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ORIGINAL RESEARCH

Biomechanics of Aortic Dissection: A Comparison of Aortas Associated With Bicuspid and Tricuspid Aortic Valves

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BACKGROUND: Current methods for aortic dissection risk assessment are inadequate for patients with ascending aortic aneurysms associated with either bicuspid aortic valves (BAVs) or tricuspid aortic valves (TAVs). Biomechanical testing of aortic tissue may provide novel insights and biomarkers.

METHODS AND RESULTS: From March 2017 to August 2019, aneurysmal ascending aortas (BAV=23, TAV=23) were collected from elective aortic surgery, normal aortas from transplant donors (n=9), and dissected aortas from surgery for aortic dissection (n=7). These aortas underwent delamination testing in simulation of aortic dissection. Biaxial tensile testing was performed to determine modulus of elasticity (aortic stiffness), and energy loss (a measure of efficiency in performing the Windkessel function). Delamination strength (S_d) was lowest in dissected aortas (18±6 mN/mm) and highest in normal aortas (58±16 mN/mm), and aneurysms fell in between, with greater S_d in the BAV group (37±10 mN/mm) than the TAV group (27±10 mN/mm) (P<0.001). Bicuspid aortopathy was associated with greater stiffness (P<0.001), while aneurysms with TAV demonstrated greater energy loss (P<0.001). S_d decreased by 7.8±1.2 mmol/L per mm per decade of life (r^2=0.45, P<0.001), and it was significantly lower for patients with hypertension (P=0.001). S_d decreased by 6.1±2.1 mmol/L per mm with each centimeter increase in aortic diameter (r^2=0.15, P=0.007). Increased energy loss was associated with decreased S_d (r^2=0.41), whereas there was no relationship between S_d and aortic stiffness.

CONCLUSIONS: Aneurysms with BAV had higher S_d than those with TAV, suggesting that BAV was protective. Energy loss was lower in aneurysms with BAV, and inversely associated with S_d, representing a potential novel biomarker.

Key Words: aortic aneurysm ■ aortic dissection ■ bicuspid aortic valve ■ biomechanics

Bicuspid aortic valve (BAV), affecting 1% to 2% of the general population, is the most common congenital heart disease. Nearly half of these patients will experience dilatation of the proximal aorta,[1] increasing their risk of aortic dissection 8-fold.[2] However, the relative risk of dissection for aneurysms associated with BAV, compared with aneurysms associated with tricuspid aortic valves (TAVs), is controversial. In turn, the appropriate threshold for surgical intervention on BAV-associated aortic aneurysms is unclear. This is in the context of poor natural history data and limited evidence for the currently recommended size thresholds for elective repair of aortic root and ascending aortic aneurysms.[3,4] The majority of patients presenting for aortic dissection have aortic diameters below the threshold for elective surgical repair.[5,6] There is therefore a pressing need to identify alternative biomarkers for the risk of aortic dissection in patients with aortic aneurysms with BAVs and TAVs.

Aortic biomechanics may help clarify risk prediction in aortic aneurysms. Aortic distensibility, pulse wave velocity, and other imaging-based surrogates

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CLINICAL PERSPECTIVE

What Is New?

- Aortic aneurysms associated with bicuspid aortic valves demonstrate greater resistance to dissection compared with those associated with tricuspid aortic valves in ex vivo testing.
- Energy loss, a biomechanical parameter that measures the Windkessel function, is better preserved in aortic aneurysms associated with bicuspid aortic valves.
- Energy loss correlates well with susceptibility to dissection ex vivo.

What Are the Clinical Implications?

- Aortic tissue biomechanics support aneurysms associated with bicuspid aortic valves as having decreased risk of aortic dissection compared with those associated with tricuspid aortic valves.
- Energy loss is a potential novel biomarker of dissection risk that requires further investigation.

Nonstandard Abbreviations and Acronyms

AI	anisotropy index
BAV	bicuspid aortic valve
IC	inner curvature
IRAD	International Registry of Acute Aortic Dissection
OC	outer curvature
S_d	delamination strength
TAV	tricuspid aortic valve
ΔU_L	energy loss

of aortic stiffness have been shown to be altered in Marfan syndrome, and studies have demonstrated an association of these metrics with increased aortic growth rates in patients with connective tissue disease.[7–9] In the population with nonconnective tissue disease, carotid-femoral pulse wave velocity, a global measure of arterial stiffness, has been correlated with faster rate of growth in aneurysm size for women and those with BAV.[10,11]

Through direct biomechanical testing of aneurysmal aortic tissue, we have reported a novel biomechanical parameter "energy loss" (ΔU_L) that was more predictive of elastin and collagen composition of the aortic wall than the traditional mechanical parameter of aortic stiffness or aortic diameter.[12,13] This metric is reflective of aortic health in that it corresponds to the efficiency with which the aorta performs the Windkessel function, storing and returning energy over the cardiac cycle. It remains unknown, however, whether any biomechanical parameter of intact aortic aneurysms translates into predicting clinically relevant aortic events, namely aortic dissection. Delamination testing, which measures the strength required to peel apart the layers of the aortic wall, has been proposed to simulate aortic dissection.[14,15] We hypothesize that patients with aneurysms associated with BAV will have biomechanically healthier aortas and require greater forces to dissect than those with TAV.

The aim of our study was 2-fold: (1) to thoroughly characterize the mechanical phenotype of bicuspid aortopathy, including its delamination strength (S_d), and contrast that to the mechanical phenotype of aortas associated with TAVs; and (2) to determine the association of the strength required to dissect apart aortic tissue as measured by S_d with clinical variables and other biomechanical metrics of aortic tissue quality. Tying a biomechanical metric to the strength required to dissect apart aortic tissue would identify a potential biomarker for risk of dissection.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Patient Population

This study was approved by the research ethics board of the University Health Network, Toronto, and participants (or substitute decision makers) provided written informed consent. Aortic samples from 62 patients were collected for this study. Aneurysmal ascending aortas (n=46) were collected from patients during elective aortic surgery from March 2017 to August 2019. This population included 23 patients with BAVs and 23 patients with TAVs. Normal aortas, serving as negative controls (n=9), were collected from transplant donors. Dissected aortas, serving as positive controls (n=7), were collected from patients who were undergoing surgery for aortic dissection of the ascending aorta. All control samples were associated with TAV.

The aortic specimens were transferred directly from the operating room on excision in a Ringer Lactate solution to the laboratory, which is a 5-minute walk (the specimens stay indoors at all times as the buildings are connected). The specimens were then kept on ice while awaiting testing. This was usually performed right away, but if there was any delay in testing, the specimens were placed in a 4°C fridge until testing was performed, which was always within 24 hours. The specimens were never frozen. After testing, the

specimens were fixed in formalin for further histological analysis.

Charts were reviewed prospectively to collect clinically pertinent patient information. Diameters of the ascending aortas were taken from preoperative computed tomography scans primarily. If computed tomography scans were unavailable, magnetic resonance imaging scans were used, and echocardiography measurements were taken if magnetic resonance imaging was unavailable.

