Cardiovascular Pathology 80 (2026) 107782

Contents lists available at ScienceDirect

Cardiovascular Pathology

journal homepage: www.elsevier.com/locate/cvp

Aortic valve morphology rather than aortic valve function, aortic dilatation, and age interferes with ascending aortic structural and biomechanical properties

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ARTICLE INFO

Keywords:
Ascending aorta
Aortic valve malformation
Aortic wall properties
Aortic valve function
Age
Ascending aortic dilatation

ABSTRACT

Aortic valve (AV) malformation and AV malfunction have been linked to aortic wall degeneration. Studies concomitantly assessing AV morphology, AV function, age, ascending aortic dilatation, and aortic biomechanical properties are lacking. This exploratory study aims to close this gap. Surgical samples of the ascending aorta (n=102) were histologically assessed. Based on echocardiographic studies, the elastic modulus (slope stress-strain curve) was calculated. Patient characteristics were collected from the patient charts. Samples obtained during autopsy (n=10) served as reference for the microscopic analysis. The patient characteristics, structural aortic wall changes, and biomechanical wall properties were statistically explored using comparative analyses and a Spearman correlation matrix. Marked medial degeneration was found significantly earlier in life for unicuspid AV morphology compared to bicuspid and tricuspid AV. Significantly fewer lamellar units and thinner aortic walls were found in surgical samples compared to the reference group regardless of AV morphology, AV function, age, and aortic dilatation. Adventitial structural impairment was associated with stiffer aortic walls. Hints were found that AV morphology (rather than AV function, age, and presence/absence of aortic dilatation) affects structural and functional ascending aortic wall properties. Additionally, the observations suggest more advanced aortic degeneration in association with unicuspid AV, underpin the need for non-surgical control samples in surgical pathological studies, and highlight the importance of the adventitial layer for aortic biomechanics.

1. Introduction

Aneurysms of the ascending aorta (AA, summary of abbreviations **Supplemental File S1 Appendix A**) are associated with relevant morbidity and mortality due to associated complications like aortic rupture or dissection [1]. The known underlying causes of AA aneurysm formation are acquired or congenital/hereditary [2]. A frequent congenital cause is aortic dilatation in association with aortic valve (AV) malformation [2] such as bicuspid (BAV) [3,4] or unicuspid AV (UAV) morphology [5,6]. Additionally, both AV malformations are associated with degeneration of the AA wall [5,7,8], alterations of AV function, such as aortic regurgitation (AR) and/or AV stenosis (AS) [6,9,10], and altered blood flow in the AA [11,12]. This, in turn, interferes with the stress distribution [9,13] and the biomechanical properties of the AA wall [14]. The basis of these AA biomechanical properties is the microstructure of the AA wall with its three layers, i.e., the inner tunica intima, the outer tunica adventitia, and the tunica media as middle layer [14]. Degeneration of these structures, especially of the lamellar units as basic functional units of the aortic wall [15,16], was also found in association with AV malfunction [17]. Although most diseases usually affect more than one layer (e.g., atherosclerotic vascular disease (ASVD) alters all three wall layers [18–20]) most histological studies on AA wall

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https://doi.org/10.1016/j.carpath.2025.107782
Received 26 February 2025; Received in revised form 7 August 2025; Accepted 17 September 2025
Available online 18 September 2025

degeneration in different AV morphologies focus on the tunica media in aneurysmatic aortic tissue and are lacking control groups (e. g., [5]). Comprehensive studies analyzing AA wall degeneration in dilated and non-dilated aortas considering the presence/absence of AV dysfunction, aortic wall's biomechanical properties, and age in individuals with UAV, BAV, and tricuspid AV (TAV) are absent (literature search see **Supplemental File S1 Appendix B**). Thus, the present study intends to characterize concomitantly the AA wall degeneration and AA wall's biomechanical properties associated with AA dilatation, AV malformation and function in different age groups. Therefore, all three aortic wall layers were histologically analyzed in surgically obtained samples of individuals with UAV, BAV, and TAV. The biomechanical aortic wall properties were approximated by the calculation of the elastic modulus (i.e., slope of the stress-strain curve) based on pre-operative echocardiographic measurements. Tissue collected during autopsies with macroscopic normal hearts and AA served as reference for the histomorphological assessment. The aims of the present study were (A) to explore the AA structural changes and (B) to compare the AA biomechanical properties in dilated and non-dilated aortas, different AV morphologies, different age groups, and functional AV abnormalities simultaneously.

2. Material and methods

The workflow of the present study is summarized in Fig. 1.

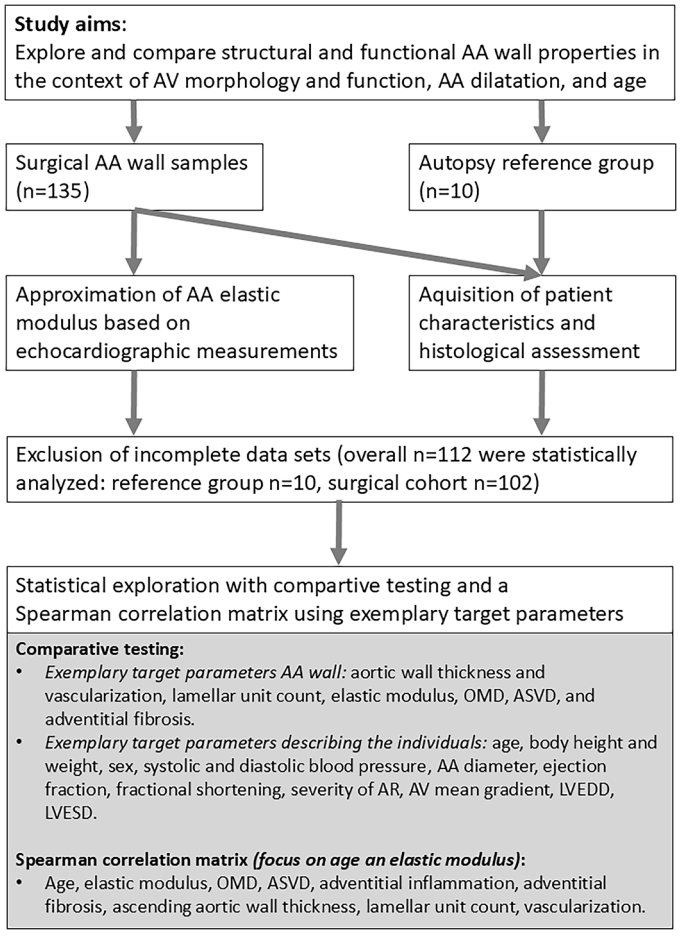

Fig. 1. Flow chart of the present exploratory study. Summary of the work flow applied for the present exploratory study. For further details, please see **Material and methods** section. ***Abbreviations:*** AA – ascending aorta, AR – aortic regurgitation, ASVD – atherosclerotic vascular disease, AV – aortic valve, LVEDD – left ventricular end-diastolic diameter, LVESD – left ventricular end-systolic diameter, OMD – overall medial degeneration.

2.1. Ethics

The present study was approved by the local ethics committee (vote number 47/14). Surgical samples were obtained after written informed consent was given.

2.2. Applied definitions

A TAV was seen if all three commissures were of equal height without any fusion of the cusps [21]. A BAV was present if one commissure was lower and at least a partial fusion of two cusps was observed [22]. A UAV was seen if two commissures were hypoplastic and if two cusps were fused [22]. Acommissural UAVs were not encountered.

Different factors influence what is termed a *normal* AA size [23]. Additionally, several approaches for normalization [24] or the calculation of the upper limit of normal of AA size [25] are available. Thus, for the purpose of this exploratory study the threshold for dilatation was set to 40 mm based on past recommendations for surgical replacement of the aorta in individuals with BAV [26].

2.3. Samples and cohort

2.3.1. Sampling and patient characteristics

Samples from the anterior aspect of the AA 5-10 mm above the sinotubular junction were analyzed. The samples measured approximately 0.4 cm in cranio-caudal direction and between 1 to 1.5 cm in the frontal plane. Overall, 135 AA specimens were obtained during cardiac surgery requiring aortotomy (e.g., AV surgery and/or AA replacement; study cohort). Samples collected at the same height during autopsy served as reference (n=10). In these cases, the autopsy revealed no signs of AV disease and absence of AA dilatation.

For the study cohort, basic characteristics (i.e., age, sex, body height and weight, and AA diameter) were obtained from the patient charts. For the reference cohort only sex, age, body height and weight were available. Echocardiographic data were collected from the pre-operative echocardiography (only available for the study cohort). As basic parameters, the ejection fraction (EF), fractional shortening (FS), left ventricular end-systolic (LVESD) and end-diastolic diameter (LVEDD) were collected. The aforementioned parameters were measured or calculated based on measurements in the M-Mode in the parasternal long axis view. The description of the AV function was based on the mean AV gradient captured by the continuous wave Doppler measurement in the apical five chamber view and the AR classification according to Lancellotti et al. [27]. Based on that, the AV disease was classified as "non-significant", "predominant AS", or "predominant AR".

Study cohort and reference group are statistically described in Table 1.

2.3.2. Biomechanical properties of the AA wall

The biomechanical AA wall properties of the surgical samples were approximated based on pre-operative echocardiographic measurements and the calculation of the elastic modulus [28] (i.e., the slope of the stress-strain curve under loading conditions [29]) according to Cho and Kim [28] (Formula 1 and 2). In these calculations, the blood pressure measured prior to pre-operative echocardiography was used. The minimum and maximum diameter of the tubular AA were captured in the parasternal long axis view. The measurement was performed orthogonally to the AA wall approximately 5-10 mm above the sinotubular junction corresponding to the sampling site. A higher elastic modulus means that less strain results in relatively more aortic wall stress. Thus, a higher elastic modulus indicates a higher stiffness of the aortic wall.

2.3.3. Handling of missing values

Missing values led to the exclusion of the complete case in the study cohort. Finally, 112 cases were included in the statistical analyses (study

Table 1
Reference group and surgical cohort.

