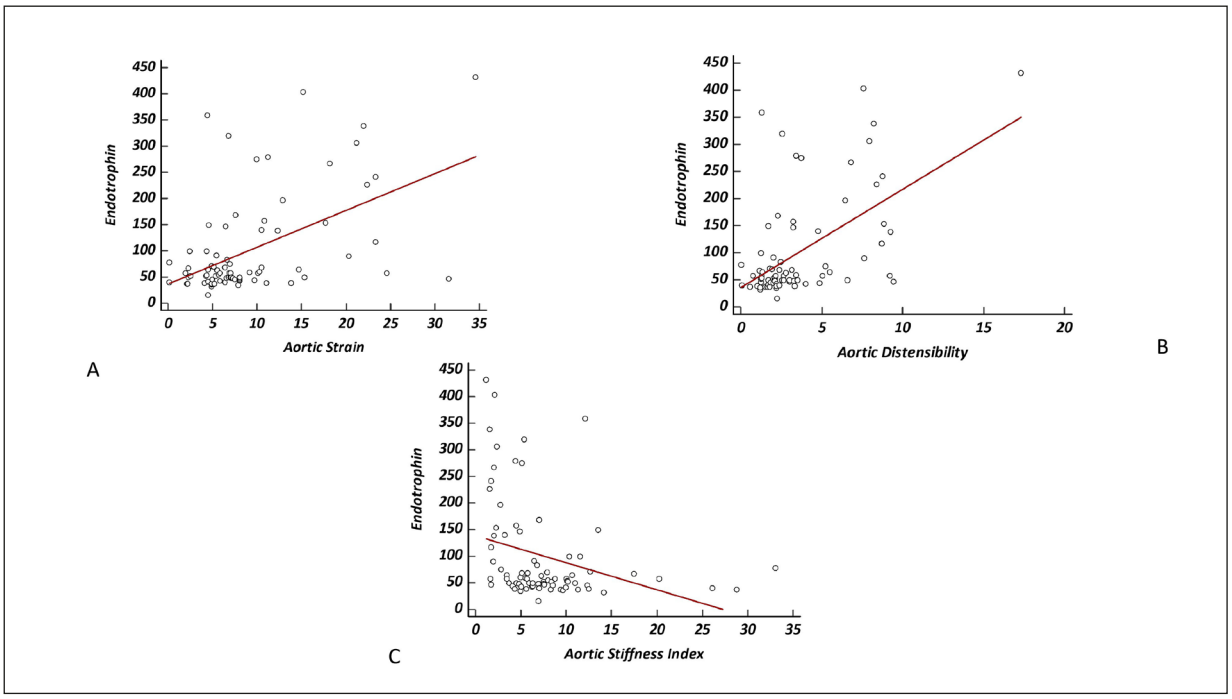

Figure 5. **A**, Correlation of endotrophin with aortic strain. **B**, Correlation of endotrophin with aortic distensibility. **C**, Correlation of endotrophin with aortic stiffness index.

in areas with high shear stress[11]. The activity of MMP-2 has been demonstrated to be correlated with the presence of important hemodynamic wall stress11. Indeed, BAV patients with aortic dilatation have been reported to manifest increased degradation of elastin and collagen fibers in the aortic wall mediated by MMP-2, which has been shown to be strongly expressed in the proximal aortas of patients with BAV[30].

On the other hand, guiding the optimal time for surgery based solely on imaging methods can lead to understatement. In this regard, reliable biomarkers are needed for BAV or HTN aortopathy to determine risk profiles, optimal timing of surgery, and to properly monitor the dilatation of aortic wall[5]. A novel inflammatory biomarker called endotrophin is a cleavage fragment of the type VI collagen alpha-3 chain that has been shown to be released from mature collagen VI following secretion[12,13,32,33]. Previous studies[13,32-34] have reported that endotrophin can play an active role in various biological processes, including inflammation, transforming growth factor-β (TGF-β) production, adipose tissue fibrosis, and increased insulin resistance. Fenton et al[34] reported that endotrophin provides structural support for cells in connective tissues. Endotrophin also has cytoprotective functions, such as the inhibition of apoptosis and oxidative damage and the regulation of cell differentiation[35]. Moreover, endotrophin has been shown to be highly expressed in a variety of cancers and plays a significant role in cancer progression[36].