Mechanical Tests: Biaxial Tensile Testing

ΔU_L and modulus of elasticity were derived from the results of biaxial tensile testing. ΔU_L is a measure of the efficiency with which the aorta performs the Windkessel function. Higher values of ΔU_L mean that a higher proportion of energy absorbed by the aorta during loading is dissipated including into the tissue itself, and less of it is returned to the circulation. Modulus of elasticity measures intrinsic tissue stiffness, with higher values meaning greater stiffness. Less healthy tissue would therefore have higher levels of both ΔU_L and modulus of elasticity.

Aortic tissue was collected as complete rings from the ascending aorta and was cut using custom-made cutters to 14×14-mm square samples from both the outer curvature (OC) and inner curvature (IC) of the aorta (Figure 1A). Up to 3 samples, depending on the size of the specimen, were collected longitudinally from the OC and 1 square was collected from the IC. The thickness of the samples was measured using a high-magnification (12×) zoom lens (Navitar) before mechanical testing. Each sample was immersed in 37°C Ringer lactate solution and subjected to 10 preconditioning stretch cycles followed by 3 analyzed cycles under 25% equibiaxial strain using a BioTester (CellScale) (Figure 1C). From the generated stress-strain curve, ΔU_L and the tangent modulus of elasticity at 10% strain were calculated in both the longitudinal and circumferential directions of loading (Figure 2A).

Biomechanical variables, such as ΔU_L and tangent modulus of elasticity at 10% strain, when measured in the longitudinal versus the circumferential direction, are not necessarily equal. The anisotropy index (AI), is a measure of this type of asymmetry (Figure 2B). The more negative the AI, the greater the biomechanical variable in the circumferential direction; while the more positive the AI, the greater the longitudinal direction. When the AI is close to zero, the variable is equal in the circumferential and longitudinal directions. We previously found that healthy aortic tissue demonstrated a natural asymmetry (a negative AI), which was no longer present with medial degeneration.[13]

Mechanical Tests: Delamination Testing

For delamination testing, 6×30-mm rectangles were cut out from the areas immediately adjacent to the square samples (Figure 1B). These rectangles were delaminated by peeling apart the medial layer proximally to distally along the aorta. An incision was manually introduced in the media and then the specimen manually peeled back to 14 mm before being mounted on the BioTester for controlled delamination while immersed in 37°C Ringer lactate solution (Figure 1D). The average force to peel through the strip was normalized by the width of the specimen to determine S_d (Figure 2C). Aortic tissue that is resistant to dissection has a higher S_d than aortas that are prone to dissection.

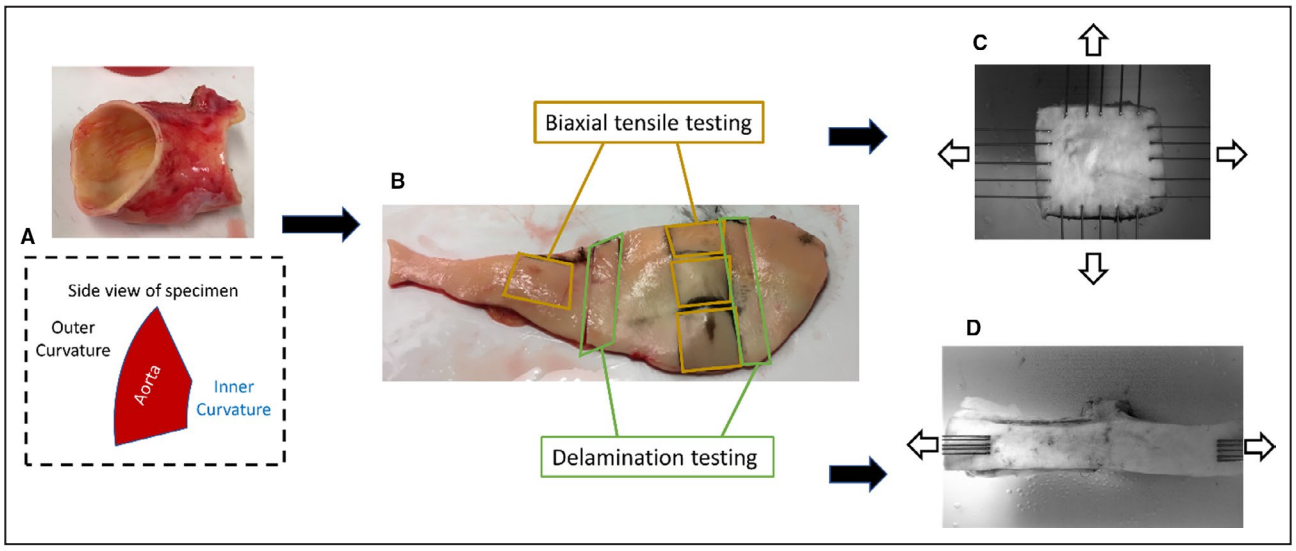

Figure 1. Aortic specimens excised at the time of surgery are taken for ex vivo biomechanical testing.
A, Specimen sampled at both inner and outer curvatures. **B**, Squares are sampled for (**C**) biaxial tensile testing and rectangular strips are sampled for (**D**) delamination testing.

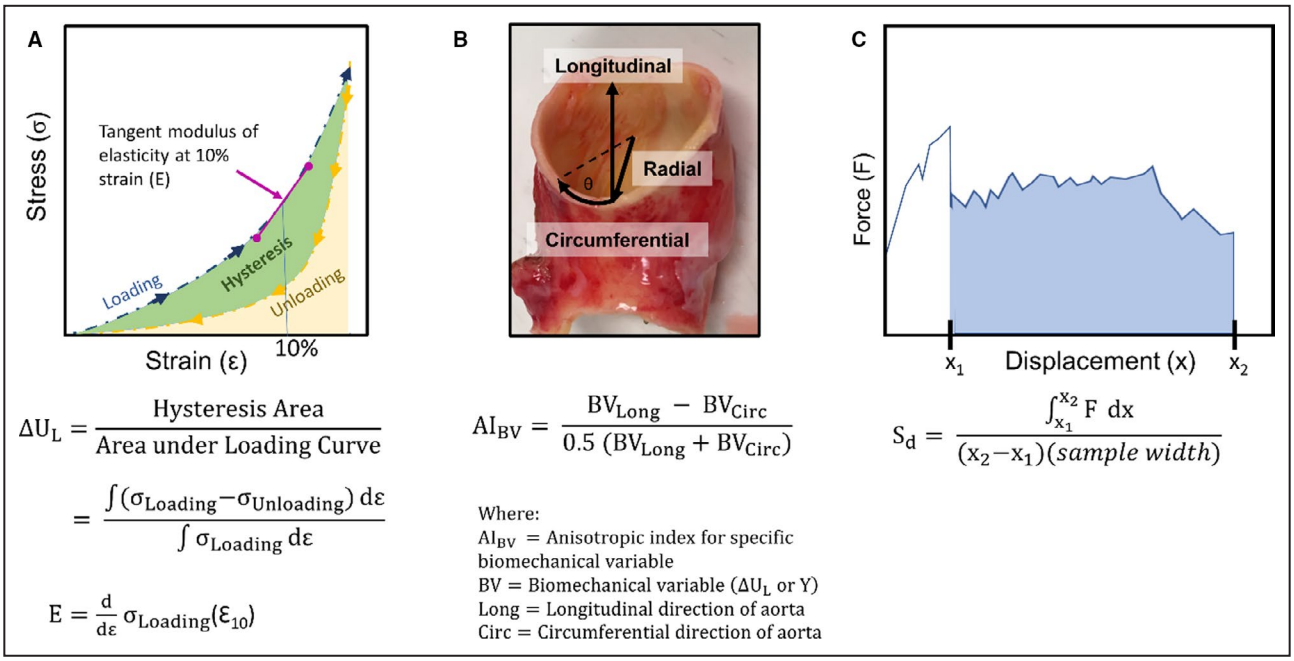

Figure 2. Definitions of biomechanical parameters.
A, Tangent modulus of elasticity and energy loss is derived from the stress-strain curve. **B**, Anisotropy index quantifies the relationship between parameters measured in the longitudinal vs circumferential directions. **C**, Delamination strength is derived from the average force after the first peak during delamination testing.