	Reference group		Surgical cohort	
	n	%C	n	%C
	10	8.9%	102	91.1%
Continuous variables	*M*	*SD*	*M*	*SD*
Age [years]	39	6	50	18
Body height [cm]	176	12	176	8
Body weight [kg]	77.20	15.27	81.27	13.17
Systolic blood pressure [mmHg]	NA	NA	134	13
Diastolic blood pressure [mmHg]	NA	NA	73	12
AA diameter [mm]	NA	NA	38	11
AA wall thickness [μm]	2801	641.09	1834.51	514.61
EF [%]	NA	NA	60	10
FS [%]	NA	NA	31	7
LVEDD [mm]	NA	NA	55	10
LVSED [mm]	NA	NA	39	9
AV mean gradient [mmHg]	NA	NA	16	18
VV/mm^2	4	1.79	2.91	1.94
Elastic modulus [mmHg]	NA	NA	1201.08	686.20
Lamellar units	69.3	11.6	46.0	14.4
Categorial variables [a]	*n*	*%G*	*N*	*%G*
Sex				
Male	8	80.0%	84	82.4%
Female	2	20.0%	18	17.6%
AR grade				
None	0	0.0%	6	5.9%
Mild	0	0.0%	12	11.8%
Moderate	0	0.0%	15	14.7%
Severe	0	0.0%	69	67.6%
OMD				
Mild	1	10.0%	8	7.8%
Moderate	6	60.0%	61	59.8%
Severe	3	30.0%	33	32.4%
ASVD				
No significant	0	0.0%	2	2.0%
Mild	3	30.0%	28	27.5%
Moderate	7	70.0%	42	41.2%
Severe	0	0.0%	30	29.4%
Adventitial fibrosis				
Present	5	50,0%	56	54,9%
Absent	5	50,0%	46	45,1%

Annotations: a – The percentage per variable and column are reported (%G).
Abbreviations: AA – ascending aorta, AR – aortic regurgitation, ASVD – atherosclerotic vascular disease, EF – ejection fraction, FS – fractional shortening, M – mean, NA – not available due to study design, SD – standard deviation, VV – vasa vasorum.

cohort: n=102, reference group: n=10).

2.3.4. Previous cardiovascular intervention and/or surgery

Of the 102 clinical cases included in the final statistical analysis, six individuals had prior vascular intervention and/or surgery (n=1 surgery 29 years before sampling due to congenital pulmonary valve and AV stenosis; n=1 stenting of the right coronary artery 3 years before sampling; n=1 surgical Tetralogy of Fallot correction approximately 40 years prior to sampling; n=2 surgical correction of isthmic stenosis of the aorta 33 or 17 years, respectively, before sampling; n=1 surgical correction of aortic arch stenosis and isthmic stenosis of the aorta with a patch plastic 42 years prior to sampling).

2.4. Tissue processing

For each sample, the cranial margin was marked with a suture to allow for paraffin embedding in a transversal plane. This allows for sectioning perpendicular to the inner lining with subsequent analysis of the full thickness of the aorta following current recommendations [30].

After placement of the suture, the tissue was immediately placed in 4% phosphate-buffered formalin. Following dewatering, the tissue was paraffin embedded and sectioned at 3 μm thickness. A panel of standard stains allowing for cross-validation of the findings was applied (hematoxylin-eosin-, alcian-blue-, Masson-Goldner-trichrome-, elastica-hematoxylin-eosin-, Movat-pentachrom according to Verhöff-stain, and toluidine blue-histochemistry). Immunohistochemical labelling of cluster of differentiation 31 (CD31, endothelial marker [31]) was performed to support histomorphometric assessment of the vascularization of the AA wall (primary antibody: ab76533, Abcam, Cambridge, UK, dilution 1:100; secondary antibody Peroxidase-conjugated Affini Pure Goat Anti-Rabbit IgG (H+L), Jackson Immuno Research Laboratories, INC., PA, USA, code number 111-035-144, dilution 1:100). Following staining, the tissue was mounted using Roti-Histokitt II (Carl Roth, Karlsruhe, Germany). Further details regarding the staining protocols and reagents are provided in **Supplemental File S1 Appendix C and D**.

With this panel of stains applied, seven sections of the aortic wall were forwarded to the histological assessment meeting the requirements of current recommendations in surgical pathology [30].

2.5. Histological assessment

The microscopic examiners (JMF, PT) were trained, supervised, and spot-checked during the evaluation process by an experienced pathologist (see **Acknowledgments**). The examiners were blinded during the microscopic analysis. The microscopic analyses were performed using an Olympus BX60 microscope, Olympus DP37 camera, and Olympus "cellSens Dimensions 1.15" software (Olympus Corporation, Tokyo, Japan; see **Acknowledgments**).

2.5.1. Histomorphological assessment

The ASVD primarily affecting the tunica intima was graded as recommended by the consensus statement on the surgical pathology of the aorta Part I (inflammatory diseases). No significant ASVD was graded if intimal thickening with some scattered foam cells was observed. Extracellular lipid deposits without associated fibrosis were graded as mild ASVD. Extracellular lipid deposits with associated fibrosis and destruction of less than one third of the adjacent tunica media were rated as moderate ASVD. Severe ASVD was seen if one third or more of the tunica media was affected by a fibrotic lesion with extracellular lipid deposits [18].

For the assessment of the tunica media, the histological grading was adapted from the current recommendations by the consensus statement on the surgical pathology of the aorta Part II (noninflammatory degenerative diseases) [8] (Table 2). Following parameters were assessed to describe the medial degeneration: mucoid extracellular matrix accumulation (MEMA), elastic fiber fragmentation, elastic fiber loss, elastic fiber disorganization, elastic fiber thinning, smooth muscle cell nuclei loss, smooth muscle cell disorganization, laminar medial collapse, and

Table 2
Grading of medial lesions.

Criterion	Consensus statement	Presented study
Severity of a lesion		
Up to 3 lamellar units affected	"mild"	A
Lesion affecting 4 to 10 lamellar units	"moderate"	B
More than 10 lamellar units are affected	"severe"	C
Distribution of a lesion		
Lesion type not present	"Absent"	Absent
Up to 10 % of medial area affected	"Focal"	1
Between 10 to 30 % of medial area are affected	"Multi-focal"	2
Between 30 to 50 % of medial area are affected	"Extensive"	3
Between 50 to 75 % of medial area are affected	"Extensive"	4
More than 75% of the medial area are affected	"Extensive"	5

The grading was adapted from the consensus statement on surgical pathology of the aorta II (noninflammatory degenerative diseases) [8].

medial fibrosis [8] (schematic drawing of the lesions are provided in **Supplemental File S1 Appendix E**). To describe the overall medial degeneration (OMD) the MEMA, elastic fiber fragmentation and loss, smooth muscle cell nuclei loss, and laminar medial collapse were integrated based on the grading scheme put forward by Halushka et al. [8].

Regarding the adventitial layer, the presence and absence of adventitial fibrosis and inflammatory cell infiltrates (Fig. 2) were noted. Adventitial fibrosis was noted if the loose, lamellar and wavy or seashore-like structure of adventitial layer was replaced by a dense and uniform connective tissue indicated by a connective tissue stain (Fig. 3). For the purpose of this exploratory study no further differentiation of any inflammatory infiltrates was carried out.

2.5.2. *Histomorphometric assessment*

First, the section was screened in a 12.5x and 50x magnification to identify areas of different thicknesses visually. In five areas of different

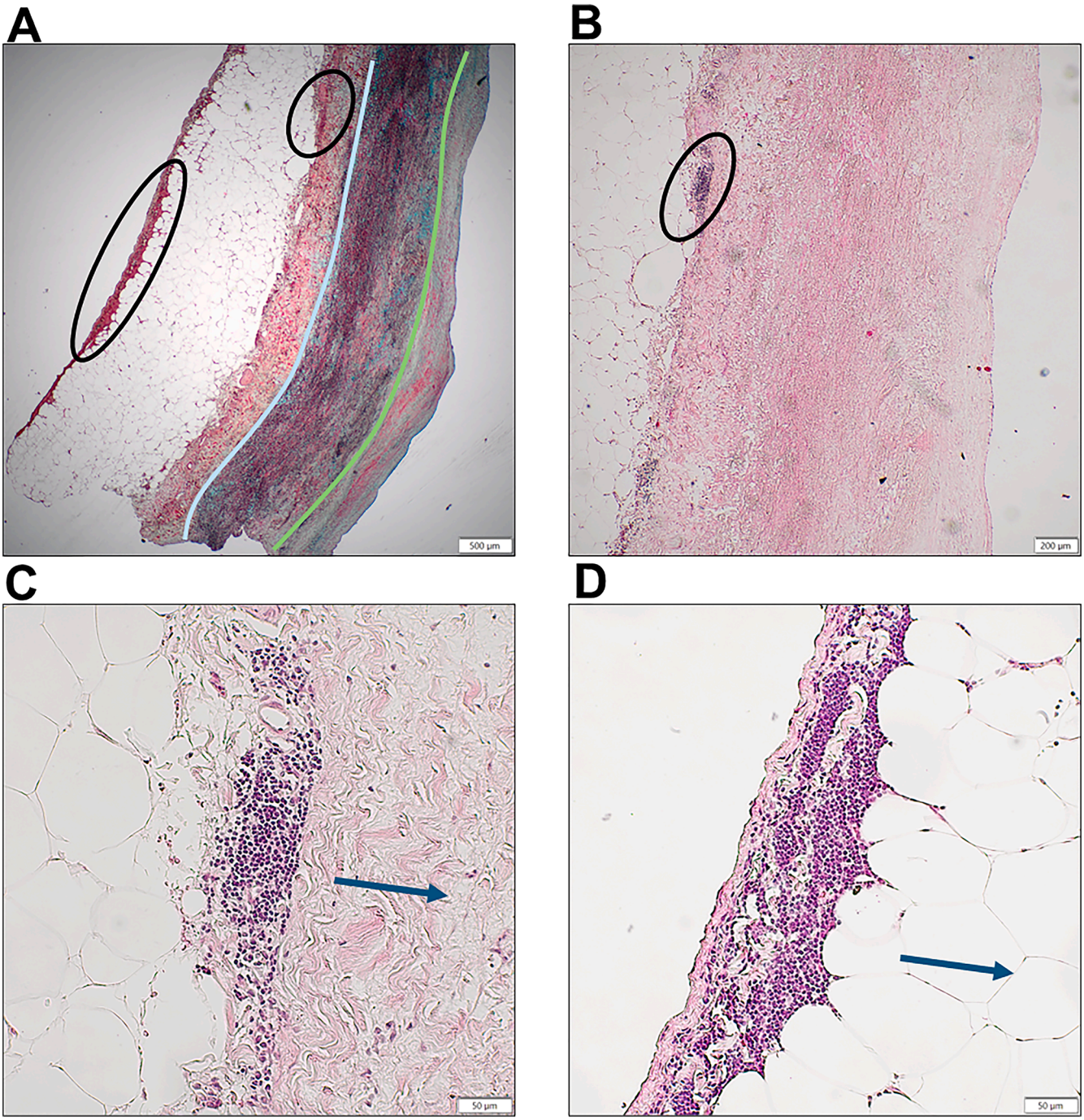

Fig. 2. Adventitial inflammatory lesions. All four panels display the ascending aorta of the same patient: female, 64 years, BMI 39,95 kg/m^2, no re-do surgery, BAV, predominant AR, dilated proximal aorta (basal diameter 42 mm, sinus diameter 46 mm, sinotubular junction diameter 39 mm, ascending aorta diameter 42 mm). (A) shows an overview of the aortic wall in a Movat-pentrachrome stain (20x magnification). (B) to (D) show hematoxylin eosin stained tissue (B 40x magnification; C and D 200x magnification). The black circles mark the inflammatory cell infiltrates in the tunica adventitia displayed in panel (C) and (D). (A) The green line shows the transition zone of the tunica intima and the tunica media. The bright blue line marks the transition zone of the tunica media and tunica adventitia. The arrows in panel (C) and (D) point towards the aortic lumen. Abbreviations: AR – aortic regurgitation; BAV – bicuspid aortic valve; BMI – body mass index.

