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Comparison of microstructural alterations in the proximal aorta between aortic stenosis and regurgitation

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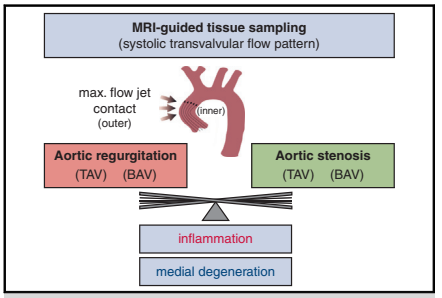
ABSTRACT

Objective: We aimed to analyze the association among flow patterns, gene expression, and histologic alterations of the proximal aorta in patients with aortic valve disease.

Methods: A total of 131 patients referred for aortic valve replacement were grouped by valve dysfunction (aortic stenosis vs aortic regurgitation) and valve morphology (bicuspid vs tricuspid). On the basis of magnetic resonance imaging, aortic tissue from outer and inner curvature was collected for gene expression and histologic analysis. To identify differences in aortic remodeling, age- and sex-adjusted data for inflammation (*CCL2*, *VCAM1*, inflammation and atherosclerosis) and medial degeneration (*COL1A1*, *ELN*, fibrosis, elastin fragmentation, and cystic medial necrosis) were compared.

Results: First, we compared all patients with aortic regurgitation (n = 64) and patients with aortic stenosis (n = 67). In patients with aortic regurgitation, *COL1A1* expression and all histologic markers were significantly increased. With respect to aortic diameter, all subsequent analyses were refined by considering only individuals with aortic diameter 40 mm or greater. Second, patients with bicuspid aortic valve were compared, resulting in a similar aortic diameter. Although patients with aortic regurgitation were younger, no differences were found in gene expression or histologic level. Third, valve morphology was compared in patients with aortic regurgitation. Although aortic diameter was similar, patients with regurgitant bicuspid aortic valve were younger than patients with regurgitant tricuspid aortic valve. Inflammatory markers were similar, whereas markers for medial degeneration were increased in patients with regurgitant tricuspid aortic valve.

Conclusions: Our results indicate that the proximal aorta in patients with aortic regurgitation showed an increased inflammation and medial degeneration compared with patients with aortic stenosis. Refining both groups by valve morphology, in patients with bicuspid aortic valve, no difference except age was detected between aortic regurgitation and aortic stenosis. In patients with aortic regurgitation, tricuspid aortic valve revealed increased markers for medial degeneration but no differences regarding inflammatory markers. (J Thorac Cardiovasc Surg 2021;162:1684-95)



Aortic inflammation and medial degeneration differ between regurgitation and stenosis.

CENTRAL MESSAGE

Compared with stenosis, aortic tissue from patients with regurgitation revealed increased inflammation and even more medial degeneration, which was aggravated in patients with tricuspid valve morphology.

PERSPECTIVE

Compared with patients with stenosis, aortic tissue derived from patients with regurgitation presented more severe vascular remodeling, which was even more pronounced in those patients with tricuspid valve morphology. Severe vascular remodeling may result in faster aortic dilation; therefore, regurgitation should be considered as a possible risk factor to prevent future complications.

See Commentaries on pages 1696 and 1698.

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Abbreviations and Acronyms

AD = aortic diameter
AR = aortic regurgitation
AS = aortic stenosis
BAV = bicuspid aortic valve
CMN = cystic medial necrosis
MRI = magnetic resonance imaging
TAV = tricuspid aortic valve
WSS = wall shear stress

Scanning this QR code will take you to the table of contents to access supplementary information.

Ascending aortic dilation is the most common aortic pathological condition associated with an elevated risk of dissection or rupture.[1] Because of its silent nature, there exists an urgent need of better understanding of risk factors and pathophysiology. Previous studies suggested that structural alterations of the aortic wall are mainly caused by a variable interaction between genetic predisposition and altered hemodynamics.[2-5]

Genetic predisposition is usually associated with congenital aortic wall weakness, such as in Loeys–Dietz and Marfan syndrome.[6] However, the hemodynamics in the proximal aorta may exhibit variable flow patterns and is influenced by functional aortic root elements, the aortic valve being one of the most important. A normal tricuspid aortic valve (TAV) induces steady laminar flow pattern in the proximal aorta, as demonstrated by 4-dimensional flow magnetic resonance imaging (MRI) analysis.[7] In contrast, patients with an aortic valve dysfunction (aortic stenosis [AS] or aortic regurgitation [AR]) exhibit different flow and wall shear stress (WSS) patterns in the proximal aorta.[7]

Recent data indicate that elevated WSS due to aortic valve dysfunction can alter gene expression in the aortic wall and further induce microstructural lesions, which finally lead to changes in vessel geometry. This process is also known as ''aortic remodeling.''[8,9] In the present study, we aim to analyze the association among transvalvular flow patterns, gene expression, and histologic alterations of the proximal aorta in patients with aortic valve disease. Because of the marked heterogeneity of the study population, age- and sex-adjusted comparisons were made on the basis of valve dysfunction and morphology.

MATERIAL AND METHODS

Study Population

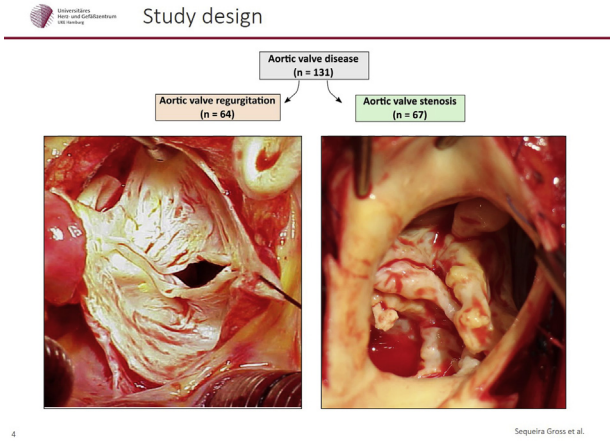
We prospectively identified 131 consecutive patients who were referred for aortic valve surgery with or without proximal aortic surgery from 2012 to 2016. All patients who underwent urgent surgical procedures (eg, acute aortic dissection or endocarditis) were excluded from this study. We excluded all patients who were diagnosed with congenital connective tissue disorders. The diagnosis of valve dysfunction and morphology was based on echocardiographic and cardiac MRI.

Our study design is presented in Figure 1. On the basis of valve dysfunction (AR and AS) and valve morphology (BAV and TAV), several comparisons adjusted for age and sex were analyzed. First, in comparison 1a, all patients with AR (n = 64) were compared with all patients with AS (n = 67). Likewise, in comparison 1b, patients with AR (n = 58) were compared with patients with AS (n = 44) refined by aortic diameter (AD) 40 mm or greater. Comparison 2 used patients with AR and patients with AS refined by bicuspid aortic valve (BAV) morphology and AD 40 mm or greater (AR-BAV, n = 18 and AS-BAV, n = 40). In comparison 3, patients with AR with BAV (AR-BAV, n = 18) were compared with patients with AR with TAV (AR-TAV, n = 40) (Video 1).

The present study conformed to the principles outlined in the Declaration of Helsinki. All patients provided their written informed consent, and the protocol was approved by the Thuringian Chamber of Physicians Ethics Committee (23333/2014/146).

Aortic Tissue Samples Based on Magnetic Resonance Imaging

All patients underwent a noncontrast cardiac MRI (Avanto 1.5T scanner; Siemens, Erlangen, Germany), including phase-velocity encoded imaging of the left ventricular outflow tract and the proximal aorta. Proximal AD was determined as the largest cross-section observed perpendicular to the aortic axis curve in a mid-vessel slice. Structural breath-held, steady-state free precession images were acquired to visually identify the turbulent flow jet in stenotic or regurgitant aortic valves. Using steady-state free precession images, we determined the area of proximal aorta exposed to maximal flow-jet, mostly the outer curvature, as well as the contralateral ''low-flow'' area, mostly the inner curvature. In patients without a jet, aortic samples were obtained from standard aortotomy height before closure. A specific description of samples collection is presented in the Online Data Supplement (Figure E1).