For specimens collected from cases of aortic dissection (6 acute aortic dissection and 1 subacute dissection), only sections of the aorta where all 3 layers remained intact were tested. In all of the dissected specimens in our series, the intact portion was found on the IC of the aorta and rectangular samples were then collected for delamination testing. All mechanical testing calculations were performed using in-house script in Matlab r018b (The MathWorks, Inc).

Histology

After mechanical testing, square aortic samples were fixed in 10% formalin. Each sample was then paraffin-embedded and cut in 4-μm thick sections for Movat pentachrome staining. Using an Aperio ImageScope version 12.4.0.5043 (Leica Biosystems), 3 random locations from each digitally scanned slide of aortic media were captured at 20×. For quality control, a randomly selected subset of these magnified images was reviewed with a cardiovascular pathologist to ensure that the aortic wall was accurately captured for analysis. Images were saved and colorimetric analysis was performed using ImageJ version 1.52p (National Institutes of Health).

The YUV color threshold of each image was manually adjusted to quantify the proportion of the following components: collagen shown as yellow, smooth muscle cell shown as red, and mucopolysaccharide shown as blue. Images were then converted to 32-bit grey scale to determine the composition percentage of elastin, shown as black. For each slide, measurements from the 3 random locations were averaged before use in analysis.

Statistical Analysis

All variables were summarized using mean±SD. The α level used was 0.05. Linear regression was used to compare pairs of continuous variables (combinations of clinical, biomechanical, and histological factors). For categorical comparisons between groups (eg, sex across groups), chi-square test was used, while *t* tests were used to compare continuous variables between 2 groups. One-way ANOVA was performed to compare variables between multiple groups, and Tukey multiple comparison test was used for post hoc analysis. When both IC and OC values were available, 2-way ANOVA was performed to compare variables between multiple groups, and the method was used for multiple comparison tests. The analyses were conducted using Prism 5 (GraphPad). Multivariable regression modeling was performed using R in RStudio version 1.2.5033 (RStudio) to assess for the independent contributions of age and the presence of BAV on S_d.

RESULTS

The clinical characteristics of our patient population and controls are presented in the Table. As expected,

Table. Baseline Characteristics of Patients Whose Aortic Tissue Underwent Biomechanical Testing

Variable	Controls		Aneurysm			
	Normal (n=9)	Dissection (n=7)	Total (n=46)	BAV (n=23)	TAV (n=23)	*P* Value (BAV vs TAV)
Age, y	45±11	66±10	64±12	58±11	70±9	<0.001
Women, %	33.3	14.3	34.8	39.1	30.4	0.76
Hypertension, %	0	71.4	56.5	43.5	69.6	0.14
Diabetes mellitus, %	0	0	0	0	4.4	>0.99
Renal failure, %	0	14.3	8.9	8.7	8.7	>0.99
Coronary artery disease, %	0	0	11.1	8.7	13.0	>0.99
Connective tissue disease, %	0	0	4.44	0	8.7	0.49
History of Smoking, %	33.3	0	41.3	43.5	39.1	>0.99
COPD, %	0	0	8.7	4.4	13.0	0.61
Aortic regurgitation (moderate or more), %	0	57.1	45.6	30.4	60.9	0.07
Aortic stenosis (moderate or more), %	0	0	28.3	52.2	4.4	<0.001
β-Blocker use, %	0	28.6	41.3	43.5	39.1	>0.99
ACEI/ARB use, %	0	14.3	39.1	30.4	47.8	0.37
Aortic diameter, mm	31.0±5.3	57.0±9.3	53.1±8.4	50.7±6.6	55.6±9.4	0.048

ACEI indicates angiotension-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BAV, bicuspid aortic valve; COPD, chronic obstructive pulmonary disease; and TAV, tricuspid aortic valve.

Normal aortas were taken from cardiac transplant donors, while dissection specimens were taken from patients who had aortic dissection.

The aneurysm group consists of patients who underwent elective surgery for aortic aneurysms. Data are represented as mean±SD. Chi-square test was used to compare categorical variables, while *t* test was used to compare continuous variables.

patients with ascending aortic aneurysms associated with BAV were younger than those associated with TAV (58±11 versus 70±9 years, $P<0.001$). Clinical characteristics were otherwise similar, with low incidence of diabetes mellitus, renal failure, coronary artery disease, or connective tissue disease (1 patient with Marfan syndrome and 1 patient with Loeys-Dietz syndrome). The mean aortic diameter at the time of elective repair was smaller for patients with BAV compared with those with TAV (50.7±6.6 mm versus 55.6±9.4 mm, $P=0.048$).

Comparison of Biomechanical Parameters Between Patient Groups

S_d, or the force required to mechanically tear apart the aortic sample, was lowest in the dissection group and highest in the controls with normal aortas. The S_d of aneurysmal aortas fell in between the 2 extremes (normal: 58±16 mN/mm, dissection: 18±6 mN/mm, BAV: 37±10 mN/mm, TAV: 27±10 mN/mm; $P<0.001$) (Figure 3A). It required greater strength to tear apart aortas from patients with BAV than those from patients with TAV ($P<0.05$). IC samples were used for this analysis as it was only the inner curve of the aorta that was available for delamination testing in dissected aortas. S_d of the IC was not significantly different from that of the OC in the normal, BAV, and TAV groups ($P=0.07$, Figure 3B).

Stiffness, as measured by the modulus of elasticity, was significantly different across groups when it was measured both circumferentially ($P<0.001$) and longitudinally ($P<0.001$) (Figure 3C and 3D). There was no difference in stiffness of the IC versus the OC of the ascending aorta (circumferential: $P=0.13$, longitudinal: $P=0.98$). Bicuspid aortopathy was associated with greater stiffness than normal aortas when measured circumferentially (OC: 167±41 kPa versus 121±11 kPa, $P<0.01$) and greater stiffness than both normal aortas and aneurysms with TAV when measured longitudinally (IC:154±40 kPa versus 113±10 kPa versus 121±36 kPa, $P<0.05$).