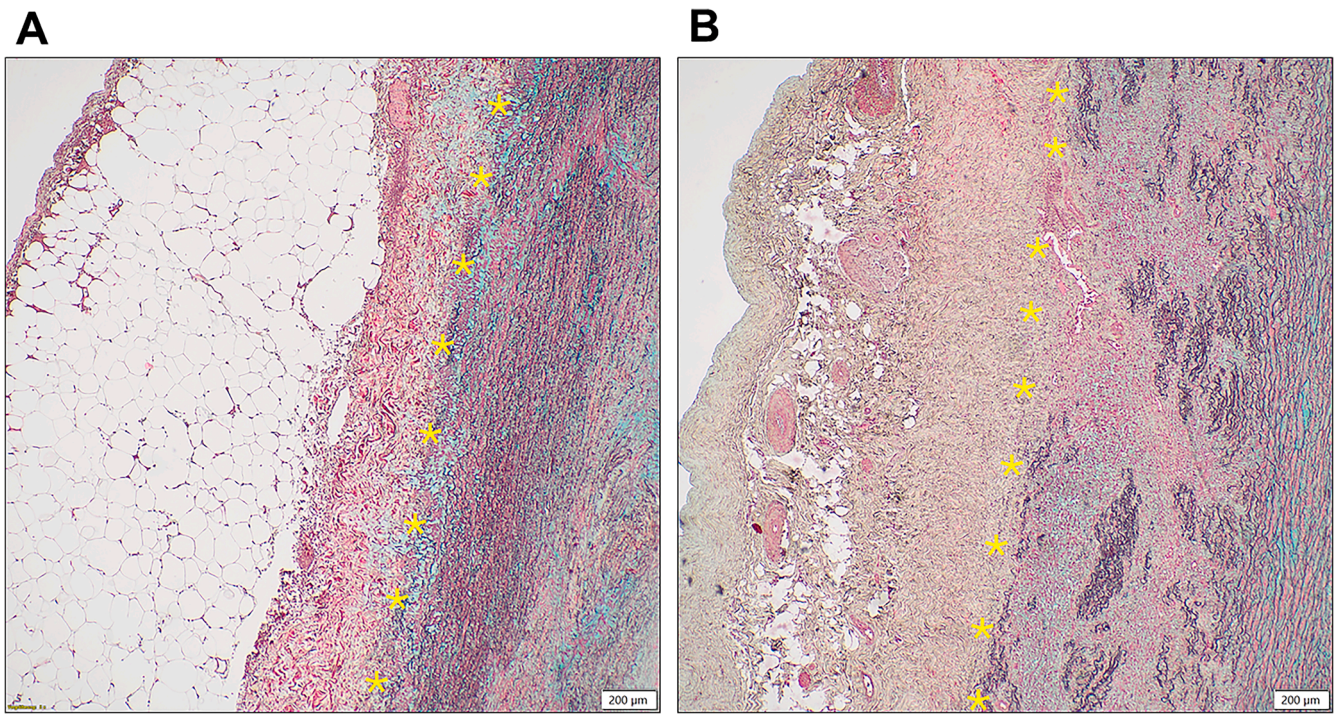

Fig. 3. Adventitial fibrosis. In both panels the yellow asterisks mark the transition zone from tunica adventitia (left from the asterisks) to tunica media (right from the asterisks). A Movat-pentachrome stain is displayed in both photographs (40x magnification). (A) shows a non-fibrotic tunica adventitia with fatty tissue and loose and wavy structured connective tissue adjacent to the tunica media. The connective tissue is mainly red- and yellow stained, a hint for collagen I and collagen III content of the tunica adventitia. (B) shows a fibrotic tunica adventitia. The structure is solid. The compacted tissue mainly exhibits a yellow stain serving as a hint for a increased collagen I content of the tunica adventitia.

thicknesses, the complete aortic wall thickness was measured orthogonally to the tunica intima (50x magnification). The mean aortic wall thickness was calculated and reported. Afterwards, the intact lamellar units, i.e., units with morphologically delineable borders of elastic fibers, were counted in these five orthogonal lines passing the AA wall. The average lamellar unit count was calculated and reported. Third, complete specimen area was measured in 12.5x magnification in five different slides. The average area of the respective sample was calculated and reported. To describe the vascularization of the aortic wall, the Vasa vasorum (VV) count per mm^2 was determined (VV/mm^2). To do so, the CD31-labelled slides were assessed using 200x and 400x magnification. Positive signals that simultaneously resembled a vascular structure were counted across the whole sample. Afterwards the numeric density, i.e. the number of $CD31^+$-VV/mm^2, was calculated with the average sample area.

2.6. Data preparation and statistical analyses

Data framing was done using Microsoft® Excel (Version 2410). IBM SPSS Version 29 was used for descriptive statistics. Further statistical analyses were carried out using R (Version 4.4.3) and RStudio (2024.12.1 Build 563). Normal distribution of continuous variables was assumed. Because of the explorative approach and the associated high number of statistical tests, a significance level of $\alpha=0.01$ was defined, i.e., p-values<0.01 were rated as "statistically significant". Categorical variables were described by absolute and relative frequencies. The relative frequency relates to the respective group it is reported for. Continuous variables are described by mean (M) and standard deviation (SD). The case characteristics, results of the histological and echocardiographic analyses were statistically explored by comparative analyses. Differences in categorical data were comparatively explored with the Kruskal-Wallis test. An analysis of variance (ANOVA) was used to explore differences in metric data. If these screening tests showed a $p<0.01$ comparing more than two groups, post-hoc testing was performed (metric variables: Games-Howell test with Tukey's method for p-adjustment; non-metric variables: Dunn test with Benjamini-Hochberg correction for p-adjustment). To allow for these comparative analyses, the surgical cohort was grouped by the AV morphology (Table 3), presence/absence of AA dilation (Table 4), and AV function (Table 5). Additionally, age groups were defined (i.e., individuals $\leq$29 years, age from 30–59 years, individuals $\geq$60 years; Table 6). These age groups were derived from the findings of a large epidemiological study, that assigned a relative risk of 1 for thoracic aortic aneurysm and dissection to individuals $\leq$29 years. In this epidemiological analysis, in different age groups, i.e., 30-39 years, 40-49 years and 50-59 years, an increasing relative risk for thoracic aortic aneurysm and dissection was observed. A sudden rise of the relative risk to 65.2 was observed in individuals between 60-69 years [32]. Thus, individuals $\leq$29 years have a low, individuals 30-59 years a moderate, and individuals $\geq$60 years a high relative risk for thoracic aortic aneurysms and dissections.

To complement the comparative analysis with particular focus on the influence of age on the findings, especially the elastic modulus, a Spearman correlation matrix was computed with age as a continuous variable (rather than participants being categorized into age groups). Regarding the correlations, Spearman correlation coefficient ρ and the p-values are reported.

The parameters to describe medial degeneration claimed by the international consensus statements on the surgical pathology of the aorta result in around 20 statistical variables. To avoid excess α-error inflation, only a panel of exemplary target parameters was included in the statistical analyses. To describe the individuals, age, body height and weight, sex, systolic and diastolic blood pressure, AA diameter, EF, FS, LVEDD, LVESD, AV mean gradient and AR severity were included. To summarize the aortic wall properties, OMD, ASVD, presence/absence of adventitial fibrosis, AA wall thickness, lamellar unit count, VV/mm^2 and elastic modulus were statistically analyzed.

Table 3
Comparisons by AV morphology.

	Ref		UAV		BAV		TAV		p
	n	%C	n	%C	n	%C	n	%C	
	10	8.9%	22	19.6%	46	41.1%	34	30.4%	
Continuous variables	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	
Age [years]	39	6	37	13	51	21	57	12	<0.001[a]
Body height [cm]	176	12	177	7	175	9	175	7	0.894
Body weight [kg]	77.20	15.27	81.55	16.03	81.85	13.22	80.29	11.29	0.776
Systolic blood pressure [mmHg]	NA	NA	129	12	133	13	138	13	0.036
Diastolic blood pressure [mmHg]	NA	NA	72	10	73	12	73	12	0.983
AA diameter [mm]	NA	NA	38	13	38	9	39	12	0.897
AA wall thickness [µm]	2801.00	641.09	2051.46	560.55	1634.74	369.13	1964.39	566.00	<0.001[b]
Ejection fraction [%]	NA	NA	59	11	59	12	62	7	0.488
Fractional Shortening [%]	NA	NA	30	8	31	7	30	6	0.885
LVEDD [mm]	NA	NA	54	12	57	9	55	8	0.59
LVSED [mm]	NA	NA	38	11	41	9	38	7	0.423
AV mean gradient [mmHg]	NA	NA	28	17	17	20	6	4	<0.001[c]
VV/mm^2	4.00	1.79	3.06	2.38	3.80	1.68	1.61	1.13	<0.001[d]
Elastic modulus [mmHg]	NA	NA	786.51	425.56	1166.52	541.46	1516.08	841.22	<0.001[e]
Lamellar units	69.3	11.6	46.9	12.3	46.6	7.7	44.4	21.3	<0.001[f]
Categorial variables [h]	*n*	*%G*	*n*	*%G*	*n*	*%G*	*n*	*%G*	
Sex									
Male	8	80.0%	18	81.8%	40	87.0%	26	76.5%	0.684
Female	2	20.0%	4	18.2%	6	13.0%	8	23.5%	
AR grade									0.622
None	0	0.0%	0	0.0%	4	8.7%	2	5.9%	
Mild	0	0.0%	3	13.6%	7	15.2%	2	5.9%	
Moderate	0	0.0%	6	27.3%	4	8.7%	5	14.7%	
Severe	0	0.0%	13	59.1%	31	67.4%	25	73.5%	
OMD									0.571
Mild	1	10.0%	0	0.0%	7	15.2%	1	2.9%	
Moderate	6	60.0%	13	59.1%	23	50.0%	25	73.5%	
Severe	3	30.0%	9	40.9%	16	34.8%	8	23.5%	
ASVD									<0.001[g]
No significant	0	0.0%	1	4.5%	0	0.0%	1	2.9%	
Mild	3	30.0%	14	63.6%	7	15.2%	7	20.6%	
Moderate	7	70.0%	6	27.3%	24	52.2%	12	35.3%	
Severe	0	0.0%	1	4.5%	15	32.6%	14	41.2%	
Adventitial fibrosis									0.215
Present	5	50.0%	16	72.7%	21	45.7%	19	55.9%	
Absent	5	50.0%	6	27.3%	25	54.3%	15	44.1%	