VIDEO 1. Summary of the presented study: Aortic inflammation and medial degeneration differ between regurgitation and stenosis. Video available at: https://www.jtcvs.org/article/S0022-5223(20)30548-1/fulltext.

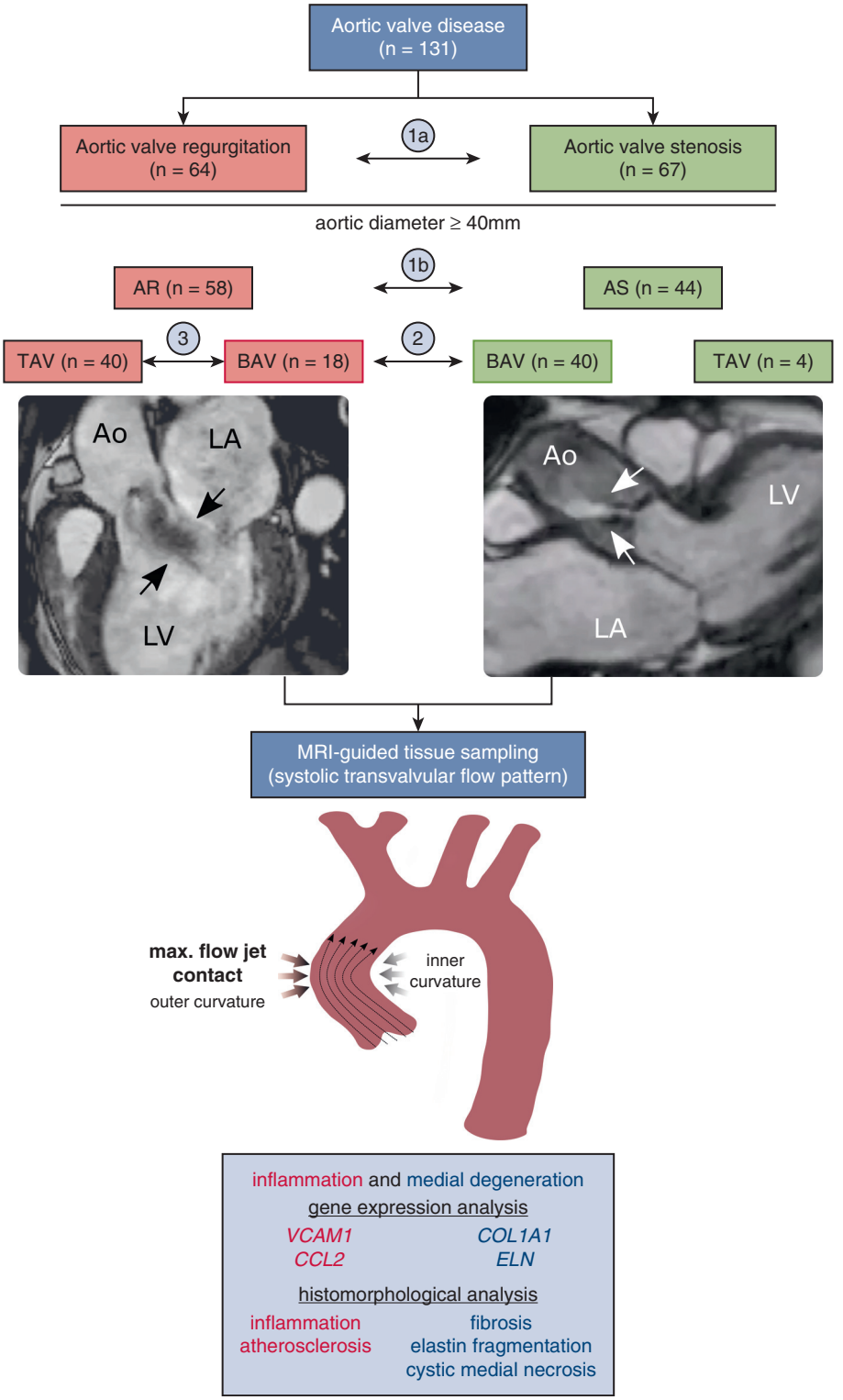
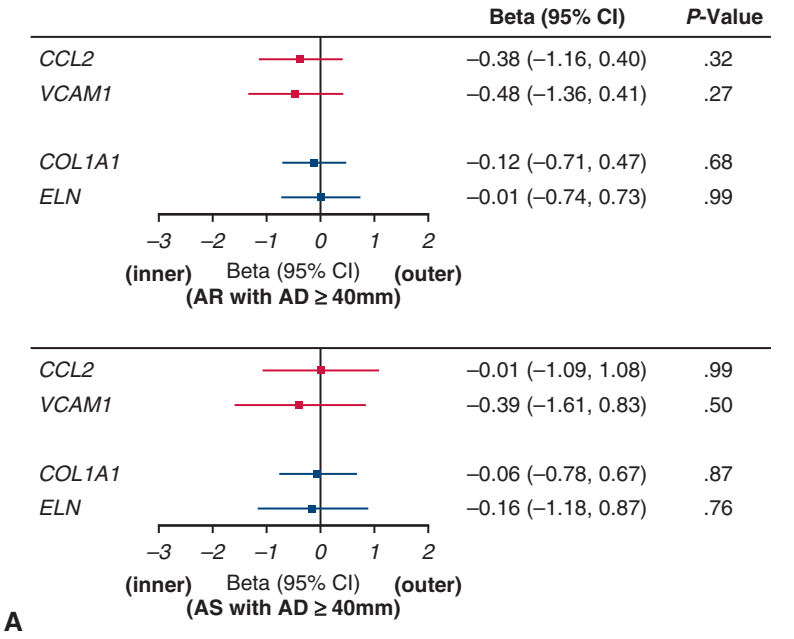
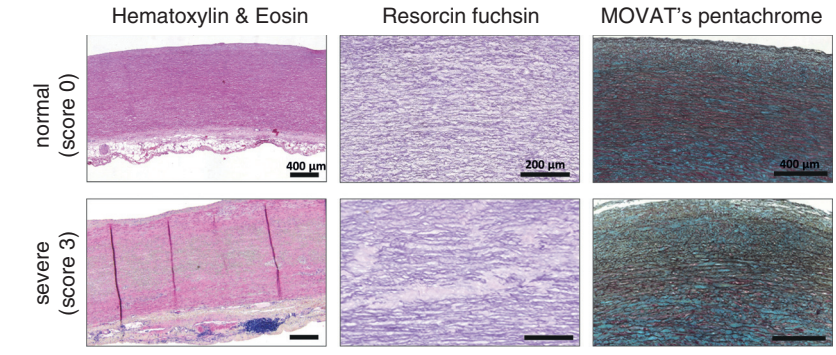


FIGURE 1. Scheme of the study design. A total of 131 patients diagnosed with aortic valve diseases were included in this study. According to valve dysfunction and morphology, 4 comparisons between subgroups were performed. Representative steady-state free precession images demonstrate AR (backflow highlighted with *black arrows*) or AS (eccentric jet highlighted with *white arrows*). With the use of MRI, maximal jet impact area was determined in the proximal aorta to guide the collection of aortic samples. Intraoperatively, 1 sample was obtained from the aortic area exposed to jet and another from the contralateral aortic wall. Both samples were investigated regarding markers for inflammation and medial degeneration using gene expression and histomorphologic analysis. *AR,* Aortic regurgitation; *TAV,* tricuspid aortic valve; *BAV,* bicuspid aortic valve; *Ao,* aorta; *LA,* left atrium; *LV,* left ventricle; *MRI,* magnetic resonance imaging.