ΔU_L, which is the proportion of energy dissipated or absorbed by aortic tissue during relaxation after deformation, also differed across groups whether measured circumferentially ($P<0.001$) or longitudinally ($P<0.001$), without a difference between the IC or OC (circumferential: $P=0.33$, longitudinal: $P=0.06$). The TAV group demonstrated greater ΔU_L compared with normal aortas, both when measurements were made circumferentially (IC: 0.093±0.026 versus 0.065±0.019 $P<0.01$; OC: 0.101±0.029 versus 0.064±0.023, $P<0.001$) and longitudinally (IC: 0.071±0.029 versus 0.037±0.006, $P<0.001$; OC: 0.085±0.032 versus 0.041±0.011, $P<0.001$). The TAV group also demonstrated greater ΔU_L than the BAV group (circumferential IC: 0.093±0.026 versus 0.063±0.019 [$P<0.001$], circumferential OC: 0.101±0.029 versus 0.071±0.016 [$P<0.001$]; longitudinal IC: 0.071±0.029 versus 0.046±0.017 [$P<0.001$], longitudinal OC: 0.085±0.032 versus 0.055±0.014 [$P<0.001$]).

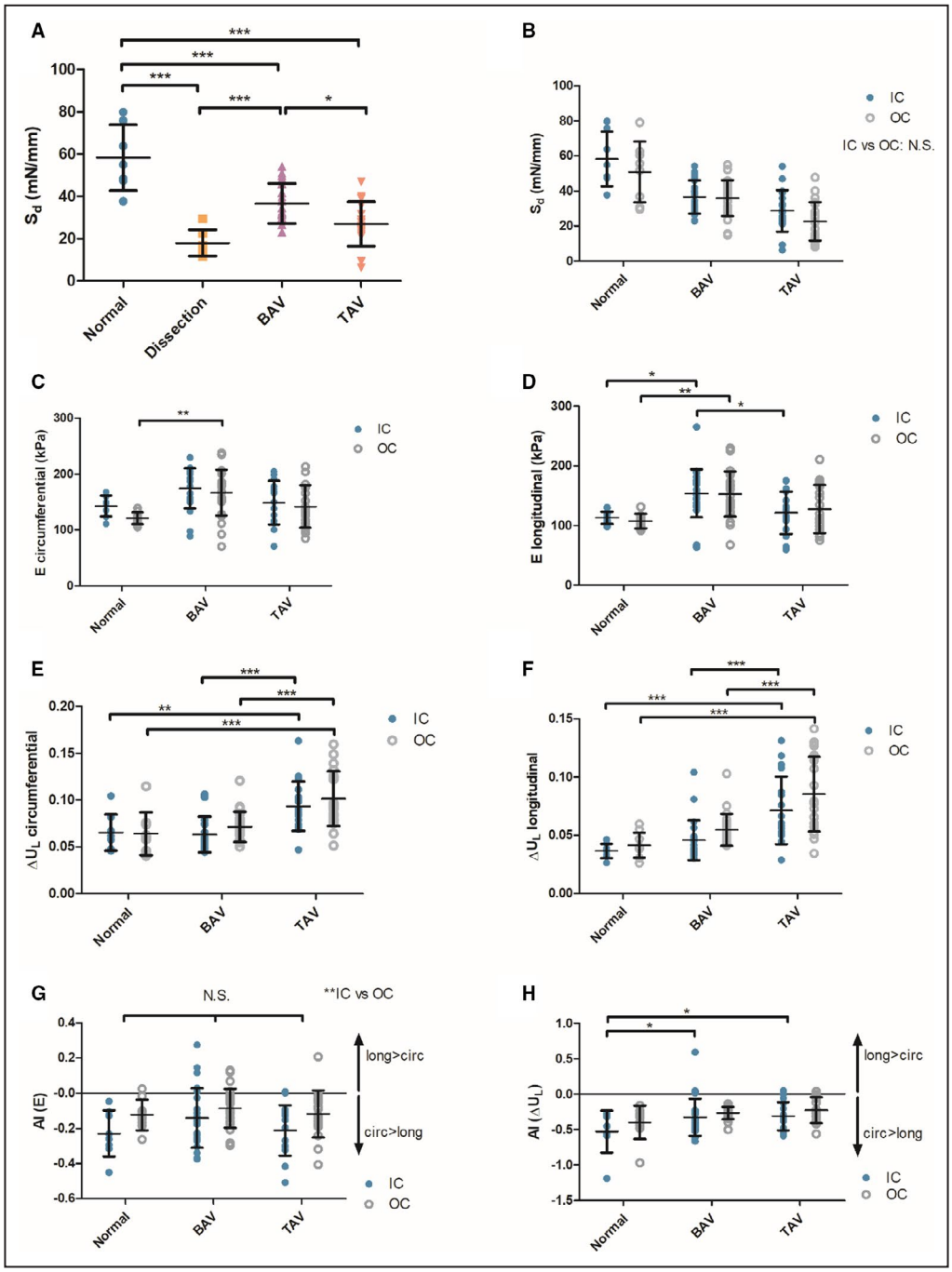

Figure 3. Biomechanical properties across patient groups.
A, Delamination strength (S_d) of aneurysms is significantly lower than normal aortas and higher than dissected aortas. Inner curvature (IC; blue solid circles) samples were used for this analysis as it was only the inner curve of the aorta that was available for delamination testing in dissected aortas. **B**, S_d is the same on the IC vs the outer curvature across patient groups. **C** and **D**, Aneurysms with bicuspid aortic valves have higher modulus of elasticity (**E**) than normal aortas and aneurysms with tricuspid aortic valves, especially when measured in the longitudinal direction. **E** and **F**, Aneurysms with tricuspid aortic valves have higher energy loss (ΔU_L) than either normal aortas or aneurysms with bicuspid aortic valves as measured in both the circumferential and longitudinal directions. **G**, The anisotropy index (AI) of the modulus of elasticity (**E**), was the same across groups. **H**, The AI of ΔU_L was more negative for normal aortas than aneurysmal aortas. BAV indicates bicuspid aortic valve; circ, circumferential; long, longitudinal; OC, outer curvature (grey open circles); and TAV, tricuspid aortic valve. Statistics shown are 1-way ANOVA for (**A**) and 2-way ANOVA for the rest: *P<0.05, **P<0.01, ***P<0.001.

AIs evaluating the directional dependency of both aortic stiffness and ΔU_L were evaluated. Aortas across groups were stiffer in the circumferential direction than the longitudinal (Figure 3G). This asymmetry in stiffness was greater in the IC than the OC (P=0.006), but there was no difference between patient groups (P=0.14). Aortas across all groups had greater ΔU_L in the circumferential direction than the longitudinal (Figure 3H). The asymmetry in ΔU_L was greater in the IC than the OC (P=0.049), and it was greatest for normal aortas compared with aneurysms with BAV (IC: −0.53±0.30 versus −0.33±0.26, P<0.05) or aneurysms with TAV (IC: −0.53±0.30 versus −0.31±0.20, P<0.05).