Annotations: a – Post-hoc testing Age: Ref vs. TAV p<0.001; UAV vs. BAV p=0.005; UAV vs. TAV p<0.001. b – Post-hoc testing Aortic wall thickness: Ref. Vs. BAV p=0.001. c – Post-hoc testing Mean AV gradient: UAV vs. TAV p<0.001; BAV vs. TAV p=0.004. d – Post-hoc testing VV/mm^2: Ref vs. TAV p=0.009; BAV vs. TAV p<0.001. e – Post-hoc testing Elastic modulus: UAV vs. TAV p<0.001. f – Post-hoc testing Lamellar unit count: Ref vs. UAV p<0.001; Ref vs. BAV p<0.001; Ref vs. TAV p<0.001. g – Post-hoc testing ASVD severity: UAV vs. BAV p<0.001; UAV vs. TAV p<0.001. h – The percentage per variable and column are reported (%G). ***Abbreviations:*** AA – ascending aorta, AR – aortic regurgitation, ASVD – atherosclerotic vascular disease, BAV – bicuspid aortic valve, EF – ejection fraction, FS – fractional shortening, M – mean, NA – not available due to study design, SD – standard deviation, TAV – tricuspid aortic valve, UAV – unicuspid aortic valve, VV – vasa vasorum.

3. Results

The results of the exploratory statistical analyses, i.e., comparative testing (Tables 3–6) and Spearman correlation matrix (Fig. 4), are outlined in the following. The findings for each individual histomorphological parameter underlying the chosen exemplary target parameters are detailed in **Supplemental File 1 Appendix F**.

3.1. Aortic wall properties surgical cohort

Overall, moderate OMD was the most frequent grade of medial alteration (61 individuals, 59.8%). Severe aortic wall degeneration was found in 33 cases (32.4%). An example of severe elastic fiber disorganization is shown in Fig. 5. Predominantly, individuals were affected by mild ASVD (n=28, 27.5%). Adventitial inflammatory lesions were present in 11 individuals (10.8%; Fig. 2). Adventitial fibrosis was found in 46 cases (45.1%, Fig. 3). Across the surgical cohort, a mean AA wall thickness of 1834.5 µm (SD=514.6) with a mean lamellar unit count of 46 (SD=14.4) was observed. In terms of vascularization, a mean of 2.9 vessels per mm^2 (SD=1.9) was found. Such aortic wall structure translated to a mean elastic modulus of 1201.1 mmHg (SD=686.2).

3.2. Comparisons by AV morphology

In the following, the main findings are summarized, all comparisons are displayed in Table 3.

Individuals with TAV (M=57 years / SD=12) were statistically significantly older compared to the reference group (M=39 years / SD=6, p<0.001) and individuals with UAV (M=37 years / SD=13, p<0.001). Individuals with BAV (M=51 years / SD=21) were statistically significantly older than those with UAV (p=0.005). In terms of sex, no difference between the groups was observed. The AA wall thickness was significantly thinner in BAV individuals compared to the reference group (p=0.001). No other statistically significant differences between the groups were observed regarding the aortic wall thickness. The AA wall of individuals with a TAV showed the least VV/mm^2 (M=1.61 / SD=1.13) resulting in statistically significant differences compared to the reference group (M=4 VV/mm^2 / SD=1.79, p=0.009) and individuals with BAV (M=3.8 VV/mm^2 / SD=1.68, p=0.001). With regard to the aortic wall vascularization no other statistically significant differences were observed. Individuals with TAV showed the stiffest AA indicated by the highest elastic modulus (TAV: M=1516.05 mmHg / SD=841.22) with a statistically significant difference compared to individuals with UAV (M=786.51 mmHg / SD=425.56, p<0.001). No

Table 4
Comparisons by presence of absence of aortic dilatation.

	Ref		Non-dilated AA		Dilated AA		p
	n	%C	n	%C	n	%C	
	10	8.9%	52	46.4%	50	44.6%	
Continuous variables	*M*	*SD*	*M*	*SD*	*M*	*SD*	
Age [years]	39	6	48	20	53	16	0.053
Body height [cm]	176	12	175	8	176	9	0.839
Body weight [kg]	77.20	15.27	78.23	11.91	84.42	13.78	0.041
Systolic blood pressure [mmHg]	NA	NA	134	14	134	11	0.789
Diastolic blood pressure [mmHg]	NA	NA	70	10	75	13	0.047
AA diameter [mm]	NA	NA	31	6	45	10	<0.001[a]
AA wall thickness [μm]	2801.00	641.09	1769.87	443.21	1901.73	576.50	<0.001[b]
Ejection fraction [%]	NA	NA	61	9	60	11	0.67
Fractional Shortening [%]	NA	NA	31	7	30	7	0.694
LVEDD [mm]	NA	NA	55	10	56	9	0.414
LVSED [mm]	NA	NA	39	9	40	9	0.453
AV mean gradient [mmHg]	NA	NA	18	19	13	16	0.145
VV/mm^2	4.00	1.79	2.72	1.76	3.11	2.12	0.143
Elastic modulus [mmHg]	NA	NA	1160.86	616.75	1242.90	755.76	0.549
Lamellar units	69.3	11.6	49.6	11.4	42.2	16.2	<0.001[c]
Categorial variables [d]	*n*	*%G*	*n*	*%G*	*n*	*%G*	
Sex							0.633
Male	8	80.0%	41	78.8%	43	86.0%	
Female	2	20.0%	11	21.2%	7	14.0%	
AR grade							0.771
None	0	0.0%	5	9.6%	1	2.0%	
Mild	0	0.0%	5	9.6%	7	14.0%	
Moderate	0	0.0%	7	13.5%	8	16.0%	
Severe	0	0.0%	35	67.3%	34	68.0%	
OMD							0.842
Mild	1	10.0%	5	9.6%	3	6.0%	
Moderate	6	60.0%	31	59.6%	30	60.0%	
Severe	3	30.0%	16	30.8%	17	34.0%	
ASVD							0.061
No significant	0	0.0%	1	1.9%	1	2.0%	
Mild	3	30.0%	18	34.6%	10	20.0%	
Moderate	7	70.0%	22	42.3%	20	40.0%	
Severe	0	0.0%	11	21.2%	19	38.0%	
Adventitial fibrosis							0.093
Present	5	50.0%	34	65.4%	22	44.0%	
Absent	5	50.0%	18	34.6%	28	56.0%	

Annotations: a – No post-hoc testing performed as only two groups were compared. b – Post-hoc testing Aortic wall thickness: Ref vs. Non-dilated AA p=0.001; Ref vs. Dilated AA p=0.004. c – Post-hoc testing Lamellar unit count: Ref vs. Non-dilated AA p<0.001; Ref vs. Dilated AA p<0.001. d – The percentage per variable and column are reported (%G). ***Abbreviations:*** AA – ascending aorta, AR – aortic regurgitation, ASVD – atherosclerotic vascular disease, EF – ejection fraction, FS – fractional shortening, M – mean, NA – not available due to study design, SD – standard deviation, VV – vasa vasorum.

other significant differences were encountered with regard to the elastic modulus. Regarding the lamellar unit count, each surgical AV morphology group, i.e., UAV (M=46.9 / SD=12.3, p<0.001), BAV (M=46.6 / SD=7.7, p<0.001), and TAV (M=44.4 / SD=21.3, p<0.001), showed statistically significant fewer lamellar units compared to the reference group (M=69.3 / SD=11.6). Individuals with UAV (63.6% no significant ASVD) showed statistically significant less ASVD alteration of the AA compared to individuals with BAV (52.2% moderate ASVD, p<0.001) and those with TAV (41.2% with severe ASVD, p=0<0.001). With regard to OMD and adventitial fibrosis, the screening tests did not indicate significant differences between the groups.

3.3. Comparisons by AA dilatation status

All comparisons by presence or absence of AA dilatation are displayed in Table 4. No differences in terms of sex and age were encountered between the groups. Overall, only for the average count of lamellar units, statistically significant differences were found. With regard to the AA wall thickness, both surgical samples from dilated (M=1769.87 μm / SD=443.21, p<0.001) and non-dilated AAs (M=1901.73 μm / SD=576.5, p=0.004) were thinner compared to the reference group (M=2801 μm / SD=641.09). The AA wall thickness was comparable between samples from dilated and non-dilated AAs (post-hoc testing p≥0.01). Correspondingly, the lamellar unit count was significantly lower in surgical AA samples from non-dilated (M=49.6 / SD=11.4, p<0.001) and dilated aortas (M=42.2 / SD=16.2; p<0.001) compared to the reference group (M=69.3 / SD=11.6). There was no significant difference regarding the lamellar unit count between surgical samples from non-dilated (M=49.6 / SD=11.4) and dilated aortas (post-hoc testing p≥0.01). The screening tests did not indicate significant differences between the groups regarding OMD, ASVD, and adventitial fibrosis.

3.4. Comparisons by AV disease

All comparisons by the AV function are shown in Table 5. In the following, the significant differences are summarized.

Individuals affected by predominant AR presented with significantly larger left ventricular cavities (LVEDD M=58 mm; LVESD M=41 mm) compared to those with predominant AS (LVEDD M=45 mm, p<0.001; LVSED=M 32 mm, p=0.006). At the same time, ejection fraction and fractional shortening of individuals with predominant AS (ejection fraction M=63%, fractional shortening M=33%) and predominant AR (ejection fraction M=60%, p=0.547; fractional shortening M=30%, p=0.353) were comparable.