In our study, we evaluated and compared plasma endotrophin and MMP-2 levels in the study groups and controls. We aimed to assess whether there is a possible relationship between serum endotrophin levels and ascending aortic dilatation in BAV and HTN patient groups. We found that endotrophin levels were significantly reduced in study groups compared to the controls. However, endotrophin levels did not differ between the BAV-associated aortopathy and HTN-associated aortopathy groups. Endotrophin was also found to be an independent predictor of ascending aortic dilatation. Endotrophin was significantly positively correlated with aortic strain and aortic distensibility, and inversely associated with the aortic stiffness index. Additionally, endotrophin was positively associated with the LS parameters of the proximal ascending aorta by STE. Furthermore, values below 82.38 ng/mL for endotrophin predicted ascending aortic dilatation, with a sensitivity of 80.3% and a specificity of 78.5%. However, MMP-2 levels did not dif-

Table VI. Correlation of endotrophin and matrix metalloproteinase-2 with aortic deformation parameters by 2D-STE imaging.

Aortic deformation parameters (2D-Speckle-tracking imaging)	Matrix metalloproteinase-2		Endotrophin	
	r	*p*-value	r	*p*-value
Longitudinal strain (%)				
Anterior wall				
Endo	-0.195	0.153	0.198	0.151
Myo	-0.223	0.101	0.236	0.086
Epi	-0.208	0.128	0.281	0.040*
Posterior wall				
Endo	-0.190	0.165	0.218	0.113
Myo	-0.261	0.054	0.276	0.043*
Epi	-0.343	0.010*	0.286	0.036*
Average				
Endo	-0.154	0.323	0.257	0.100
Myo	-0.204	0.189	0.311	0.045*
Epi	-0.231	0.136	0.420	0.006*
Longitudinal velocity (cm/s)				
Anterior wall				
Endo	0.105	0.443	0.308	0.024*
Myo	0.087	0.530	0.305	0.025*
Epi	0.051	0.711	0.275	0.044*
Posterior wall				
Endo	0.068	0.620	0.187	0.175
Myo	0.043	0.756	0.174	0.209
Epi	0.041	0.766	0.146	0.291
Average				
Endo	0.081	0.606	0.283	0.069
Myo	0.069	0.659	0.272	0.082
Epi	0.067	0.670	0.250	0.111
Longitudinal displacement (mm)				
Anterior wall				
Endo	0.337	0.012*	-0.050	0.719
Myo	0.285	0.035*	-0.109	0.432
Epi	0.221	0.105	-0.154	0.266
Posterior wall				
Endo	0.083	0.547	-0.050	0.719
Myo	0.151	0.270	-0.107	0.442
Epi	0.144	0.293	-0.132	0.341
Average				
Endo	0.177	0.256	-0.054	0.732
Myo	0.207	0.183	-0.079	0.620
Epi	0.212	0.173	-0.103	0.516
Transverse strain (%)				
Anterior wall	0.100	0.465	-0.231	0.093
Posterior wall	-0.059	0.668	-0.105	0.449
Average	0.037	0.795	-0.161	0.253
Transverse displacement (mm)				
Anterior wall				
Endo	-0.144	0.310	0.106	0.459
Myo	-0.067	0.635	0.119	0.405
Epi	-0.070	0.622	0.086	0.549
Posterior wall				
Endo	-0.076	0.589	0.003	0.983
Myo	-0.045	0.751	0.033	0.815
Epi	0.026	0.855	0.124	0.382
Average				
Endo	-0.127	0.416	0.006	0.972
Myo	-0.119	0.447	-0.025	0.874
Epi	-0.044	0.780	-0.006	0.968

2D-STE: two dimensional-speckle tracking echocardiography, Endo: endocardium, myo: myocardium, epi: epicardium; *$p<0.05$.