Association of Clinical Variables With S_d

S_d decreased as age increased (IC: r^2=0.18, P<0.002; OC: r^2=0.45, P<0.001). In the OC, there was a decrease of S_d by 7.8±1.2 mmol/L per mm per decade of life. S_d was similar between men and women (IC: 33±16 mN/mm versus 35±15 mN/mm, P=0.72; OC: 32±14 mN/mm versus 31±15 mN/mm, P=0.80). For patients with aneurysms, S_d was significantly lower for patients with hypertension versus those without (IC: 27±11 mN/mm versus 39±13 mN/mm, P=0.006; OC: 26±11 mN/mm versus 39±16 mN/mm, P=0.001), implying that less force is needed to dissect aortas of patients with aneurysms and preexisting hypertension. There was no relationship with S_d and history of smoking (IC: 32±12 mN/mm versus 35±17 mN/mm, P=0.46; OC: 32±13 mN/mm versus 34±17 mN/mm, P=0.60). Increasing aortic diameter correlated with decreasing S_d (Figure 4C) (IC: r^2=0.10, P=0.02; OC: r^2=0.15, P=0.007), suggesting that larger aortas required less force to dissect. In the OC, there was a decrease of S_d by 6.1±2.1 mmol/L per mm per centimeter increase in aortic diameter. Indexing the diameter by body surface area demonstrated a similar correlation (Figure 4D) (IC: r^2=0.11, P=0.02; OC: r^2=0.14, P=0.01). Presence of aortic stenosis or regurgitation, and the use of β-blockers or angiotensin-converting enzyme inhibitors/angiotensin receptor blockers did not correlate with S_d (P=NS). Given the significant relationship between age and S_d, as well as the difference in age between the BAV and TAV groups, multivariable linear regression was used to evaluate whether the protective effect of BAV on S_d was secondary to younger age alone. With respect to the other baseline differences between the 2 groups, aortic valve stenosis did not influence S_d and was not included in the model. Aortic diameter was only slightly different between the 2 groups, and diameter in the aneurysm cohort alone did not significantly correlate with S_d (r^2=0.022, P=0.33), suggesting that diameter alone is an inadequate metric of propensity for dissection. Multivariable linear regression revealed that both age (P=0.02) and BAV (P=0.01) were independent predictors of S_d (Table S1).

Association of Biomechanical Variables With S_d

ΔU_L and aortic stiffness in both the longitudinal and circumferential directions, and their respective AIs, were next correlated with S_d. There was a negative relationship between ΔU_L and S_d (Figure 5A and 5D), and the strongest relationship was found in the OC when measured longitudinally (r^2=0.41). The data were fit with a 1-phase decay model, as neither S_d nor ΔU_L can be <0. In other words, the ascending aorta tears apart more easily as it becomes less efficient in performing the Windkessel function. Aortic stiffness did not correlate with S_d in either the IC (circumferential: r^2=0.015, P=0.39; longitudinal: r^2=0.007, P=0.56) or the OC (circumferential: r^2<0.001, P=0.91; longitudinal: r^2=0.001, P=0.79) (Figure 5B and 5E). The anisotropic index for ΔU_L in the OC of the ascending aorta correlated with S_d such that the more isotropic aortas required less strength to tear (r^2=0.20, P<0.001). Other measurements of AI did not correlate with S_d (Figure 5C and 5F).

Histological Analysis

S_d positively correlated with elastin content (OC: r^2=0.26, P<0.001), suggesting that greater forces were required to peel apart the aortic wall in aortas with higher contents of elastin in the media (Figure 6). S_d did not significantly correlate with the collagen, mucopolysaccharide, or smooth muscle cell contents of the aortic wall (Table S2). Increased ΔU_L was associated with decreased elastin (longitudinal, OC: r^2=0.24, P<0.001) and increased collagen content (longitudinal OC: r^2=0.22, P=0.001). Greater aortic stiffness was associated with increased collagen content (longitudinal OC: r^2=0.24, P<0.001). Anisotropy did not correlate significantly with either elastin or collagen (Table S2).

DISCUSSION

This large series of biomechanical testing on aneurysms of the ascending aorta is the first to systematically compare S_d (force required to dissect the aortic wall) and other clinical and biomechanical variables. The 2 most important novel observations were as follows: (1) aneurysms associated with TAV had lower S_d than those associated with BAV, even after adjusting for the effect of age; and (2) a novel biomechanical parameter, ΔU_L (the amount of energy dissipated or absorbed by aortic tissue while performing its Windkessel function), was shown to be associated with the strength required to dissect apart aortic tissue.

The first finding suggests that aneurysms with TAV may in fact be relatively more vulnerable to aortic dissection than aneurysms with BAV. These results are in line with 2 previous studies demonstrating higher ultimate tensile strength for aortas with BAV compared with TAV.[16,17] These studies, however, examined the strength of the intact aorta and did not consider the clinically meaningful end point of aortic dissection. Pasta et al,[18] in their study of S_d in ascending aortas, found the opposite effect of valve morphology. The regional sampling of the strips of aortic tissue for delamination was not specified in their study, whereas our significantly larger study systematically sampled from the ICs and the OCs of the aorta. This may account