In terms of aortic wall thickness, both predominant AS (M=1684.16 μm / SD=569.22, p=0.002) and predominant AR (M=1808.18 μm / SD=465.69, p=0.003) were associated with thinner AA walls compared

Table 5
Comparisons by AV malfunction.

	Ref		No significant		Predominant AS		Predominant AR		p
	n	%C	n	%C	n	%C	n	%C	
	10	8.9%	8	7.1%	16	14.3%	78	69.6%	
Conitnous variables	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	
Age [years]	39	6	58	10	55	21	48	18	0.065
Body height [cm]	176	12	177	8	174	7	176	9	0.801
Body weight [kg]	77.20	15.27	87.63	10.62	75.38	13.05	81.82	13.13	0.115
Systolic blood pressure [mmHg]	NA	NA	131	9	132	10	135	14	0.567
Diastolic blood pressure [mmHg]	NA	NA	82	10	73	11	72	12	0.059
AA diameter [mm]	NA	NA	47	10	34	10	38	11	0.019
AA wall thickness [µm]	2801.00	641.09	2391.85	571.56	1684.16	569.22	1808.18	465.69	<0.001[a]
Ejection fraction [%]	NA	NA	61	8	63	14	60	9	0.547
Fractional Shortening [%]	NA	NA	30	5	33	10	30	6	0.353
LVEDD [mm]	NA	NA	53	8	45	9	58	9	<0.001[b]
LVSED [mm]	NA	NA	37	7	32	10	41	8	<0.001[c]
AV mean gradient [mmHg]	NA	NA	5	3	51	13	9	8	<0.001[d]
VV/mm^2	4.00	1.79	1.97	0.97	2.68	1.74	3.05	2.04	0.146
Elastic modulus [mmHg]	NA	NA	1340.94	675.15	1227.10	552.17	1181.39	717.11	0.814
Lamellar units	69.3	11.6	46.9	21.5	45.1	9.2	46.1	14.6	<0.001[e]
Categorial variables	*n*	*%G*	*n*	*%G*	*n*	*%G*	*n*	*%G*	
Sex									0.511
Male	8	80.0%	8	100.0%	12	75.0%	64	82.1%	
Female	2	20.0%	0	0.0%	4	25.0%	14	17.9%	
AR grade									<0.001[f]
None	0	0.0%	1	12.5%	5	31.3%	0	0.0%	
Mild	0	0.0%	4	50.0%	6	37.5%	2	2.6%	
Moderate	0	0.0%	3	37.5%	4	25.0%	8	10.3%	
Severe	0	0.0%	0	0.0%	1	6.3%	68	87.2%	
OMD									0.789
Mild	1	10.0%	0	0.0%	1	6.3%	7	9.0%	
Moderate	6	60.0%	6	75.0%	8	50.0%	47	60.3%	
Severe	3	30.0%	2	25.0%	7	43.8%	24	30.8%	
ASVD									0.726
No significant	0	0.0%	0	0.0%	0	0.0%	2	2.6%	
Mild	3	30.0%	2	25.0%	5	31.3%	21	26.9%	
Moderate	7	70.0%	4	50.0%	6	37.5%	32	41.0%	
Severe	0	0.0%	2	25.0%	5	31.3%	23	29.5%	
Adventitial fibrosis									0.957
Present	5	50.0%	5	62.5%	9	56.3%	42	53.8%	
Absent	5	50.0%	3	37.5%	7	43.8%	36	46.2%	

Annotations: a – Post-hoc testing Aortic wall thickness: Ref vs. Predominant AS p=0.002; Ref vs. Predominant AR p=0.003. b – Post-hoc testing LVEDD: Predominant AS vs. Predominant AR p<0.001. c – Post-hoc testing LVESD: Predomonant AS vs. Predominant AR p=0.006. d – Post-hoc testing Mean AV gradient: No significant AV disease vs. Predominant AS p<0.001; No significant AR vs. Predominant AR p<0.001; Predominant AS vs. Predominant AR p<0.001. e – Post-hoc testing Lamellar units: Ref vs. Predominant AS p<0.001; Ref vs. Predominant AR p<0.001. f – Post-hoc testing AR Grade: Predominant AR vs. Predominant AS p<0.001; No significant AV disease vs. Predominant AR p<0.001. g – The percentage per variable and column are reported (%G). ***Abbreviations:*** AA – ascending aorta, AR – aortic regurgitation, AS – aortic stenosis. ASVD – atherosclerotic vascular disease, EF – ejection fraction, FS – fractional shortening, M – mean, NA – not available due to study design, SD – standard deviation, VV – vasa vasorum.

to the reference group (M=2801 / SD=641.09). No significant difference between both types of AV disease was found (post-hoc testing p≥0.01). These findings are mirrored by the results regarding the lamellar unit count. The AA wall of individuals with predominant AR (M=46.1 / SD=14.6, p<0.001) and individuals with predominant AS (M=45.1 / SD9.1, p<0.001) exhibited statistically significant fewer lamellar units compared to the reference group. Both types of AV disease were comparable regarding the lamellar unit count. With regard to OMD, ASVD, and adventitial fibrosis, the screening tests did not indicate significant differences between the groups.

3.5. Comparisons by age group

All comparisons by age group are displayed in Table 6. In the following, the main findings are summarized.

Older individuals showed larger AA diameter with statistically significant differences comparing individuals ≤29 years (M=30 mm / SD=9) to individuals of 30-59 years (M=39 mm / SD=12, p=0.006) and to individuals ≥60 years (M=40 mm / SD=9, p<0.001). Other significant differences regarding the AA diameter were not observed during post-hoc testing. In terms of AA wall thickness, each age group of the surgical samples, i.e., ≤29 years (M=1816.94 µm / SD=420.67, p=0.004), 30-59 years (M=1917.75 µm / SD=574.79, p=0.008), and ≥60 years (M=1766.01 µm / SD=490.94, p=0.002) showed a statistically significant thinner aortic wall compared to the reference group (M=2801 µm / SD=641.09). These findings regarding the AA wall thickness are mirrored by the findings regarding the lamellar unit count. The reference group showed statistically significant more lamellar units (M=69.3 / SD=11.6) compared to individuals ≤29 years (M=44.3 / SD=6.4, p<0.001), individuals 30-59 years (M=45.9 / SD=17.8, p<0.001) and individuals ≥60 years (M=46.7 / SD=13.5, p<0.001). With regard to OMD, ASVD, and adventitial fibrosis, the screening tests did not indicate significant differences between the groups.

3.6. Spearman correlation matrix

The Spearman correlation matrix assessing the associations of the age for the whole cohort, i.e., surgical and reference samples, is displayed in Fig. 4**A** and **B**. This analysis showed significant positive correlations between age and severity of ASVD (p=0.008; ρ=0.246), severity of ASVD and the presence of adventitial inflammatory lesions (p<0.001; ρ=0.447), and AA wall thickness and the lamellar unit count (p≤0.001; ρ=0.405). Statistically significant negative correlations were observed between AA wall thickness and the presence of adventitial

Table 6
Comparisons by age group.

	Ref		</= 29 years		30-59 years		>/= 60 years		p
	n	%C	n	%C	n	%C	n	%C	
	10	8.9%	18	16.1%	50	35.7%	44	39.3	
Continous variables	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	
Age [years]	39	6	23	3	43	8	68	6	<0.001[a]
Body height [cm]	176	12	179	8	177	7	173	8	0.017
Body weight [kg]	77.20	15.27	78.77	13.12	80.55	11.90	82.94	14.31	0.522
Systolic blood pressure [mmHg]	NA	NA	133	15	133	14	135	11	0.761
Diastolic blood pressure [mmHg]	NA	NA	69	10	75	12	72	12	0.186
AA diameter [mm]	NA	NA	30	9	39	12	40	9	0.001[b]
AA wall thickness [µm]	2801.00	641.09	1816.94	420.67	1917.75	574.79	1766.01	490.94	<0.001[c]
Ejection fraction [%]	NA	NA	61	8	60	9	60	11	0.937
Fractional Shortening [%]	NA	NA	32	7	30	6	30	8	0.704
LVEDD [mm]	NA	NA	52	8	57	10	56	9	0.233
LVSED [mm]	NA	NA	37	8	40	9	40	9	0.568
AV mean gradient [mmHg]	NA	NA	20	20	15	15	15	19	0.612
VV/mm^2	4.00	1.79	3.43	1.65	3.00	2.13	2.61	1.86	0.149
Elastic modulus [mmHg]	NA	NA	1028.72	633.50	1207.68	750.15	1265.58	648.26	0.47
Lamellar units	69.3	11.6	44.3	6.4	45.9	17.8	46.7	13.5	<0.001[d]
Categorial variables [e]									
Sex									0.533
Male	8	80.0%	17	94.4%	32	80.0%	35	79.5%	
Female	2	20.0%	1	5.6%	8	20.0%	9	20.5%	
AR grade									0.772
None	0	0.0%	2	11.1%	2	5.0%	2	4.5%	
Mild	0	0.0%	1	5.6%	5	12.5%	6	13.6%	
Moderate	0	0.0%	3	16.7%	4	10.0%	8	18.2%	
Severe	0	0.0%	12	66.7%	29	72.5%	28	63.6%	
OMD									0.783
Mild	1	10.0%	2	11.1%	1	2.5%	5	11.4%	
Moderate	6	60.0%	8	44.4%	27	67.5%	26	59.1%	
Severe	3	30.0%	8	44.4%	12	30.0%	13	29.5%	
ASVD									0.149
No significant	0	0.0%	1	5.6%	0	0.0%	1	2.3%	
Mild	3	30.0%	6	33.3%	13	32.5%	9	20.5%	
Moderate	7	70.0%	9	50.0%	15	37.5%	18	40.9%	
Severe	0	0.0%	2	11.1%	12	30.0%	16	36.4%	
Adventitial fibrosis									0.381
Present	5	50.0%	12	66.7%	24	60.0%	20	45.5%	
Absent	5	50.0%	6	33.3%	16	40.0%	24	54.5%	

Annotations: a – Post-hoc testing Age: only ref vs. 30-60 years yielded a p=0.439; all other comparisons showed p<0.001, i.e., Ref vs. (</=29), Ref vs. (>/= 60), (</= 29) vs. (30 - 59), (</= 29) vs. (>/= 60), (30 - 59) vs. (>/= 60). b – Post-hoc testing AA diameter: (</= 29) vs (30 - 59) p=0.006; (</=29) vs. (>/= 60) p≤0.001. c – Post-hoc testing AA wall thickness: Ref vs. (</= 29) p=0.004; Ref vs. (30 - 59) p=0.008; Ref vs. (>/= 60) p=0.002. d – Post-hoc testing Lamellar unit count: Ref vs. (</= 29) p<0.001; Ref vs. (30 - 59) p<0.001; Ref vs. (>/= 60) p<0.001. e – The percentage per variable and column are reported (%G). ***Abbreviations:*** AA – ascending aorta, AR – aortic regurgitation, ASVD – atherosclerotic vascular disease, EF – ejection fraction, FS – fractional shortening, M – mean, NA – not available due to study design, SD – standard deviation, VV – vasa vasorum.

fibrosis (p≤0.001; ρ=-0.337), lamellar unit count and the presence of adventitial fibrosis (p≤0.001; ρ=-0.317), presence of adventitial inflammatory lesions and lamellar unit count (p=0.007; ρ=-0.253), and the vascularization and the presence of adventitial inflammatory lesions (p=0.001; ρ=-0.299).