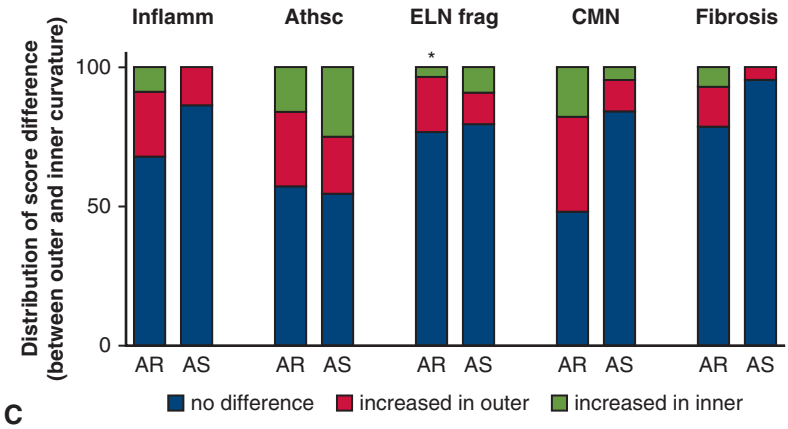


FIGURE 2. Paired differences between outer and inner curvature. A, Gene expression of patients with AD 40 mm or greater were compared between outer and inner curvatures (upper graph: AR, n = 58) (lower graph: AS, n = 44). No differences in the gene expression of *CCL2*, *VCAM1*, *COL1A1,* and *ELN* were detected. Paired comparison of log-transformed gene expression data between outer and inner curvatures was performed using a linear mixed model adjusted for age and sex. The regression coefficient beta is plotted as forest plots for genes associated with inflammation (*red*) or medial degeneration (*blue*). B, Representative histologic images of hematoxylin–eosin-stained, resorcin fuchsin–stained, and MOVAT's pentachrome–stained aortic samples. C, Histologic scores of patients with AD 40 mm or greater were compared between outer and inner curvature for patients with AR and AS (comparison 1b). The 5

TABLE 1. Baseline characteristics of study cohort

Comparison 1a	Aortic valve regurgitation (n = 64)	Aortic valve stenosis (n = 67)	*P* value AR vs AS
Male gender, n (%)	46 (71.9)	45 (67.2)	.58
BMI (kg/m^2)	27.1 (23.7-31.2)	28.3 (25.5-32.6)	.28
Comorbidities			
Hyperlipidemia, n (%)	6 (9.4)	3 (4.5)	.69
History of smoking, n (%)	14 (21.9)	16 (23.9)	.81
Diabetes, n (%)	5 (7.8)	11 (16.4)	.92
Arterial hypertension, n (%)	40 (62.5)	45 (67.2)	.47
Bicuspid valve, n (%)	21 (32.8)	59 (88.1)	<.001
Age (y)	59.0 (49.8-66.0)	61.0 (55.5-68.0)	.058
Proximal AD (mm)	52.0 (47.0-58.0)	43.0 (38.5-49.5)	<.001
Comparison 1b (AD ≥40 mm)	**AR ≥ 40 mm (n = 58)**	**AS ≥40 mm (n = 44)**	***P* value AR vs AS**
Male gender, n (%)	40 (69.0)	34 (77.3)	.38
Age (y)	59.0 (49.5-66.0)	62.5 (55.8-69.0)	.041
Proximal AD (mm)	52.5 (49.0-58.8)	47.5 (43.0-52.2)	<.001
Comparison 2 (AD ≥40 mm)	**AR-BAV (n = 18)**	**AS-BAV (n = 40)**	***P* value AR-BAV vs AS-BAV**
Male gender, n (%)	12 (66.7)	32 (80.0)	.33
Age (y)	51.0 (47.2-57.0)	63.0 (55.8-69.0)	<.001
Proximal AD (mm)	51.0 (49.0-55.0)	48.5 (43.8-53.0)	.052
Comparison 3 (AD ≥40 mm)	**AR-BAV (n = 18)**	**AR-TAV (n = 40)**	***P* value AR-BAV vs AR-TAV**
Male gender, n (%)	12 (66.7)	28 (70.0)	1.00
Age (y)	51.0 (47.2-57.0)	61.0 (54.8-69.0)	.002
Proximal AD (mm)	51.0 (49.0-55.0)	53.0 (49.0-60.0)	.46

Continuous variables are given as median (25th percentile, 75th percentile). Binary variables are given as absolute number (relative frequency). *P* values are calculated using Mann–Whitney test for continuous variables and Fisher exact test for binary variables. *AR,* Aortic regurgitation; *AS,* aortic stenosis; *BMI,* body mass index; *AD,* aortic diameter; *BAV,* bicuspid aortic valve; *TAV,* tricuspid aortic valve.

Both collected tissue samples were divided to perform gene expression analysis and histologic staining. Samples for histopathologic analysis were fixed in neutral-buffered formalin, and samples for gene expression analysis were snap-frozen in liquid nitrogen. As indicated in Figure 1, subsequent analysis was designed to address inflammatory markers such as the endothelial adhesion molecule *VCAM1*, the chemo-attractive chemokine *CCL2* on gene expression level, and the infiltrated inflammatory cells and atherosclerosis on histologic tissue sections. Furthermore, we focused on markers for medial degeneration. Therefore, we measured gene expression of the extracellular matrix proteins *COL1A1* and *ELN* and fibrosis, elastin fragmentation, and cystic medial necrosis (CMN) on histologic tissue sections.

Histopathologic Analysis

The 5 histologic parameters were semiquantitatively graded according to the guidelines of the Society for Cardiovascular Pathology in 4 degrees: 0 = normal, 1 = mild, 2 = moderate, and 3 = severe.[10,11] Representative images are shown in Figure 2, *B*.

Gene Expression Analysis

Total RNA was isolated using QIAzol followed by miRNeasy Kit (Qiagen, Hilden, Germany). Details regarding isolation of total RNA are shown in the Online Data Supplement. Reverse transcription of RNA was carried out using the High-Capacity cDNA Kit (Life Technologies, Carlsbad,

histologic variables (inflammation, atherosclerosis, elastin fragmentation, CMN, and fibrosis) were semiquantitatively evaluated. *P* values < .05 are considered as significant and marked with an *asterisk*. Although differences between outer and inner curvatures were more pronounced with respect to atherosclerosis and CMN than fibrosis, the majority of patients with AR and even more patients with AS had no differences (*blue* bar). In a few patients, a tendency of increased scores in the outer curvature was detected and reached significant levels for elastin fragmentation. In the group of patients with AR, paired comparison of unadjusted histologic data was performed between outer and inner curvatures using the Stuart–Maxwell test. The corresponding contingency table is shown in Figure E6. The percentage of patients with no difference between outer and inner curvatures is plotted in *blue*, the percentage of patients with increased histologic scores in the outer curvature is plotted in *red* and in *green* in the inner curvature. *CI,* Confidence interval; *AR,* aortic regurgitation; *AD,* aortic diameter; *AS,* aortic stenosis; *CMN,* cystic medial necrosis.