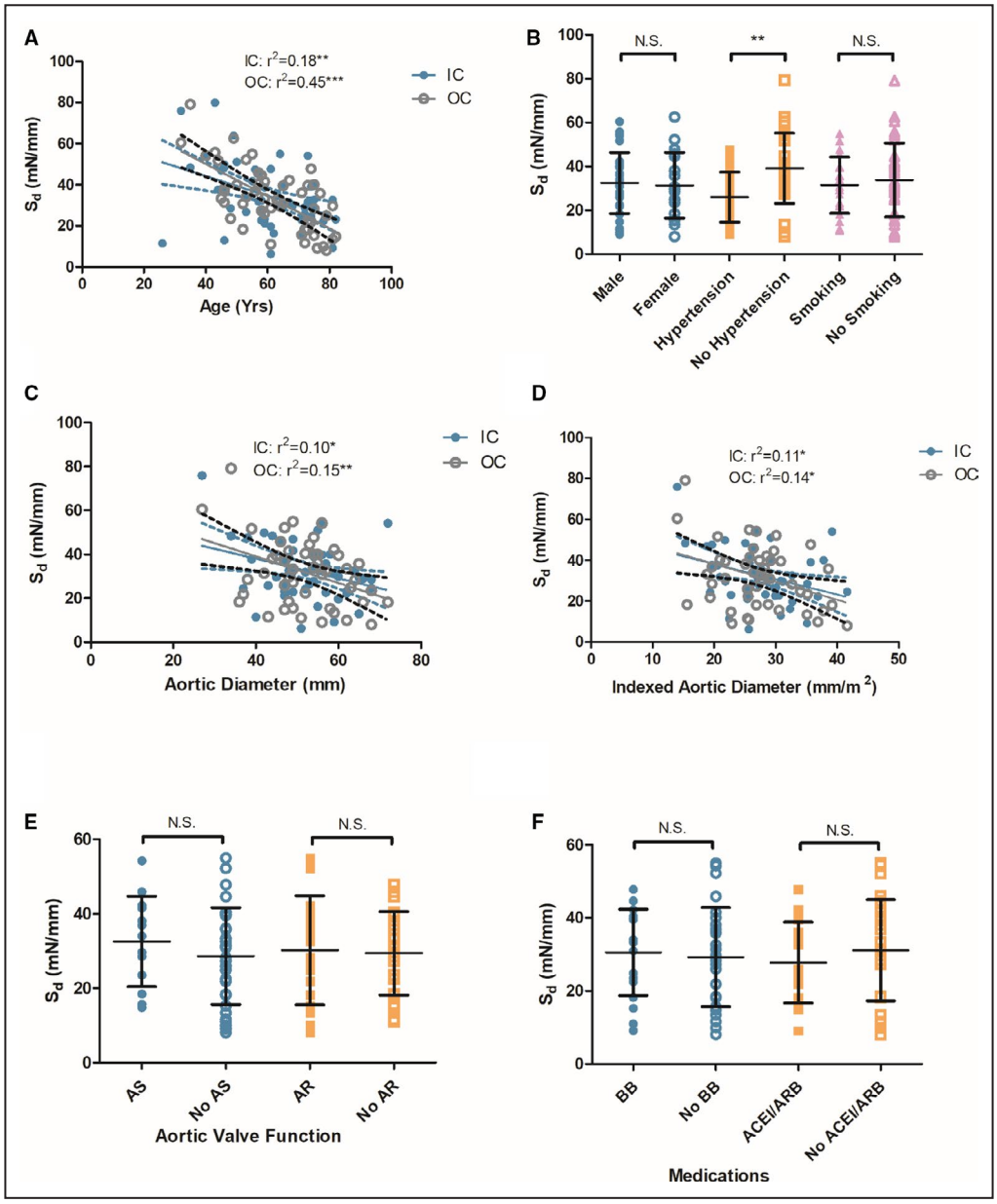

Figure 4. The relationship between delamination strength (S_d) of the ascending aorta and clinical parameters.

A, S_d decreases with age. **B**, S_d is the same between sexes and whether there is a positive smoking history. S_d is lower in patients with hypertension. **C**, S_d decreases as aortic diameter increases, **D**, and this relationship is similar when indexing aortic size to body surface area (BSA). **E**, Neither aortic stenosis (AS) or aortic regurgitation (AR) had a relationship with S_d. **F**, No relationship was seen with either β-blockers (BB) or angiotensin-converting enzyme (ACE) inhibitors/angiotensin receptor blockers (ARBs) and S_d. **B** through **F**, outer curvature (OC; grey open circles) data shown as inner curvature (IC; blue solid circles) data similar. Statistics shown are linear regression and 2-way ANOVA post-test results (Bonferroni): *P<0.05, **P<0.01, ***P<0.001.

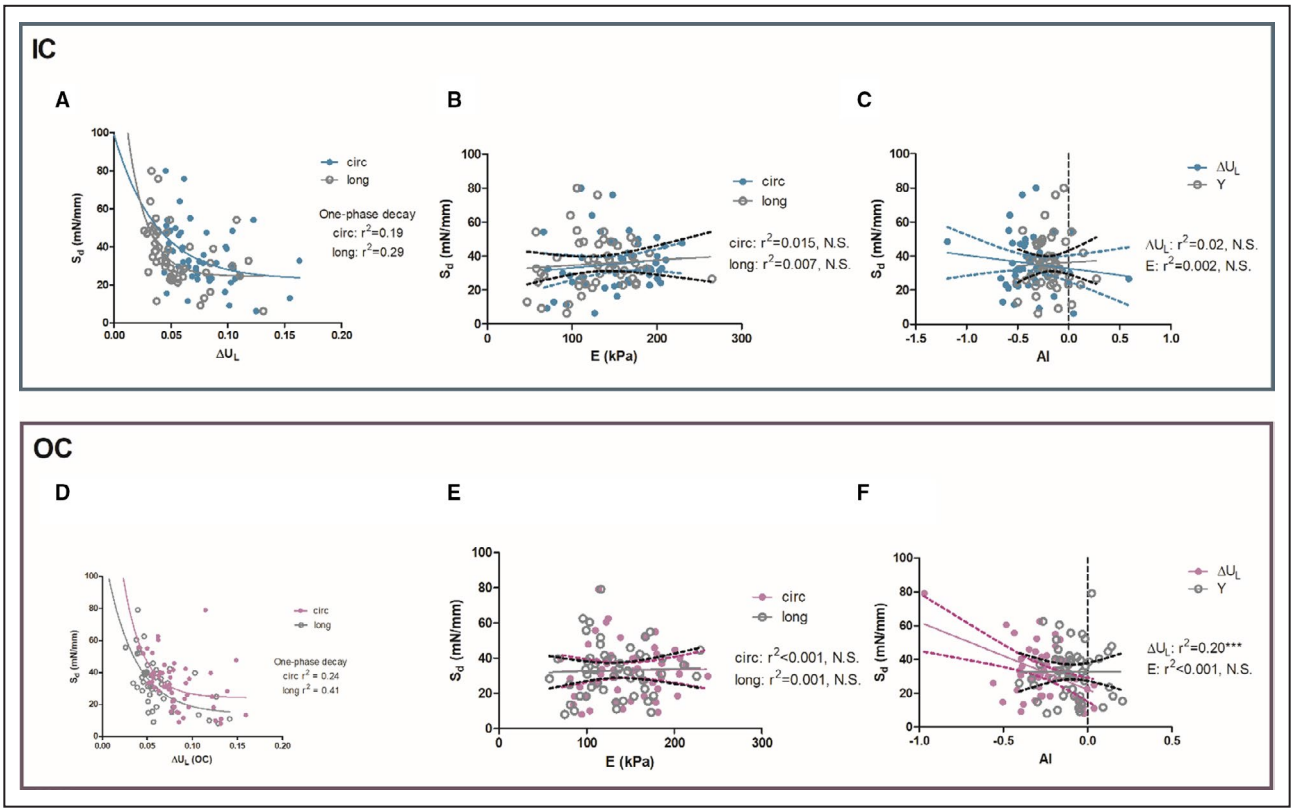

Figure 5. Biomechanical parameters were evaluated for their associations with delamination strength (S_d) of the ascending aortic tissue.

The inner curvature (IC) panel includes the relationships between S_d and (**A**) energy loss (ΔU_L), (**B**) aortic stiffness (modulus of elasticity, (**E**) and (**C**) anisotropy index (AI) on the inner curve of the aorta. The outer curvature (OC) panel includes the relationships between S_d and (**D**) ΔU_L, (**E**) aortic stiffness, and (**F**) AI on the outer curve of the aorta. S_d decreased as ΔU_L of the tissue increased. No relationship was seen between aortic stiffness and S_d. There was a significant relationship between AI of ΔU_L measured on the OC of the ascending aorta and S_d. Statistics shown are 1-phase decay for (**A** and **D**), and linear regression for the rest: ***P<0.001. circ indicates circumferential; and long, longitudinal.

for the discordant findings. Our findings agree with recent guidelines, which have moved towards a more conservative approach to BAV aortopathy, given the overall low incidence of aortic dissection in this population, by increasing the operative threshold from 50 to 55 mm.[4,19]

This difference in S_d between valve types may be attributed to our finding that aortic aneurysms had distinct mechanical phenotypes depending on valve morphology. Bicuspid aortopathy was associated with greater stiffening of the aortic wall on biaxial tensile testing, corroborating a previous study using uniaxial tensile testing.[17] Meanwhile, aortic aneurysms associated with TAV did not demonstrate this stiffening, but rather had higher levels of ΔU_L, a decreased efficiency in performing the Windkessel function.