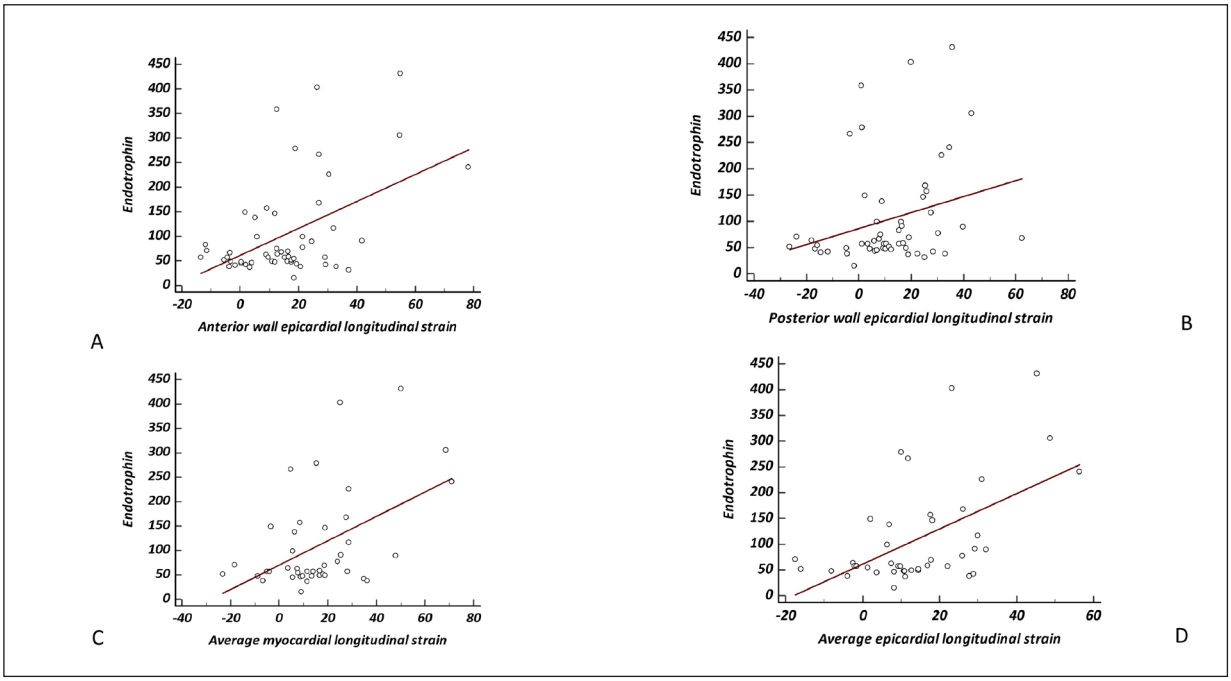

Figure 6. **A**, Correlation of endotrophin with anterior wall epicardial longitudinal strain in proximal aorta. **B**, Correlation of endotrophin with posterior wall epicardial longitudinal strain. **C**, Correlation of endotrophin with average myocardial longitudinal strain of anterior and posterior aortic wall. **D**, Correlation of endotrophin with average epicardial longitudinal strain of anterior and posterior aortic wall.

fer among the study population. In addition, MMP-2 did not show significant correlations with aortic stiffness parameters. To the best of our knowledge, this is the first study to demonstrate that low serum endotrophin levels are an independent predictor of ascending aorta dilatation in BAV- and HTN-associated aortopathy. It is difficult to reach a precise conclusion; however, we can speculate that the production of type VI collagen or its cleavage fragment of the type VI collagen alpha-3 chain may be suppressed in the presence of increased hemodynamic stress on the aortic wall or the process of extracellular matrix degradation. Similar to our results, a study by Simsek et al[19] has reported that a deficiency of apelin, which is an adipokine, caused the development of aneurysms in patients with BAV. In this study, the related mechanism has been reported by alterations in endothelial nitric oxide synthase levels. Again, a negative correlation was found between endothelial nitric oxide levels and ascending aorta diameters[37,38]. Although endotrophin plays pivotal roles in fibrosis, and inflammation of various tissues, and tumor progression as described earlier, another possible explanation we could suggest is that it may be a protective indicator of aortic elasticity and aortic dilatation. Additionally, we suggest that endotrophin may be a more sensitive biomarker than MMP-2 for detecting aortic elasticity and ascending aortic dilatation in BAV and HTN populations.

Limitations and Strengths

There are several limitations to this study. First, it is limited by the relatively small sample size, and because it is a single-center study. Second, the present study has a cross-sectional design; therefore, it is difficult to comment on the cause-effect relationship of endotrophin levels and ascending aortic dilatation. Nevertheless, these data can provide possible evidence of the relationship of endotrophin with aortic dilatation in patients with BAV and HTN, and that endotrophin may be a potential therapeutic target in BAV- and HTN aortopathy. Further multicenter studies with larger samples are needed to provide more evidence. The present study also has some strong points. No studies have yet investigated the association of endotrophin with aortic elastic properties and deformation parameters in BAV- and HTN-associated aortopathy. We evaluated aortic elasticity not only by conventional echocardiography, such as M-mode,