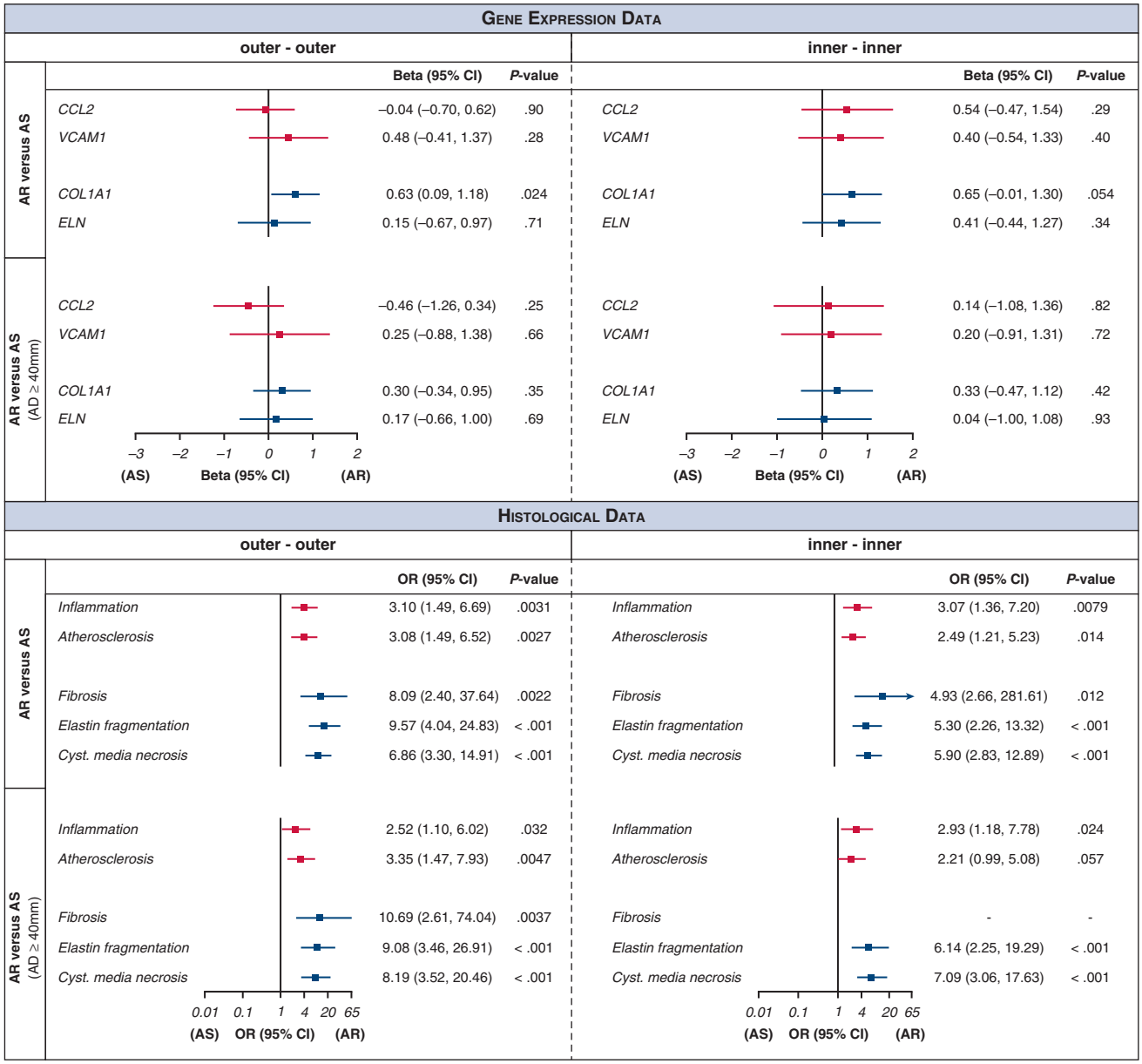


FIGURE 3. Comparison between all patients with AR and patients with AS and further refined by AD 40 mm or greater (comparison 1a and 1b). Gene expression of patients with AR (n = 64) were compared with gene expression of patients with AS (n = 67) and further refined by AD 40 mm or greater (AR, n = 58/AS, n = 44). The comparison of the outer curvatures is shown on the left and of the inner curvatures on the right. Comparison of log-transformed gene expression was performed using linear regression adjusted for age and sex. The regression coefficient beta is plotted as forest plots for genes associated with inflammation (*red*) or medial degeneration (*blue*). The comparison of histologic data of the outer curvatures is shown on the left and of the inner curvatures on the right. Comparison was performed using proportional odds regressions adjusted for age and sex. The odds ratio is plotted as forest plots for histologic parameter associated with inflammation (*red*) or medial degeneration (*blue*). In some cases, the model could not be computed because of lack of variability in the histologic score (eg, most values being equal to 0). *AR,* Aortic regurgitation; *AS,* aortic stenosis; *CI,* confidence interval; *AD,* aortic diameter; *OR,* odds ratio.

Calif), and resulting cDNA was finally used for real-time polymerase chain reaction as described in the Online Data Supplement.

Statistical Analysis

Adjusted comparisons of gene expression between different groups of patients were done using linear regression. Adjusted comparisons of histologic scores between different groups of individuals were done similarly but exchanging linear regression by the proportional odds model. Further details are provided in the Online Data Supplement.

RESULTS

Characteristics of the Study Cohort

As shown in Table 1, comparison of all patients with AR with all patients with AS revealed no differences in sex,