We verified that ΔU_L correlated strongly with S_d. The interpretation of this is that aortas less efficient in performing the Windkessel function require less force to tear apart as occurs in dissections. Histological analysis suggests that this may be driven by elastin content. S_d also correlated with ΔU_L AI (a measure of dependency on direction of measurement). This is an extension of our previous work, which showed that as AI tended towards 0 (there is no longer directional dependency in measurement of ΔU_L), there is greater medial degeneration seen on histology.[13] Here, we demonstrate that this phenomenon is also associated with less force required to separate the medial layer of the aortic wall.

To our knowledge, this is the first study to compare destructive testing used as a surrogate for aortic dissection and nondestructive tests (biaxial tensile testing). Biomechanical properties derived through nondestructive testing have the potential of being measured in vivo, whereas destructive testing, although representative of the clinical event of interest, is not reproducible by clinicians monitoring patients at risk for aortic dissection. The identification of a nondestructive biomechanical property, specifically that of ΔU_L, which may be associated with risk of aortic dissection, represents an important step in

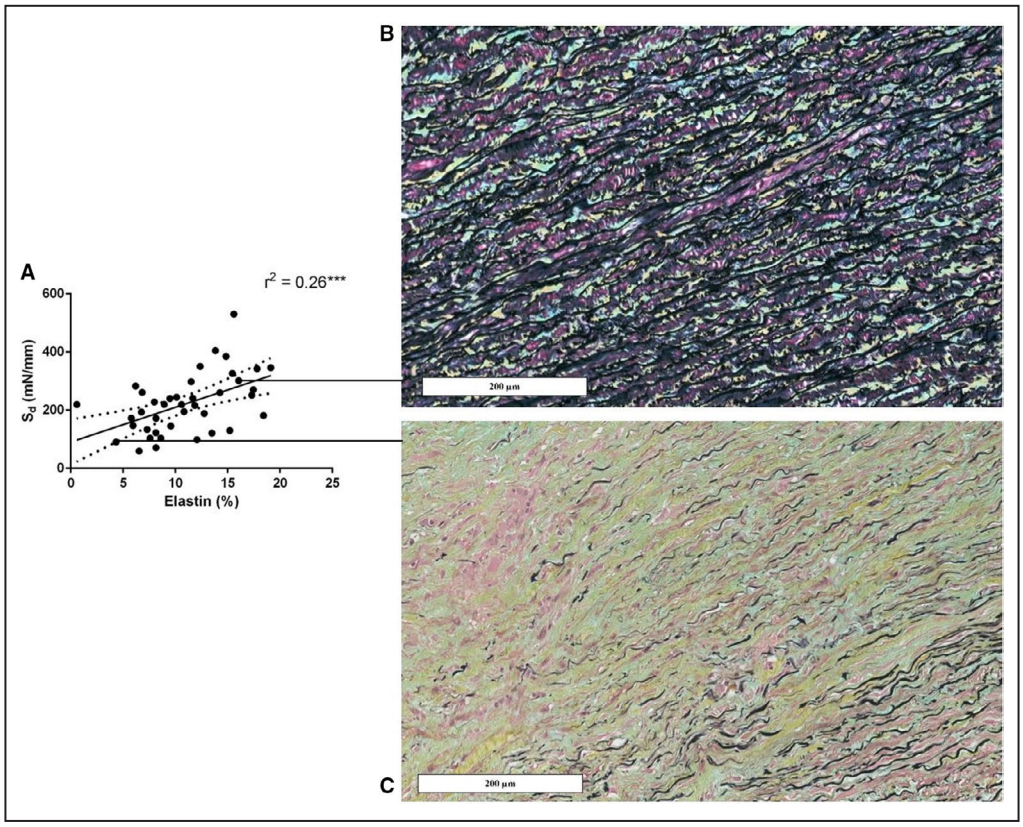

Figure 6. Aortic biomechanics is related to underlying aortic wall microstructure.
A, Relationship between delamination strength (S_d) of ascending aortic tissue during ex vivo biomechanical testing and content of elastin evaluated from colorimetric analysis of Movat pentachrome staining of histological sections of the same aortic specimens. Statistics shown are linear regression: ***P<0.001. **B**, An example of a histological section from an aortic specimen with higher S_d and higher elastin content. There were intact elastin (black) lamellae with preserved vascular smooth muscle cells (purple/pink) and relatively low contents of collagen (yellow). **C**, An example of a histological section from an aortic specimen with lower S_d and lower elastin content. There was profound medial degeneration with extensive elastin (black) fragmentation, loss of smooth muscle cells (purple/pink), and elevated content of collagen (yellow).

the development of mechanics-based biomarkers that could potentially be quantifiable noninvasively. The traditional nondestructive biomechanical property, aortic stiffness, is more frequently cited in the literature.[20–23] However, we showed that stiffness did not translate into weaker dissection properties. Aortic stiffness is limited as it does not account for the full nonlinear stress-strain behavior of aortic tissue, whereas ΔU_L does. Moreover, stiffness, and even the more comprehensive descriptor, the strain-energy density function, disregard the active diastolic unloading part of the cardiac cycle.

Several clinical variables beyond valve morphology were also associated with S_d. Aortic diameter, the current benchmark biomarker for aortic risk, demonstrated a weak association with S_d. Age demonstrated a strong association. S_d was associated with hypertension, which is the most common predisposing factor for aortic dissection, present in 72% of patients in the IRAD (International Registry of Acute Aortic Dissection).[24] We anticipate the future of aortic risk prediction will involve combining important clinical variables with mechanical variables, as they are not competing but rather complementary tools.

Our study is limited by the fact that S_d is a surrogate for aortic dissection. While we believe that this direct measurement of aortic tissue quality is closer to the actual outcome of interest of aortic dissection than other common clinical surrogates such as aneurysm growth rates, the ex vivo experimental protocol is necessarily controlled and reproducible. In contrast, a patient's aorta tears at various angles and directions, and under various loading conditions. Nonetheless, we were able to validate delamination testing for the first time against specimens from both positive and negative controls. Indeed, the force required to separate the medial layer of aortic tissue from transplant donor patients with normal aortas was significantly higher than the force required in patients who had aortic dissection. Patients with aortic

aneurysms demonstrated S_d that fell between these 2 controls. The inclusion of positive (dissection) controls and negative (normal) controls is unique to the study and strengthens it.

Another limitation lies in the observational nature of our study. The patients enrolled were naturally not randomized and so the cohort with BAVs was different from the cohort with TAVs. While using multivariable linear regression to adjust for the effect of age assists with parsing the actual effect of BAV on S_d, it cannot fully account for unmeasured confounders. We may still derive real-world implications. Of the patients who are presently being referred for and undergoing surgery, the patients with BAVs tend to have more favorable aortic biomechanics than those with TAVs.