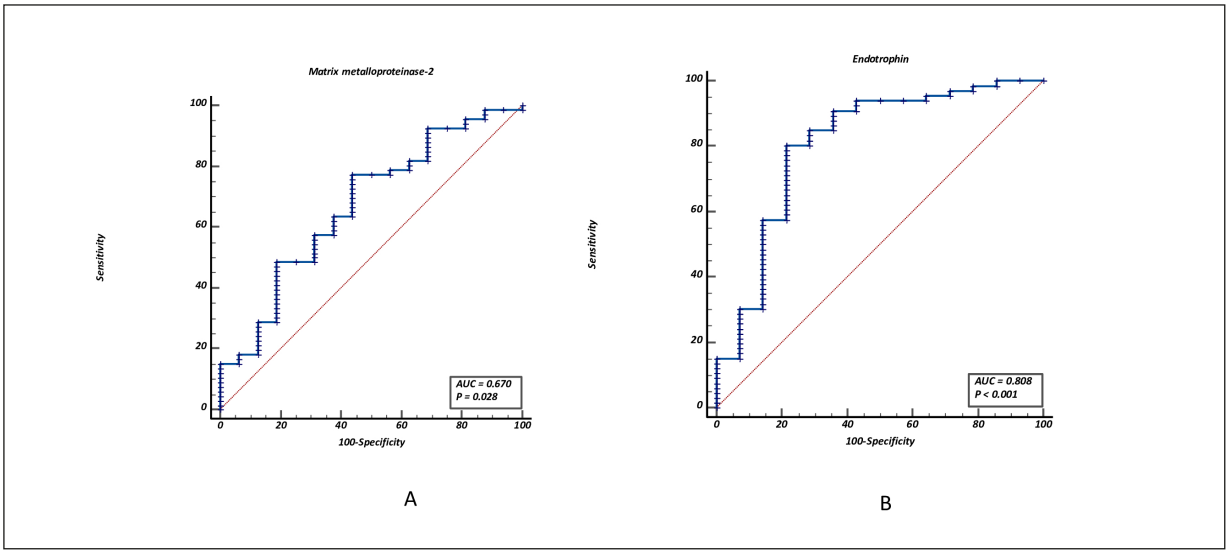

Figure 7. A, The receiver operating characteristics (ROC) curve analysis for predicting ascending aorta dilatation by matrix metalloproteinase-2 (MMP-2). **B**, ROC curve analysis for predicting ascending aorta dilatation by endotrophin.

but also by 2D-STE strain imaging methods. We were also able to compare aortic deformation and elasticity between the patients with BAV- and HTN-associated aortopathy, as well as the control subjects. Finally, we were able to compare endotrophin with MMP-2 for ascending aortic dilatation.

Conclusions

In the present study, BAV and HTN patients with ascending aortic dilatation were demonstrated to have impaired elastic properties. In addition, 2D-STE strain imaging provided a good analysis of aortic deformation. Detecting biomarkers earlier is important before the development of aortic dilatation and may be helpful in the early identification of patients at higher risk. New biomarkers can help manage the clinical course of BAV and HTN-associated aortopathy. A newly defined biomarker, endotrophin, seems to be closely related to aortic elastic properties, and a decrease in endotrophin levels is associated with ascending aortic dilatation. Our study results show that lower plasma endotrophin levels may be a promising biomarker for detecting the presence of aortic dilatation related to BAV or HTN. Future studies are needed to validate our findings.

Conflict of Interest
The authors declare that they have no conflict of interests.

Acknowledgements
None.

Availability of Data
The data and materials generated/analyzed in the present study are available from the corresponding author upon request.

Ethical Approval
This research was carried out with the permission of Istanbul University, Istanbul Faculty of Medicine, Local Ethics Committee, dated 14/05/2022 and numbered 907754.

Informed Consent
Written informed consent was obtained from all subjects according to the Declaration of Helsinki.

Authors' Contributions
D.B. is the principal author of this study, and designed the study with resources acquisition, data collection and processing data, data analysis and interpretation, writing-original draft preparation, and editing. D.B, B.U, S.U, E.A, and Z.B: conceived the idea for the article, framing the hypothesis, D.B., E.A.G., Z.G.D., M.K., M.L.Y., and Z.B: designed the methods to generate results, A.E., E.A., S.U., BU, and Z.B: supervision of the project and the manuscript, E.A.G,

Z.G.D., M.K., M.L.Y., S.E., and D.B: resources acquisition, Z.G.D., E.A.G., M.L.Y, M.K: materials and referring patients, E.A.G., Z.G.D, M.L.Y., S.E., and D.B: data collection and processing data, D.B., E.A., A.E., S.U., B.U, and Z.B: data analysis and interpretation, E.A.G., M.K., E.A, and D.B: writing-original draft preparation, A.E., E.A., S.U., B.U., and Z.B: critical review and editing. All authors have read and approved the final paper.

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