The correlation analysis with regard to the elastic modulus for the surgical cohort is visualized in Fig. 4**C** and **D**. Regarding the elastic modulus, only one statistically significant correlation with one of the assessed parameters was observed, i.e., a positive correlation between the presence of adventitial inflammatory lesions and the elastic modulus (p<0.001; ρ=0.333). Further positive correlations within the study cohort were found between the presence of adventitial inflammatory lesions and the ASVD severity (p≤0.001; ρ=0.452), the presence of adventitial fibrosis and the presence of adventitial inflammatory lesions (p=0.009; ρ=0.257), and the AA wall thickness and lamellar unit count (p=0.003; ρ=0.289). Statistically significant negative correlations were found between aortic wall thickness and the presence of adventitial fibrosis (p≤0.001; ρ=-0.375), the number of lamellar units and the presence of adventitial fibrosis (p<0.001; ρ=-0.365), and the VV/mm^2 and the presence of adventitial inflammatory lesions (p=0.003; ρ=-0.294).

4. Discussion

The present study explored the characteristics of AA wall degeneration in association with congenital AV malformation, AV disease, age and the presence or absence of AA dilatation considering all three aortic wall layers and its vascularization (Fig. 1). For this purpose, samples of the AA were obtained during surgery and underwent histological assessment. Additionally, the elastic modulus, i.e., the slope of the stress-strain curve under loading conditions [29], as an estimate of the AA biomechanical wall properties was calculated based on pre-operative echocardiographic measurements. Specimens sampled during autopsies served as a reference group for the histological assessment. The aims were (A) to explore the AA structural changes and (B) to compare the AA biomechanical properties in different AV morphologies (Table 3), presence/absence of AA dilatation (Table 4), different AV malfunctions (Table 5), and age groups (Table 6). After histomorphological assessment, exemplary target parameters to describe the structural and functional AA wall properties (i.e., ASVD, OMD, adventitial fibrosis, VV/mm^2, aortic wall thickness, and lamellar unit count, elastic modulus) were statistically explored.

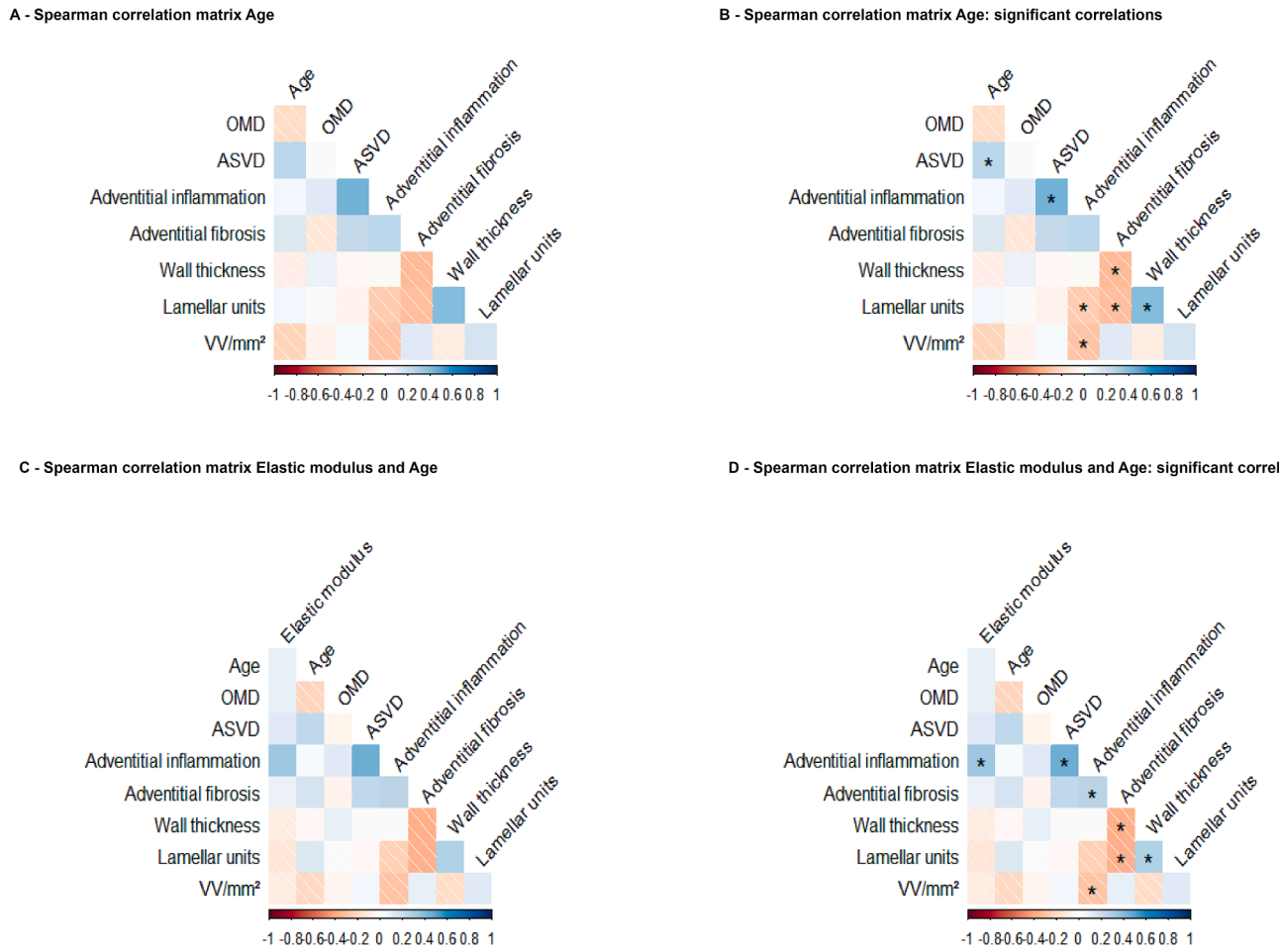

Fig. 4. Spearman correlation matrix. Each panel (A-D) shows a Spearman correlation matrix. Blue color indicates a positive correlation (i.e., positive Spearman correlation coefficient ρ). Red color indicates a negative correlation. The color intensity codes the strength of the correlation (scale at the bottom). In the panels on the right sight (B, D) statistically significant correlations are highlighted with the asterisks. The upper panels (A, B) show the Spearman correlation matrix with particular regard to the impact of age. Only variables available in all cases including the reference group were included. The lower panels (C, D) show the Spearman correlation matrix with particular regard to the elastic modulus and age. As the method chosen does not allow missing values, the reference group is not included in this sub-analysis. ***Abbreviations:*** ASVD – atherosclerotic vascular disease, OMD – overall medial degeneration.

4.1. Comparing reference group and surgical cohort

Across all comparative analyses, the reference group was found to have thicker aortic walls (M=2801 μm) and higher counts of lamellar units (M=69) compared to the surgical samples (Tables 3–6; in all screening tests $p<0.001$).

On the one hand, this underlines the need for a non-surgical control in studies on AA wall structure using surgical samples. However, many studies on AA degeneration lack non-surgical controls, like de Sa et al. [33], Pisano et al. [34] or Bechtel et al. [35]. Established non-surgical controls are AA samples obtained from whole heart specimens from heart transplant (e.g., Heng et al. [36]) or samples obtained during autopsy (for example, Balistreri et al. [37] or Roberts et al. [38]) – like in the present study.

The tunica media and lamellar units are the structural basis of the biomechanical properties of the aortic wall [15,16]. Thus, on the other hand, these striking differences between the reference group and surgical cohort in terms of aortic wall thickness and lamellar unit count might be a hint for healthy viscoelastic properties of the reference samples. However, the design of the present study with echocardiographic approximation of the biomechanical wall properties did not allow to determine the elastic modulus of the reference group. Thus, further studies are needed including biomechanical analyses of the reference group to validate this assumption.