CONCLUSIONS

The biomechanical data from our study add significantly to the ongoing debate as to whether surgeons should intervene earlier in cases of bicuspid aortopathy for elective aortic surgery. This work also provides a blueprint for evaluating candidate biomechanical parameters for their association with dissection risk, and sets the stage for further vetting of ΔU_L. This includes studying the pathophysiology that drives the association between ΔU_L and S_d. Future work will focus on development and validation of noninvasive imaging techniques and postprocessing algorithms to measure in vivo ΔU_L, which may then be used for more definitive longitudinal clinical studies for improving dissection risk assessment.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Materials

Tables S1–S2

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Supplemental Material

Table S1. Multivariable linear regression model of the effects of age and bicuspid aortopathy on the delamination strength of ascending aortic aneurysms.

Model	Unstandardized Coefficients		t-value	p-value.
	B	St. Error		
Constant	49.25	11.05	4.46	6.07e-05
Age (years)	-0.37	0.15	-2.40	0.02
BAV*	9.12	3.58	2.55	0.01

*BAV: bicuspid aortic valve.

Table S2. Comparison of biomechanical variables to percentage composition of aortic tissue in patients with bicuspid and tricuspid aortic valves.

	ALL AORTAS				BAV				TAV			
	IC		OC		IC		OC		IC		OC	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Elastin	0.09	0.06	**0.26**	**0.001**	0.08	0.27	0.07	0.30	0.01	0.67	**0.30**	**0.01**
SM	0.05	0.18	0.01	0.56	**0.24**	**0.05**	0.04	0.42	0.01	0.73	0.02	0.57
Collagen	<0.001	0.94	0.04	0.22	0.03	0.52	0.001	0.89	0.001	0.92	0.05	0.34
MPS	<0.001	0.89	0.02	0.38	0.12	0.20	0.03	0.49	0.05	0.37	0.003	0.81
E:C	0.002	0.81	**0.22**	**0.003**	0.08	0.29	0.11	0.21	0.02	0.58	0.12	0.14
	IC		OC		IC		OC		IC		OC	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Elastin	**0.22**	**0.002**	**0.23**	**0.001**	0.10	0.19	0.09	0.22	**0.38**	**0.005**	**0.37**	**0.002**
SM	**0.19**	**0.005**	0.03	0.25	**0.22**	**0.05**	0.08	0.25	0.06	0.31	0.00	0.91
Collagen	0.06	0.15	**0.13**	**0.02**	0.10	0.22	**0.25**	**0.04**	0.02	0.57	0.15	0.07
MPS	0.01	0.56	0.08	0.07	0.01	0.69	0.11	0.17	0.002	0.85	0.003	0.81
E:C	**0.11**	**0.04**	0.07	0.08	0.04	0.43	0.06	0.36	0.12	0.15	0.13	0.09
	IC		OC		IC		OC		IC		OC	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Elastin	**0.21**	**0.003**	**0.24**	**0.001**	0.08	0.25	0.11	0.17	**0.28**	**0.02**	**0.26**	**0.01**
SM	0.05	0.18	0.00	0.69	0.14	0.12	0.12	0.16	0.03	0.49	0.00	0.85
Collagen	**0.17**	**0.01**	**0.22**	**0.001**	**0.47**	**0.002**	**0.39**	**0.01**	0.09	0.22	**0.23**	**0.02**
MPS	0.001	0.81	0.06	0.10	0.14	0.14	0.21	0.06	0.04	0.40	0.001	0.91
E:C	**0.14**	**0.02**	0.07	0.09	0.19	0.08	0.07	0.30	0.13	0.13	0.09	0.16

	IC		OC		IC		OC		IC		OC	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Elastin	0.01	0.46	0.02	0.38	0.02	0.55	0.07	0.28	0.005	0.78	0.02	0.57
SM	0.02	0.42	0.02	0.42	0.10	0.21	0.09	0.24	0.03	0.45	0.01	0.59
Collagen	0.03	0.34	**0.27**	**<0.001**	0.22	0.05	**0.33**	**0.02**	0.04	0.41	0.15	0.07
MPS	0.004	0.70	0.00	0.65	0.09	0.24	0.05	0.37	0.06	0.34	0.04	0.34
E:C	0.003	0.74	0.02	0.37	0.02	0.58	**0.25**	**0.04**	0.03	0.47	0.001	0.88
	IC		**OC**		**IC**		**OC**		**IC**		**OC**	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Elastin	0.04	0.19	0.01	0.47	0.08	0.25	0.01	0.69	0.01	0.77	0.04	0.37
SM	0.06	0.12	0.01	0.63	0.18	0.08	0.02	0.56	0.02	0.58	0.002	0.85
Collagen	0.02	0.38	**0.24**	**0.001**	0.10	0.21	0.18	0.09	0.03	0.47	0.16	0.06
MPS	0.01	0.65	0.002	0.76	0.10	0.21	0.08	0.24	0.08	0.26	0.08	0.18
E:C	0.01	0.49	0.02	0.39	0.002	0.87	0.07	0.30	0.002	0.87	0.01	0.71
	IC		**OC**		**IC**		**OC**		**IC**		**OC**	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value
Elastin	0.01	0.47	0.07	0.08	0.00	0.99	0.01	0.70	0.09	0.16	0.12	0.08
SM	0.04	0.23	0.02	0.38	0.00	0.99	0.01	0.70	0.09	0.17	0.05	0.27
Collagen	0.01	0.52	0.02	0.31	0.01	0.66	0.00	0.97	0.01	0.60	**0.18**	**0.03**
MPS	0.01	0.63	0.01	0.50	0.07	0.30	0.06	0.32	0.03	0.41	0.00	0.73
E:C	0.03	0.25	0.02	0.41	0.08	0.25	0.01	0.73	0.03	0.41	0.02	0.51
	IC		**OC**		**IC**		**OC**		**IC**		**OC**	
	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value	r^2	p value

Elastin	0.06	0.19	0.001	0.86	0.08	0.24	0.12	0.16	0.03	0.45	0.02	0.48
SM	**0.10**	**0.04**	0.001	0.81	0.10	0.21	0.08	0.26	0.06	0.26	0.005	0.73
Collagen	0.01	0.51	0.002	0.76	0.03	0.46	0.001	0.89	0.00	0.98	0.04	0.33
MPS	0.005	0.67	0.001	0.83	0.04	0.41	0.01	0.71	0.01	0.69	0.02	0.43
E:C	0.06	0.12	0.00	0.96	0.003	0.84	0.11	0.17	0.11	0.12	0.01	0.68

AI Y	Anisotropy Index Young's Modulus
AI ΔUL	Anisotropy Index Energy Loss
BAV	Bicuspid Aortic Valve
S_d	Delamination strength
Y CIRC	Young's Modulus in the circumferential direction
Y LONG	Young's Modulus in the longitudinal direction
E:C	Elastin to Collagen ratio
ΔUL CIRC	Energy Loss in the circumferential direction
ΔUL LONG	Energy Loss in the longitudinal direction
IC	Inner Curvature
MPS	Mucopolysaccharide
OC	Outer Curvature
SM	Smooth Muscle
TAV	Tricuspid Aortic Valve