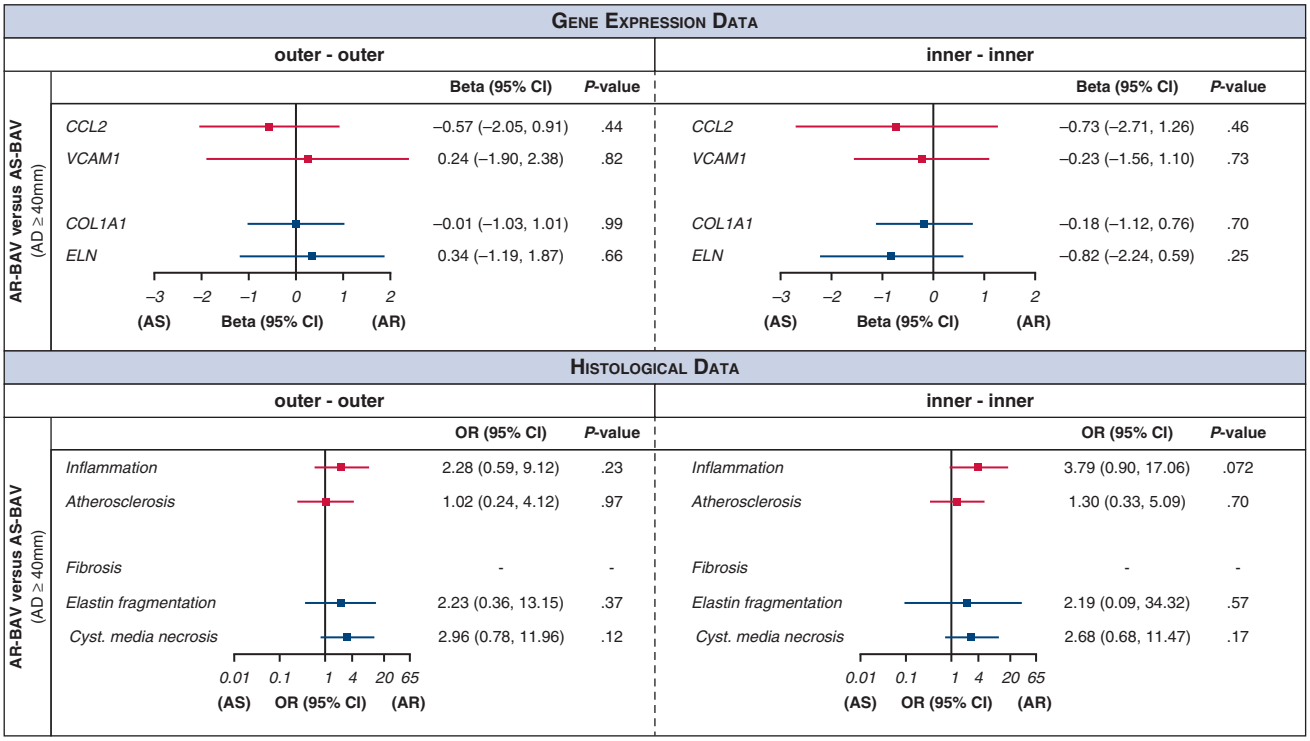


FIGURE 4. Comparison of patients with BAVs between patients with AR and patients with AS with AD 40 mm or greater (comparison 2). Gene expressions of patients with AR with an AD 40 mm or greater and BAV (AR-BAV, n = 18) were compared with gene expressions of patients with AS with an AD 40 mm or greater and BAV (AS-BAV, n = 40). The comparison of the outer curvatures is shown on the left and of the inner curvatures on the right. Comparison of log-transformed gene expression was performed using linear regression adjusted for age and sex. The regression coefficient beta is plotted as forest plots for genes associated with inflammation (*red*) or medial degeneration (*blue*). The comparison of histologic data of the outer curvatures is shown on the left and of the inner curvatures on the right. Comparison was performed using proportional odds regressions adjusted for age and sex. The odds ratio is plotted as forest plots for histologic parameter associated with inflammation (*red*) or medial degeneration (*blue*). In some cases, the model could not be computed because of lack of variability in the histologic score (eg, most values being equal to 0). *AR,* Aortic regurgitation; *AS,* aortic stenosis; *AD,* aortic diameter; *CI,* confidence interval; *OR,* odds ratio.

body mass index, and age. Furthermore, the most relevant comorbidities were similarly distributed in both groups. Significant differences were found regarding valve morphology and maximal cross-sectional proximal AD. As expected, patients with AS revealed a higher incidence of BAVs (33% vs 88%; $P < .001$) and exhibited a smaller AD (median, 52.0 vs 43.0; $P < .001$) compared with patients with AR (comparison 1a).

To reduce the effects of different AD, the study cohort was further refined by applying the cutoff for AD 40 mm or greater (comparison 1b). Consequently, the difference of AD between patients with AR and patients with AS was reduced but remained significantly different (median, 52.5 vs 47.5; $P < .001$).

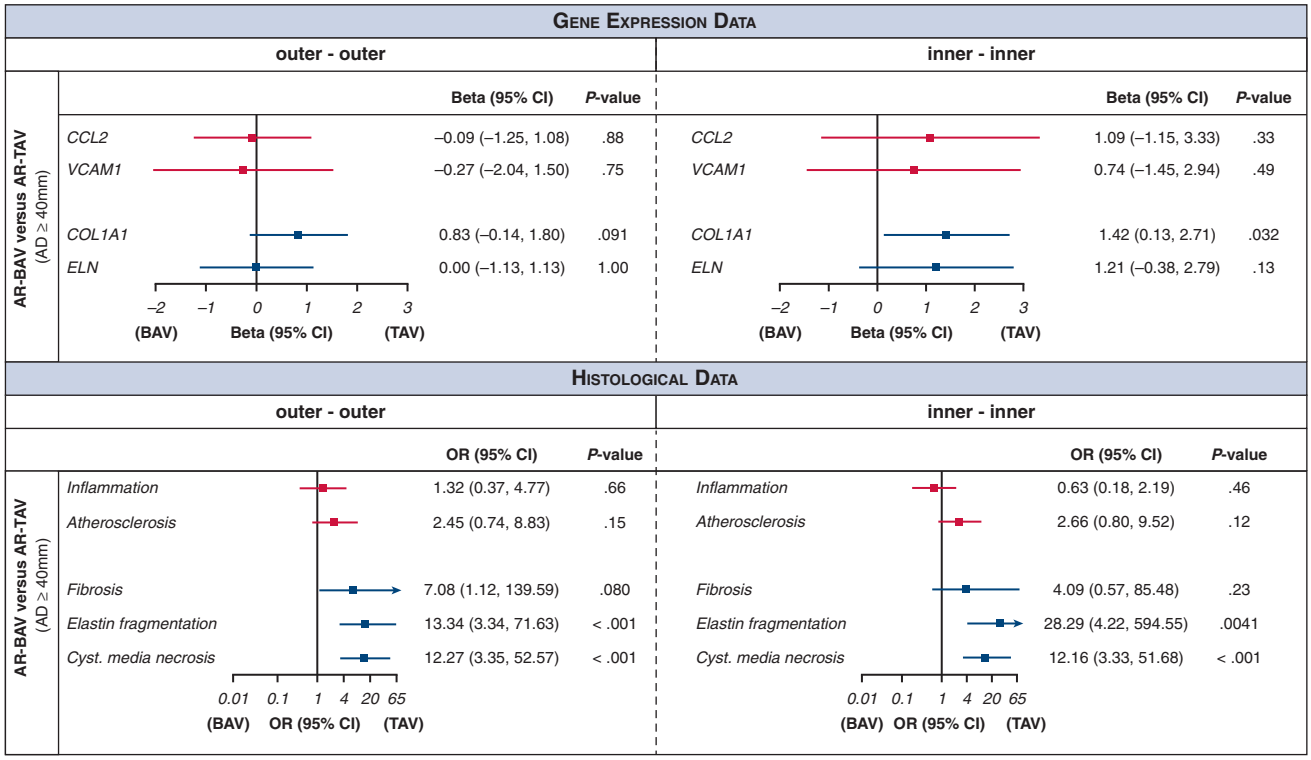
Next, to exclude effects of different valve morphologies, BAVs were used to compare patients with AR and patients with AS (comparison 2). Patients with AR-BAV were significantly younger than patients with AS-BAV (median, 51.0 vs 63.0; $P < .001$), but AD was no longer significantly different (median, 51.0 vs 48.5; $P < .052$).

To investigate the effects of the different valve morphologies, BAVs and TAVs were compared within the AR group (comparison 3). No significant difference in AD between AR-BAV and AR-TAV was detected (median, 51.0 vs 53.0; $P = .46$), whereas the patients with AR-BAV were significantly younger (median = 51.0 vs 61.0 years; $P = .002$).

Negligible Differences Between Outer and Inner Curvature Within One Patient

To uncover differences between outer and inner curvature, paired samples were compared separately for each subgroup defined in Figure 1. Gene expression data, adjusted for age and sex, revealed no differences between the outer and inner curvatures. In Figure 2, *A*, the 2 subgroups, defined for comparison 1b, are depicted. The analyses of the other subgroups are presented in Figures E2, *A*, E3, *A*, and E4, *A*.