4.2. Biomechanical and structural AA wall properties

Regarding the elastic modulus, only the comparisons of the different AV morphologies observed statistically significant differences (screening test $p<0.001$). The post-hoc testing revealed a statistically significant higher elastic modulus (i.e., stiffer aortic wall) in individuals with TAV (M=1516 mmHg) compared to individuals with UAV (M=786.51 mmHg, $p<0.001$). No statistically significant differences were observed between individuals with BAV (M=1166.52 mmHg) and individuals with TAV ($p=0.016$) or UAV ($p=0.014$). The different comparisons regarding AV malfunction, presence/absence of AA dilatation, and different age groups did not yield any statistically significant differences (all screening tests $p>0.01$; details see Tables 3–6). A conceivable explanation for this finding might be the significantly younger age of individuals with UAV (M=37 years) compared to those with BAV (M=51 years, $p=0.005$) or TAV morphology (M=57 years, $p<0.001$) with aging being associated with aortic stiffening [39,40]. However, the comparison of the different age groups did not yield statistically significant differences regarding the elastic modulus between individuals ≤29 years (M=1028,27 mmHg), 30-59 years (M=1258.13 mmHg), and ≥60 years (M=1197.77 mmHg, screening test $p=0.471$). Accordingly, no statistically significant correlation between age and elastic modulus was observed in the analyzed surgical cohort (Fig. 4). This might be an indicator for a stronger impact of the AV morphology

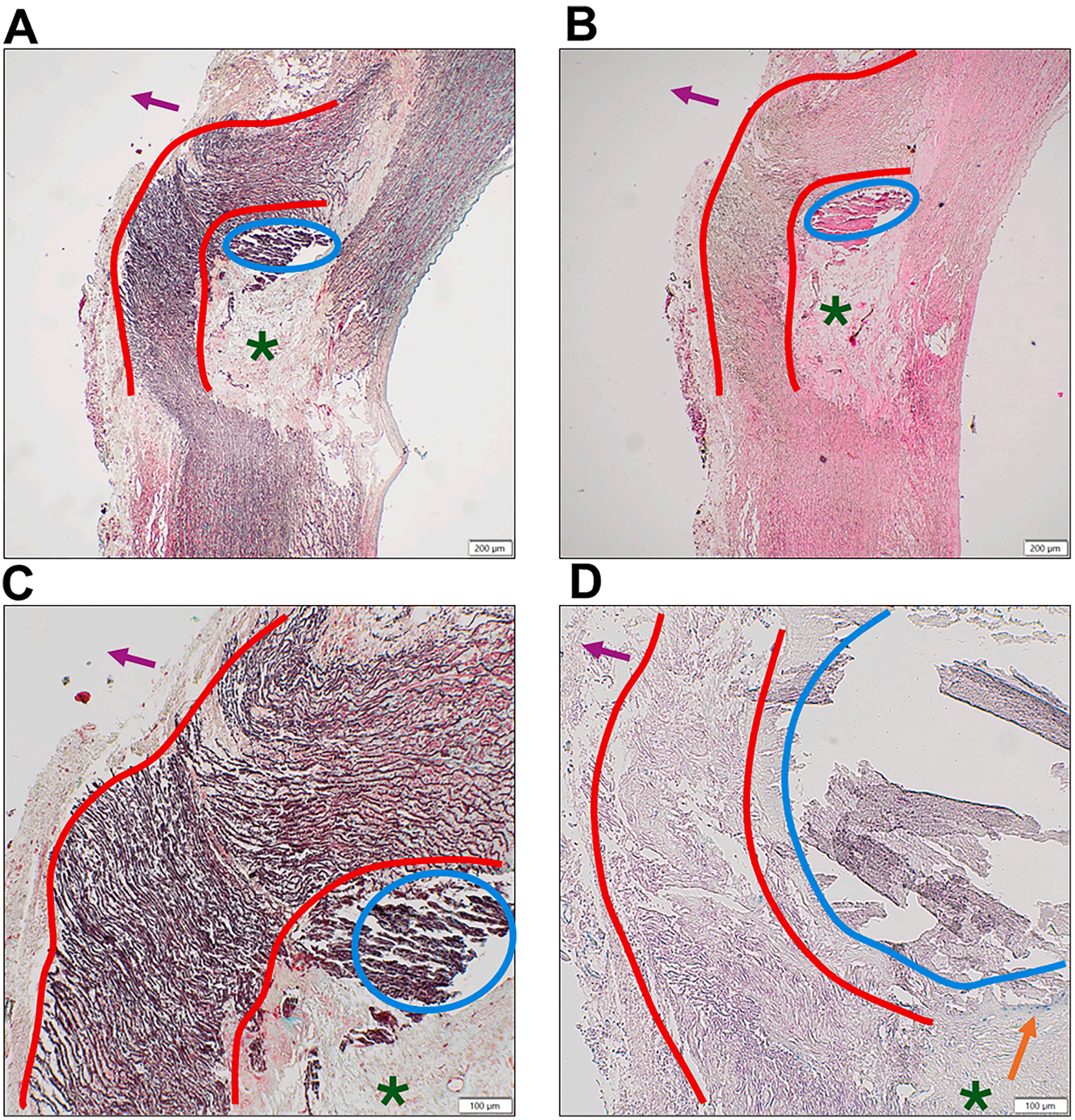

Fig. 5. Severe elastic fiber disorganization. All four panels display corresponding areas of the ascending aorta of the same patient: female, 31 years, BMI 20,9 kg/m^2, no re-do surgery, BAV, predominant AS, dilated aortic root (basal diameter 24 mm, sinus diameter 51 mm, sinotubular junction diameter 41 mm, ascending aorta diameter 30 mm). (A) Movant-pentrachrome stain, 40x magnification. (B) Hematoxylin-eosin stain, 40x magnification. (C) Movat-pentrachrome stain, 100x magnification. (D) Toluidin blue histochemistry, 100x magnification. In all for panels the purple arrow points towards the aortic lumen. The red lines surround an area of elastic fiber disorganization (course perpendicular to the lumen instead of circumferential orientation). The blue circle surrounds a former calcific lesion following decalcification (EDTA). The green asterisk indicates an area with loss of elastic fibers and vascular smooth muscle cell nuclei adjacent to the calcific lesion. (D) shows that the area of elastic fiber disorganization is affected by vascular smooth muscle cell loss also, only some nuclei in the wall of small vessel remained (orange arrow). ***Abbreviations:*** AS – aortic valve stenosis; BAV – bicuspid aortic valve; BMI – body mass index.

on the biomechanical AA wall properties in surgically pre-selected cohorts. Correspondingly, clinical studies observed aortic stiffening in association with BAV morphology [41], but with a lower impact of the BAV morphology compared to aging [42]. To the authors' knowledge, studies other than the present assessing the AA biomechanical properties in UAV are not available. Taken together, further studies are needed to systematically characterize the aortic wall biomechanical properties in individuals with UAV over time to determine the impact of UAV morphology itself on the AA biomechanics. It must be pointed out, that the present study used a rather basic approach to estimate the AA biomechanical properties. Calculations derived from echocardiographic measurements based on established formulas [28] were employed. This approach did allow for the concomitant assessment of clinical features, AA wall structure, and biomechanics at the same time. However, more

advanced methods, such as estimating AA biomechanical properties based on multiphasic computed tomography scans [43] or measurements applying a peeling test [44], are needed to validate the findings of the present exploratory study.

Another reason for the stiffer aortic wall in individuals with BAV and TAV compared to those with UAV seems to be statistically more severe ASVD in those with BAV and TAV (UAV vs. BAV $p<0.001$; UAV vs. TAV $p<0.001$, details see Table 3). Clinical studies report an association of ASVD with aortic stiffening [45]. The statistically significantly younger age of the UAV individuals might be a reason for less pronounced ASVD observed in those aortas, as ASVD is typical for age-related aortic degeneration [46]. Interestingly, no statistically significant differences between the surgical samples and the reference group were encountered in these comparisons (Reference vs. UAV $p=0.264$; Reference vs. BAV $p=0.153$; Reference vs. TAV $p=0.139$).

At this point, it must be noted that ASVD affects all three aortic layers [18–20]. Although most studies mainly focus on the degeneration of the medial layer, such as Butany et al. [5] or Roberts et al [38], the interplay of all three aortic layers is the basis of the AA wall properties [14]. Consequently, the adventitial collagen together with the elastic lamellae and medial collagen significantly impacts the tensile tissue strength and passive elastic properties [29]. For this reason, the present study included adventitial lesions, i.e., fibrosis and inflammatory infiltrates, into the correlation analysis. A positive statistically significant correlation ($p=0.009$; $\rho=0.257$) between presence of adventitial inflammation and elastic modulus was observed. This means that the presence of adventitial inflammatory lesions was associated with a stiffer aortic wall in the assessed surgical cohort. This points out the importance of the adventitial layer as functional interface of the aorta and its surroundings with regard to aortic biomechanics. Due to the exploratory study design, no in-depth analysis of the adventitial inflammatory lesions (e.g., differentiating the inflammatory cells such as $CD3^+$ T-cells versus $CD20^+$ B-cells [18]) was conducted. Thus, future studies are needed to determine the actual impact of different inflammatory lesions on the aortic wall properties.

Not only ASVD [18–20], but also the VV can be found in all three aortic layers [47–49]. In the comparisons regarding AV morphology, statistically significant differences were found in terms of the vascularization, i.e. $CD31^+$-VV/mm^2. Individuals with TAV had the least VV/mm^2 ($M=1.61$), resulting in significant differences compared to the reference group ($M=4$, $p=0.009$) and those with BAV morphology ($M=3.8$, $p<0.001$). At the same time, individuals with TAV ($M=57$ years) were older than those with BAV ($M=51$ years) and UAV ($M=37$ years; screening $p<0.001$, details see Table 3). Taken together, this might be a hint that AA wall degeneration in elderly with non-congenitally malformed AV might be associated with hypoperfusion of the aortic wall, like shown for the abdominal aorta [50] or suggested by studies analyzing VV remodeling [51]. As VV are found in association with both initiation and progression of atherosclerosis [52], this reduced vascularization fits the finding of significantly more severe ASVD in the samples of individuals with TAV compared to those with BAV or UAV (screening $p<0.001$, details Table 3).

No statistically significant differences regarding $CD31^+$-VV/mm^2 were found comparing UAV ($M=3.08$) with BAV ($p=0.56$) and TAV ($p=0.057$) valve morphology and the reference group ($p=0.606$). In the comparative analyses regarding AV malfunction ($p=0.146$), presence/absence of AA dilatation ($p=0.14$), and the age groups ($p=0.18$), the screening tests did not indicate statistically significant differences in terms of AA vascularization. However, future studies considering VV remodeling [51,53] (in addition to the pure number of $CD31^+$-VV/mm^2) are needed to determine the actual impact of the VV on the structural and biomechanical AA properties.

Reproducing earlier studies, such as Butany et al. [5], comparable OMD, i.e., an integrative measure of medial degeneration [8], was observed comparing individuals with UAV, BAV, and TAV (screening test $p=0.571$, details see Table 3). However, this shows that individuals with UAV significantly earlier in life exhibit a degree of OMD that is usually seen in significantly older individuals with BAV and TAV (i.e., individuals with more age-related degeneration, such as ASVD). This indicates that UAV is associated with an earlier onset of medial degeneration than BAV and TAV morphology, again mirroring prior observations [54]. From a clinical perspective, such a statistically significant earlier onset of UAV-associated medial degeneration might be a hint for more advanced aortic wall degeneration in individuals with UAV compared to those with TAV. The BAV is considered a “not so benign” AV morphology already [55]. With the UAV being associated with marked OMD earlier in life compared to BAV, this could suggest that the UAV morphology is an even “less benign” congenital abnormality. This assumption is further underpinned by findings of prior necropsy studies. For example, in 1984, Larson and Edwards reported a 18-times increased risk for aortic dissection in individuals with unicommissural UAV [56].