Histologic data for inflammation, atherosclerosis, elastin fragmentation, CMN, and fibrosis were scored to compare outer and inner curvatures (Figure 2, *C*). Because of the



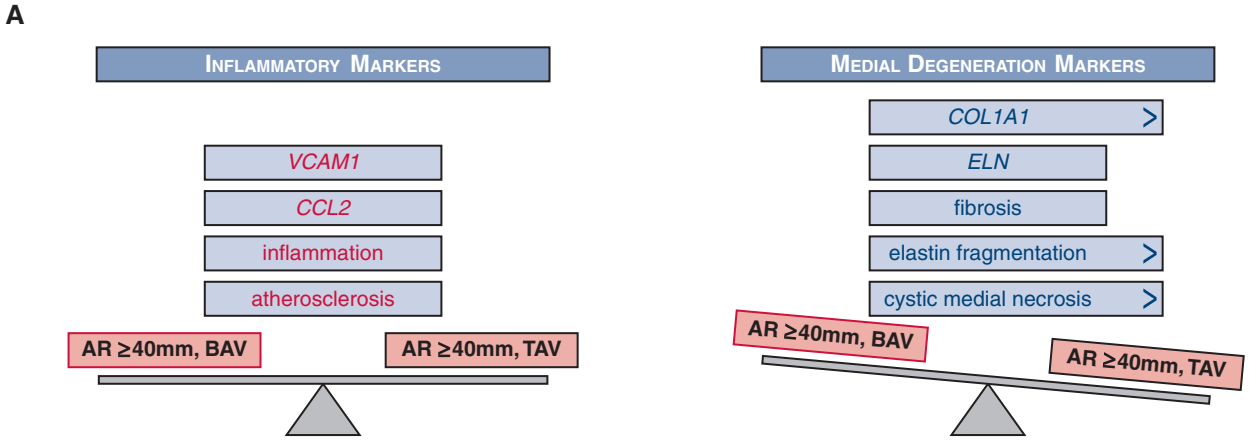


FIGURE 5. Comparison between TAV and BAV morphologies of patients with AR with AD 40 mm or greater (comparison 3). A, Gene expression between TAV and BAV morphologies of patients with AR with an AD 40 mm or greater were compared (AR-BAV, n = 18/AR-TAV, n = 40). The comparison of the outer curvatures is shown on the left and of the inner curvatures on the right. Comparison of log-transformed gene expression was performed using linear regression adjusted for age and sex. The regression coefficient beta is plotted as forest plots for genes associated with inflammation (*red*) or medial degeneration (*blue*). The comparison of histologic data of the outer curvatures is shown on the left and of the inner curvatures on the right. Comparison was performed using proportional odds regressions adjusted for age and sex. The odds ratio is plotted as forest plots for histologic parameter associated with inflammation (*red*) or medial degeneration (*blue*). B, The inflammatory markers (*red*) were similar in both subgroups. However, markers for medial degeneration (*blue*) were significantly higher in the AR-TAV compared with the AR-BAV subgroup. *AR,* Aortic regurgitation; *AD,* aortic diameter; *CI,* confidence interval; *BAV,* bicuspid aortic valve; *TAV,* tricuspid aortic valve; *OR,* odds ratio.

lack of variability of these score differences and model sample size, numeric problems were encountered in some cases when fitting age and adjusted models, and these results are not presented. For all subgroups, contingency tables were produced displaying the distribution of score differences (Figures E5-E8). In the majority of patients, scores did not differ between inner and outer curvatures. In a few patients, a tendency of increased scores in the outer curvature was

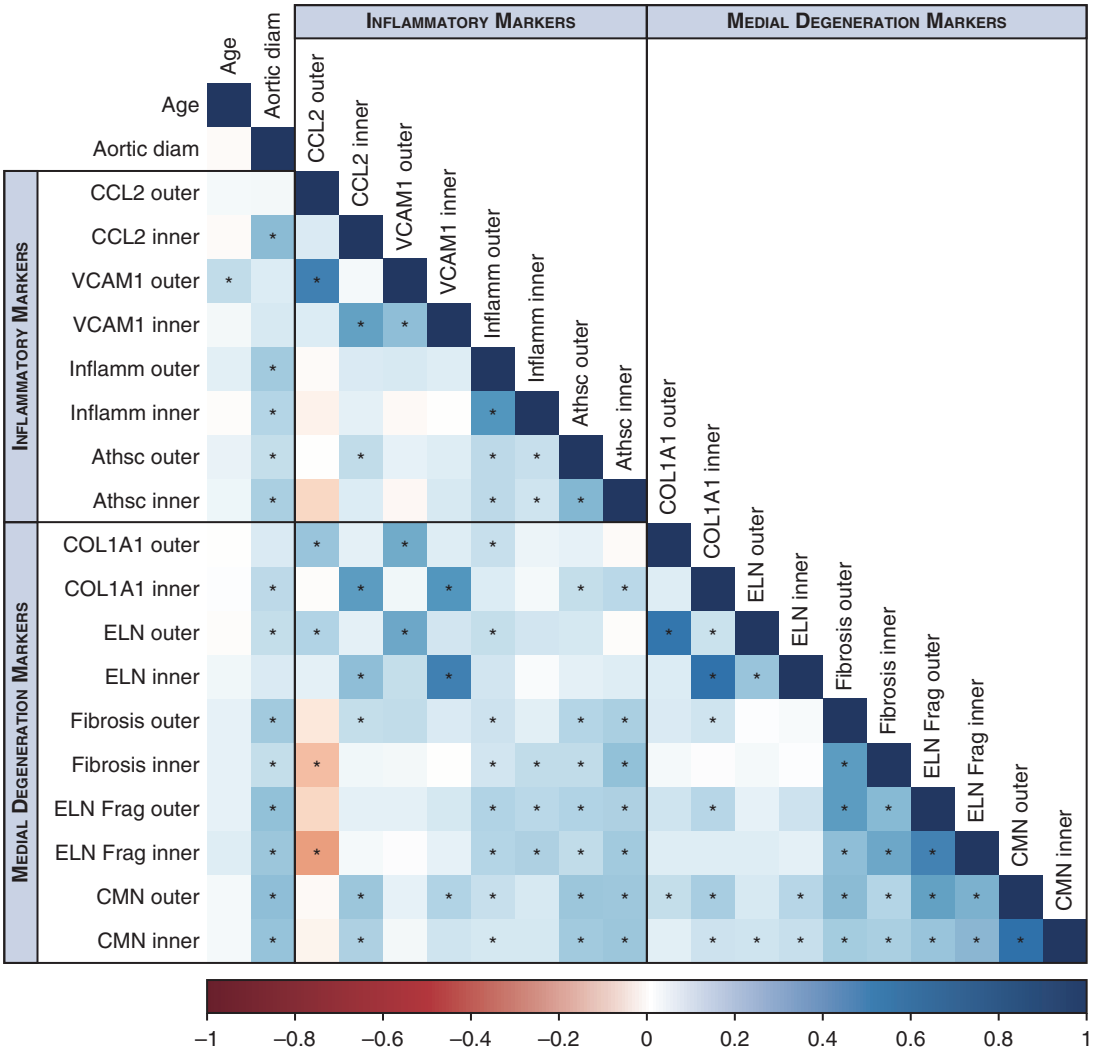


FIGURE 6. Correlogram representing Spearman correlations between inflammatory markers and medial degeneration markers for all individuals. Color indicates whether the correlation is positive (*blue*) or negative (*red*). The intensity of the color is proportional to the correlation coefficients. Correlations with a $P < .05$ are considered as significant and marked with an *asterisk*.

detected and reached significant levels for elastin fragmentation in the subgroup of patients with AR and patients with AR-TAV (Figure 2, *C*, and Figures E2, *B*, and E4, *B*).

Slightly Increased Markers for Inflammation and Strongly Increased Markers for Medial Degeneration in Patients With Aortic Regurgitation (Comparison 1a and 1b)

As shown in Figure 3, gene expression and histologic scores of all patients with AR were compared with all patients with AS (comparison 1a) and subsequently further refined by AD 40 mm or greater (comparison 1b). Both comparisons were performed for outer and inner curvatures, separately. Comparing all patients without restriction regarding AD, gene expression of *CCL2* and *VCAM1* revealed no difference, whereas inflammatory markers using histology were increased in patients with AR. With respect to markers for medial degeneration, gene expression of *COL1A1* was slightly increased and histologic data were strongly increased in patients with AR. The subsequent refinement led to similar results except that gene expression of *COL1A1* was no longer different between those with AR and those with AS. It is not clear whether the gene expression of *COL1A1* is dependent on AD or there is not enough power to detect differences because of the reduced sample size in this subgroup.