Interestingly, the screening tests did not detect any statistically significant differences regarding OMD between non-dilated and dilated AA samples ($p=0.842$, Table 4), different AV malfunctions ($p=0.789$, Table 5), and different age groups ($p=0.543$, Table 6). On the one hand, this could be attributable to the bias by selection associated with a surgical cohort, i.e., no individual with healthy proximal aorta including the aortic root and valve does need cardiac surgery in this area. On the other hand, this could be attributable to the simplifications necessary for the exploratory analyses. Due to the diversity of medial lesions (for example, MEMA, medial fibrosis, elastic fiber alterations, and smooth muscle cell alterations), it seems likely that differences between these single parameters are present (**Supplemental File S1**), as shown by Roberts et al [38]. Thus, subsequent studies assessing the impact of different lesions of the smooth muscle cells, the extracellular matrix, and the elastin on the biomechanical properties are needed.

However, the observation that the screening tests did not find significant differences comparing presence/absence of AA dilatation, AV malfunctions, and age groups in terms of OMD might indicate that AV morphology, rather than AV malfunction, presence/absence of AA dilatation and age, interferes with AA structural and biomechanical wall properties. This might be a hint that the root cause for AA degeneration in association with congenital AV malformation might trace back to the embryology of the large arteries like for example discussed by de Sa et al. [33]. Besides these developmental aspects, the aortic wall degeneration in the context of congenital AV malformation was also linked to the altered flow across a malformed valve [9], regardless of the type of AV malformation [12]. The fact that the present study observed more significant differences regarding AV morphology than AV (mal-)function could be an indicator that flow-associated wall degeneration might be secondary to a developmental root cause of the AA degeneration.

Although there were no statistically significant differences between the AV malfunction groups in terms of OMD, the left ventricular cavities in those affected by predominant AR (LVEDD $M=58$ mm) were significantly larger compared to those diagnosed with predominant AS (LVEDD $M=45$ mm, $p<0.001$). At the same time, left ventricular function was comparable between those groups in terms of ejection fraction ($p=0.547$) and fractional shortening ($p=0.353$, Table 5). Such larger ventricular cavities result in larger stroke volume in those affected by AR [57]. In line with this, AR is reportedly associated with increased aortic longitudinal strain [58]. This mechanical stress might be the reason for the notably thin AA wall in individuals affected by AR ($M=1808$ μm) compared to the reference group ($M=2801$ μm, $p=0.003$). Additionally, fewer lamellar units were found in the present study in presence of predominant AR ($M=46$) compared to the reference group ($M=69$, $p<0.001$). This could be attributable to medial degeneration observed in association with AR in prior studies [17]. Besides degenerative changes, individuals with predominant AR presented with fewer lamellar units and a thinner aortic wall (Table 5). The lamellar units are the functional units of the AA wall, i.e., the structural basis of its biomechanical viscoelastic properties [15,16]. Correspondingly, the present study

observed a lower (statistically non-significant; $p>0.01$) elastic modulus in individuals with predominant AR (M 1181=mmHg / SD=717) compared to those with predominant AS (M=1227 mmHg / SD=552) or non-significant AV disease (M=1341 mmHg / SD=675). These findings mirror clinical observations in magnetic resonance imaging studies which showed reduced aortic elasticity in association with AR [59].

Not only predominant AR, but also predominant AS was associated with a significantly thinner AA (M=1684 μm, $p=0.002$) and significantly fewer lamellar units (M=45, $p<0.001$) compared to the reference group. This might be an indicator that alterations of flow across the AV might interfere with the AA wall structure [60].

4.3. Limitations not yet discussed

This first exploratory study on the interplay of AV morphology, AV function, AA wall structure, age, and biomechanical AA wall properties did not control for accompanying diseases. Thus future studies are needed to validate the present findings, considering that diseases like arterial hypertension [61] interfere with the biomechanical properties of the vascular tree. Further, it is discussed that different pharmaceutical agents like Losartan [62] might influence the process of aortic dilatation. Thus, future studies are needed to analyze how different medications interfere with the AA wall structure and its biomechanical properties.

The chosen sampling with the acquisition of only one paraffin block does not allow for a generalization of the results for the proximal aorta. However, the following rationales underlie the presented sampling approach:

(A) Several studies indicate different structural and molecular remodeling of the proximal aorta on different heights in the cranio-caudal plane [63,64]. Also, different remodeling is described comparing convexity and concavity of the ascending aorta [63,65,66]. Potential explanations for these local differences might be the varying embryological origin of the vascular smooth muscle cells [67] or differences in wall stress due to varying exposure to flow and turbulences [9]. Because of these regional differences, the sampling site at the anterior circumference in the middle between convexity and concavity was chosen to acquire a comprehensive overview of the aortic wall structure with limited superimposition by flow- and stress-associated aortic wall changes.

(B) Usually, at our center, the non-dilated AA is only partially opened at the anterior aspect for isolated AV replacement or AV reconstruction. The chosen sampling site, however, is accessible in all procedures requiring aortotomy regardless of presence or absence of aortic dilatation. Thus, the chosen sampling allows for a consistent and comparable sampling throughout all groups. Yet, an acquisition of enough tissue to submit two cassettes to histological analyses like claimed by current recommendations [30] is not feasible.

The biomechanical properties of the aorta derive from the interplay of all three aortic layers [14] with the tunica media being the main structural basis of the aortic wall's viscoelastic properties [15,16]. The histomorphological description of the tunica media according to current standards alone requires more than 19 statistical variables already. Thus, to avoid excessive α-error inflation, a panel of variables characterizing the individuals and summarizing the AA wall structure of all three layers had to be selected for the statistical analyses. The still high number of tests associated with cumulating α-error [68] was addressed by applying a significance level of $\alpha=0.01$, and p-adjustment. Because of this approach, future studies are needed to analyze how the different individual morphological parameters (i.e., MEMA, medial fibrosis, elastic fiber alterations, etc.) interfere with the biomechanical properties of the aortic wall. However, all individual parameters describing medial degeneration are statistically described in **Supplemental File S1**.

Several different approaches are discussed to determine the actual normal aortic size of a particular individual [24,25]. Thus, to have a fixed threshold for aortic dilatation for the present exploratory study, the threshold was set to 40 mm based on past recommendations for surgical replacement of the aorta in individuals with BAV [26]. This might result in some individuals falsely classified as either "non-dilated" or "dilated" as the threshold of 40 mm might be too high or low, for example, for a particularly large or small person.

4.4. Applicability of an exploratory analysis of AA wall structure and function

Despite the limitations of the chosen methodology, the applied exploratory approach with a focus on exemplary target parameters describing the patient characteristics, AA wall structure, and biomechanical aortic wall properties, was able to replicate the findings of previous studies focusing on partial aspects only, such as aortic wall degeneration in association with AV morphology [5] and disease [38]. From the authors' perspective, this is a strong indicator of the feasibility and validity of such a broad structural and functional exploratory analysis based on pre-selected exemplary target parameters.

5. Conclusion

The present study in a surgical pathology setting is the first to concomitantly assess structural changes of all three AA wall layers, AV morphology, AA dilatation, AV function, age, and AA biomechanical properties including a non-surgical reference group. Doing so, this study reproduced the findings of prior analyses focusing on partial aspects of the assessed spectrum of parameters only. Thus, the results are an indicator of the feasibility of the applied broad explorative approach. The main findings of this exploratory analysis are the following (**Graphical abstract**): (A) A higher number of statistically significant differences was found when comparing AV morphologies than when comparing groups regarding AV function, presence/absence of aortic dilatation, and age. (B) Marked medial degeneration was found in association with UAV morphology significantly earlier in life compared to BAV and TAV morphology. (C) Significant differences regarding wall thickness and lamellar unit count between the surgical samples and the autopsy reference were found. (D) A significant positive correlation of adventitial inflammatory lesions with the elastic modulus (i.e., stiffer AA wall in presence of adventitial inflammatory lesions) was found.

From a clinical perspective, the findings are a hint that aortic wall degeneration in association with UAV might be more advanced than in TAV morphology and maybe even more pronounced than aortic degeneration associated with BAV morphology. From a biomechanical perspective, the findings underpin the importance of the adventitial layer. From a pathological perspective, the findings underpin both the need for non-surgical control samples in studies assessing AA wall structure and the need to include all three aortic layers in such studies. Additionally, hints are seen that AV morphology rather than AV function, presence/absence of AA dilatation, and age, negatively impacts aortic wall properties.

Formulas

Formula 1. Calculation of the arterial strain.

$$\text{Arterial strain} = \frac{\text{Largest arterial diameter [mm]} - \text{Smallest arterial diameter [mm]}}{\text{Smallest arterial diameter [mm]}} \quad (1)$$

Formula 2. Calculation of the elastic modulus.

$$\text{Elastic modulus [mmHg]} = \frac{\text{Systolic blood pressure [mmHg]} - \text{Diastolic blood pressure [mmHg]}}{\text{Arterial strain}} \quad (2)$$

Review board statement

The present study was approved by the local ethics committee (vote number 47/14). Surgical samples were obtained after written informed consent was given.

Informed consent statement

Informed consent was obtained from all subjects included in the study group.

Funding

No external funding was received.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used *Grammarly* (v1.2.116.1535) in order to check for spelling and grammar mistakes. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Data availability

An anonymized version of the present study's data set can be found in **Supplemental File S2**. Anonymization was needed due to German data protection requirements. The anonymization was done according to German recommendations by interchanging rows and columns.

CRediT authorship contribution statement

Jan M. Federspiel: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jan-Christian Reil:** Writing – review & editing, Methodology, Conceptualization. **Peter H. Schmidt:** Writing – review & editing, Supervision. **Paul Teping:** Writing – review & editing, Investigation. **Frank Ramsthaler:** Writing – review & editing, Formal analysis. **Tanja Schwab:** Writing – review & editing, Methodology, Investigation. **Hans-Joachim Schäfers:** Writing – review & editing, Validation, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

In mourning for Prof. P. A. Schnabel, we would like to thank him for his many years of support and active assistance in the planning and execution of the present study. Furthermore, we would like to thank Prof. M. W. Laschke for the possibility to perform investigations and stains on the premises of his institute. The authors thank the anonymous reviewers and the editors who significantly helped to improve this study.

Supplementary materials

Supplemental File S1: Appendix A: Abbreviations list; Appendix B: Systematic literature search; Appendix C: Staining protocols, Appendix D: Resources tables; Appendix E: Schemes of parameters describing medial degeneration; Appendix F: Detailed findings regarding medial degeneration.

Supplemental File S2: Anonymized data set.

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpath.2025.107782.

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