No Differences Between Patients With Aortic Regurgitation and Patients With Aortic Stenosis With Bicuspid Aortic Valve Morphology (Comparison 2)

Study cohort of comparison 1b was further refined by BAV morphology leading to comparison 2 (AR-BAV vs AS-BAV). Neither gene expression nor histologic scores revealed significant differences between both subgroups (Figure 4).

Aortic Regurgitation With Tricuspid Valves Reveal More Severe Medial Degeneration Than With Bicuspid Valves (Comparison 3)

By using the study cohort of comparison 1b, the impact of different valve morphologies was assessed leading to comparison 3 (AR-BAV vs AR-TAV). As depicted in Figure 5, data concerning inflammation displayed no differences between TAV and BAV morphology. Regarding markers for medial degeneration, gene expression of *COL1A1* was significantly increased in patients with AR-TAV in the inner curvature, whereas on the histologic level, fibrosis was not significantly different but tended toward higher expression in patients with AR-TAV. Of note, elastin fragmentation and CMN were highly increased in patients with AR-TAV compared with patients with AR-BAV.

Spearman Correlations Between Inflammatory and Medial Degeneration Markers in All Individuals

Inflammatory and medial degeneration markers were correlated to age and AD. Except for *VCAM1* gene expression, no correlation was found for age, whereas 5 of 8 inflammatory and 8 of 10 medial degeneration markers revealed significant positive correlations with AD.

Next, we correlated the different inflammatory and medial degeneration markers, and generally observed positive correlations. Between the different inflammatory markers, 36% revealed significant positive correlations, and 60% of significant positive correlations were found to correlate with the different medial degeneration makers. We observed 46% significant positive correlations between inflammation and medial degeneration markers (Figure 6). As shown in Figure E9, further correlograms were also computed for both subgroups of comparison 3.

DISCUSSION

Our results indicate that the proximal aorta in patients with AR showed an increased inflammation and medial degeneration compared with patients with AS. We further refined both groups by valve morphology. By comparing patients with bicuspid valves, patients with AR-BAV were significantly younger than patients with AS-BAV, but no further differences were identified. However, when comparing valve morphology within the subgroup of patients with AR, AR-TAV revealed increased markers for medial degeneration, but no differences regarding inflammatory markers compared with AR-BAV.

Aortic Regurgitation Exhibited Increased Markers for Inflammation and Medial Degeneration Compared With Aortic Stenosis

A previous MRI-based study revealed that patients with AS have more severe WSS in the outer curvature of the proximal aorta.[12] In contrast, a regurgitant aortic valve is associated with retrograde diastolic aortic flow leading to a disturbed flow pattern accompanied by lower WSS.[5,13] On the basis of these flow differences, we decided to compare the vascular remodeling between patients with AR and patients with AS. The histologic and gene analysis demonstrated more inflammation and medial degeneration in patients with AR. As reported by others,[14] aortopathy in regurgitation was characterized by more severe aortic dilation compared with stenosis. We also observed that AD positively correlates with inflammation and medial degeneration markers. Therefore, we subsequently refined both study groups by AD 40 mm or greater, excluding 34% of patients with AS but only 9% of patients with AR. The new refined analysis by AD 40 mm or greater confirmed our initial results regarding inflammation and medial degeneration in patients with AR.

Several studies reported that low WSS induces the expression of proinflammatory genes, thereby accelerating inflammation.[9,15-17] The aortic tissue of patients with AR revealed more inflammation, which may lead to activation of matrix metalloproteinases and subsequent elastin fragmentation, which in turn causes replacement of elastic fibers with a fibrocollagenous extracellular matrix.[18,19] These structural alterations lead to a weakening of aortic wall integrity and loss of aortic elasticity, which may further progress to aortic dilation.[1,20,21] Although hemodynamic alterations may influence the progression of aortic dilation in patients with AR, congenital factors may contribute.[22] This theory is supported by the fact that aortic dilation can also occur or progress after aortic valve surgery.[23,24]

Younger Age but No Histologic Differences in Bicuspid Aortic Regurgitation Versus Stenosis

Regarding valve morphology, we compared both aortic dysfunctional BAV subgroups. As in other studies,[24-27] patients with AR-BAV were significantly younger than patients with AS-BAV. Age- and sex-adjusted data revealed no differences in histologic or gene expression levels between both subgroups. A possible explanation for these results lies in the fact that all patients with BAV experience increased WSS over many years, which is further aggravated by a valve dysfunction as reported by Shan and colleagues[28] and Atkins and Sucosky.[29] Although both subgroups showed similar aortic wall alterations, it is extremely important to highlight that patients with AR-BAV were significantly younger, indicating that aortic remodeling in patients with AR-BAV occurs faster than in patients with AS-BAV. Wang and colleagues[24] reported that patients with AR-BAV demonstrated a faster proximal aorta dilation rate and identified AR in patients with BAV as a risk factor with increased hazard ratio.

Younger Age but Less Medial Degeneration in Bicuspid Aortic Regurgitation Versus Tricuspid Aortic Regurgitation

In the AR group, patients with BAV were younger than patients with TAV, which is in line with other studies.[30,31] This gap could be explained by a faster dilation rate in AR-BAV than in AR-TAV.[24,32] Of note, children with BAV already have an enlarged AD at birth compared with children with TAV.[33] Therefore, age plays a central role in aneurysm formation in patients with BAV and constitutes a major risk factor.[32]

Despite similar inflammation between both subgroups, makers for medial degeneration were more pronounced in patients with AR-TAV. It was previously reported that medial degeneration was more severe in patients with trileaflet aortic valve than bicuspid valve with an AD between 4 and 5 cm.[34] This marked degenerative medial differences could be due to an undiagnosed connective tissue disease in the patients with AR-TAV at the time of the surgery.

Study Limitations

Because of the small number of patients with AS-TAV, the comparison between TAV and BAV could not be performed within the AS group. Furthermore, relevant chemical parameters related to inflammation, such as lactate dehydrogenase or hemoglobin A1c, and detailed hemodynamic data, such as ejection fraction, degree of valve dysfunction, and aortic valve gradient, were not available. Furthermore, other hemodynamic factors (eg, transvalvular gradients, systolic aortic valve orifice area, left ventricle function) may have an additional impact on aortic wall changes, and a multivariate regression model incorporating complete clinical dataset would be appreciated. Nonetheless, most of the analyzed patients had normal systolic left ventricular function and transvalvular gradients in the AR cohort were negligible. Although the pathologists who read the sections were blinded, intraobserver variability was not reported.

CONCLUSIONS

Our results indicate that the proximal aorta in patients with AR showed an increased inflammation and medial degeneration compared with patients with AS. This suggests that disturbed transvalvular flow patterns, accompanied by lower WSS in the proximal aorta, may trigger severe remodeling regarding the aortic wall microstructure in patients with AR. On the basis of these findings, we should consider regurgitation as a risk factor for proximal aortic dilation. To confirm this conclusion, larger multicenter studies should be performed that give us deeper insights into disease progression. Now, we are conducting prospective studies to evaluate the value of specific circulating biomarkers that can be used to predict the progression of aortic disease. Furthermore, we are collecting longitudinal data on MRI-based transvalvular flow patterns in patients with AS and AR.

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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References

1. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE Jr, et al. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Circulation*. 2010;121:e266-369.
2. Folkersen L, Wagsater D, Paloschi V, Jackson V, Petrini J, Kurtovic S, et al. Unraveling divergent gene expression profiles in bicuspid and tricuspid aortic valve patients with thoracic aortic dilatation: the ASAP study. *Mol Med*. 2011;17:1365-73.
3. Kim HW, Stansfield BK. Genetic and epigenetic regulation of aortic aneurysms. *Biomed Res Int*. 2017;2017:7268521.
4. Maleki S, Bjorck HM, Folkersen L, Nilsson R, Renner J, Caidahl K, et al. Identification of a novel flow-mediated gene expression signature in patients with bicuspid aortic valve. *J Mol Med (Berl)*. 2013;91:129-39.
5. Martufi G, Forneris A, Appoo JJ, Di Martino ES. Is there a role for biomechanical engineering in helping to elucidate the risk profile of the thoracic aorta? *Ann Thorac Surg*. 2016;101:390-8.
6. Lindsay ME, Dietz HC. The genetic basis of aortic aneurysm. *Csh Perspect Med*. 2014;4:a015909.
7. van Ooij P, Markl M, Collins JD, Carr JC, Rigsby C, Bonow RO, et al. Aortic valve stenosis alters expression of regional aortic wall shear stress: new insights from a 4-dimensional flow magnetic resonance imaging study of 571 subjects. *J Am Heart Assoc*. 2017;6.
8. Ando J, Yamamoto K. Vascular mechanobiology: endothelial cell responses to fluid shear stress. *Circ J*. 2009;73:1983-92.
9. Bjorck HM, Renner J, Maleki S, Nilsson SF, Kihlberg J, Folkersen L, et al. Characterization of shear-sensitive genes in the normal rat aorta identifies Hand2 as a major flow-responsive transcription factor. *PLoS One*. 2012;7:e52227.
10. Halushka MK, Angelini A, Bartoloni G, Basso C, Batoroeva L, Bruneval P, et al. Consensus statement on surgical pathology of the aorta from the Society for Cardiovascular Pathology and the Association For European Cardiovascular Pathology: II. Noninflammatory degenerative diseases - nomenclature and diagnostic criteria. *Cardiovasc Pathol*. 2016;25:247-57.
11. Stone JR, Bruneval P, Angelini A, Bartoloni G, Basso C, Batoroeva L, et al. Consensus statement on surgical pathology of the aorta from the Society for Cardiovascular Pathology and the Association for European Cardiovascular Pathology: I. Inflammatory diseases. *Cardiovasc Pathol*. 2015;24:267-78.
12. Youssefi P, Gomez A, He T, Anderson L, Bunce N, Sharma R, et al. Patient-specific computational fluid dynamics-assessment of aortic hemodynamics in a spectrum of aortic valve pathologies. *J Thorac Cardiovasc Surg*. 2017;153:8-20.e23.
13. Reimold SC, Maier SE, Aggarwal K, Fleischmann KE, Piwnica-Worms D, Kikinis R, et al. Aortic flow velocity patterns in chronic aortic regurgitation: implications for Doppler echocardiography. *J Am Soc Echocardiogr*. 1996;9:675-83.
14. Falk V, Baumgartner H, Bax JJ, De Bonis M, Hamm C, Holm PJ, et al. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *Eur J Cardiothorac Surg*. 2017;52:616-64.
15. Chiu JJ, Chien S. Effects of disturbed flow on vascular endothelium: pathophysiological basis and clinical perspectives. *Physiol Rev*. 2011;91:327-87.
16. Nayak L, Lin Z, Jain MK. "Go with the flow": how Kruppel-like factor 2 regulates the vasoprotective effects of shear stress. *Antioxid Redox Signal*. 2011;15:1449-61.

17. Dhawan SS, Avati Nanjundappa RP, Branch JR, Taylor WR, Quyyumi AA, Jo H, et al. Shear stress and plaque development. *Expert Rev Cardiovasc Ther*. 2010;8:545-56.
18. Anidjar S, Dobrin PB, Eichorst M, Graham GP, Chejfec G. Correlation of inflammatory infiltrate with the enlargement of experimental aortic aneurysms. *J Vasc Surg*. 1992;16:139-47.
19. Maiellaro K, Taylor WR. The role of the adventitia in vascular inflammation. *Cardiovasc Res*. 2007;75:640-8.
20. Azadani AN, Chitsaz S, Mannion A, Mookhoek A, Wisneski A, Guccione JM, et al. Biomechanical properties of human ascending thoracic aortic aneurysms. *Ann Thorac Surg*. 2013;96:50-8.
21. Fedak PW, de Sa MP, Verma S, Nili N, Kazemian P, Butany J, et al. Vascular matrix remodeling in patients with bicuspid aortic valve malformations: implications for aortic dilatation. *J Thorac Cardiovasc Surg*. 2003;126:797-806.
22. Tadros TM, Klein MD, Shapira OM. Ascending aortic dilatation associated with bicuspid aortic valve: pathophysiology, molecular biology, and clinical implications. *Circulation*. 2009;119:880-90.
23. Padang R, Bannon PG, Jeremy R, Richmond DR, Semsarian C, Vallely M, et al. The genetic and molecular basis of bicuspid aortic valve associated thoracic aortopathy: a link to phenotype heterogeneity. *Ann Cardiothorac Surg*. 2013;2:83-91.
24. Wang Y, Wu B, Li J, Dong L, Wang C, Shu X. Impact of aortic insufficiency on ascending aortic dilatation and adverse aortic events after isolated aortic valve replacement in patients with a bicuspid aortic valve. *Ann Thorac Surg*. 2016;101:1707-14.
25. Al-Atassi T, Hynes M, Sohmer B, Lam BK, Mesana T, Boodhwani M. Aortic root geometry in bicuspid aortic insufficiency versus stenosis: implications for valve repair. *Eur J Cardiothorac Surg*. 2015;47:e151-4.
26. Benedik J, Dohle DS, Wendt D, Pilarczyk K, Price V, Mourad F, et al. Comparison of ascending aortic cohesion between patients with bicuspid aortic valve stenosis and regurgitation. *Eur J Cardiothorac Surg*. 2014;46:e89-93.
27. Girdauskas E, Rouman M, Disha K, Fey B, Dubslaff G, von Kodolitsch Y, et al. Morphologic and functional markers of aortopathy in patients with bicuspid aortic valve insufficiency versus stenosis. *Ann Thorac Surg*. 2017;103:49-57.
28. Shan Y, Li J, Wang Y, Wu B, Barker AJ, Markl M, et al. Aortic shear stress in patients with bicuspid aortic valve with stenosis and insufficiency. *J Thorac Cardiovasc Surg*. 2017;153:1263-72.e1.
29. Atkins SK, Sucosky P. Etiology of bicuspid aortic valve disease: focus on hemodynamics. *World J Cardiol*. 2014;6:1227-33.
30. Agnese V, Pasta S, Michelena HI, Mina C, Romano GM, Carerj S, et al. Patterns of ascending aortic dilatation and predictors of surgical replacement of the aorta: a comparison of bicuspid and tricuspid aortic valve patients over eight years of follow-up. *J Mol Cell Cardiol*. 2019;135:31-9.
31. Ren X, Li F, Wang C, Hou Z, Gao Y, Yin W, et al. Age- and sex-related aortic valve dysfunction and aortopathy difference in patients with bicuspid aortic valve. *Int Heart J*. 2019;60:637-42.
32. Cozijnsen L, Braam RL, Waalewijn RA, Schepens MA, Loeys BL, van Oosterhout MF, et al. What is new in dilatation of the ascending aorta? Review of current literature and practical advice for the cardiologist. *Circulation*. 2011;123:924-8.
33. Siu SC, Silversides CK. Bicuspid aortic valve disease. *J Am Coll Cardiol*. 2010;55:2789-800.
34. Heng E, Stone JR, Kim JB, Lee H, MacGillivray TE, Sundt TM. Comparative histology of aortic dilatation associated with bileaflet versus trileaflet aortic valves. *Ann Thorac Surg*. 2015;100:2095-101.

Key Words: aortic hemodynamic, aortic regurgitation, aortic remodeling, aortic stenosis, aortopathy, bicuspid aortic valve, tricuspid aortic valve

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