The layer-specific biomechanical properties of dissecting ascending aortic aneurysm (Stanford type A of dissection)

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Purpose: The aims of this paper was the analysis of the mechanical properties of dissected wall of the ascending aortic aneurysm ($n = 12$). *Methods*: All aortas were collected from men (mean age: 48 ± 12 years, mean diameter of the aneurysm: 49 mm $\pm$ 4 mm). The mechanical properties were determined based on directional tensile test. The biomechanical assay was complemented by conducting histological analysis (hematoxylin and eosin, Mallory's trichrome, Azan stain). *Results*: The highest values (median) of failure Cauchy stress, failure force, Young's modulus and stiffness coefficient were obtained for the adventitia ($\sigma_{max} = 1.40$ MPa, $F_{max} = 4.05$ N, $E = 26.11$ MPa, $k = 1.06$ N/mm). *Conclusions*: The results indicate that the mechanical function of the adventitia in healthy tissue and dissected ascending aorta aneurysm is the same, i.e., it protects the vessel against destruction. The failure Cauchy stresses found in the media and intima are comparable and amounted to 0.23 and 0.21 MPa, respectively. The results indicate that dissection affects the mechanical properties of ascending aorta wall layers. The mechanical loads are probably transferred within the dissected aneurysmal wall not only through the media, but also through the intima.

Key words: dissection, mechanical properties, human ascending aorta, aneurysm, dissecting aneurysm, aorta layers

Lists of abbreviations

GAGs – glicosaminoglycans
PGs – proteoglycans

1. Introduction

The wall of a normal aorta is composed of three layers known as the intima (inner layer), media (middle layer) and adventitia (outer layer), and its thickness ranges from 1.7 to 2 mm [9]. The intima consists of a monolayer of endothelial cells called endothelium that adhere to a thin (~80 nm) basal membrane, composed of type IV collagen and laminae and a sub-endothelial layer composed of smooth muscle cells, collageneous bundles and elastic fibrils [2]. According to Brunet et al. [2], although the endothelium does not contribute significantly to the mechanical behavior of the arterial wall, the subendothelial layer certainly does. Despite this, the effect of intima on the mechanics of a healthy vessel wall is insignificant [29]. The middle layer is media. According to MacLean et al. [14], the media consists of approximately 70 layers of alternating elastic laminae and embedded smooth muscle cells, adhesion molecules, collagen fibers (mainly types I, III, and V) and GAGs/PGs. Depending on the author, the media accounts for 77% [14] or 80% [6] of the normal aortic wall and, due to its organization, it transfers hemodynamic loads within the physiological range of arterial blood pressure in longitudinal and

circumferential direction [2]. According to MacLean et al. [14], the media is weaker in the radial direction. This is why it is prone to dissection separation [2]. The outermost layer is adventitia and consists mainly of type I collagen but also admixed elastic fibers and fibroblasts. This layer constitutes 15% or 17% [9] of the aortic wall. At the unloaded state, the collagen fibers in the adventitia are crimped. Consequently, the media mainly contributes to the mechanical behavior of the arterial wall at low pressure. At a high loads, collagen fibers in the adventitia are straightened and the arterial wall becomes extremely stiff, effectively preventing the artery from overstretching and rupture [2].

Aortic dissection is a sudden delamination of the aortic wall that occurs in the medial layer when the hemodynamic loads exerted on the aorta wall exceed bonding forces that normally hold the layers together [19]. In the most cases, dissection is initiated with an intimal tear which then enables blood to flow in the media [27]. The biomechanical events of aortic dissection are analyzed in two different mechanisms: (i) initiation and (ii) propagation, but the causes of them and the most influential factors are not well understood. According to Rajagopal et al. [20], the initiation of the aortic dissection can be related to systolic blood pressure, and the pulse pressure and heart rate can influence its propagation. Osada et al. [18] suggested an important role of the vasa vasorum in initiation of aortic dissection. Roccabianca [21] identified pooling of glycosaminoglycans/proteoglycans as a possible cause for aortic dissection initiation by creating significant stress concentrations and intra-lamellar swelling in the arterial wall.

The incidence of aortic dissection is 35 cases per year per 100,000 people among the 65–75 years of age. Risk factors include hypertension, atherosclerosis and genetic disorders that affect connective tissue like Marfan syndrome [16]. Two third of these occur in the ascending aorta along the right lateral wall, where the maximum shear stress caused by blood flow is located, whereas a smaller fractions are in the descending part of it. Type A dissection involves the ascending aorta and arch is associated with high mortality rates, and generally requires surgical interventions. Type B dissection include the descending aorta, and have lower mortality rates, and are treated using pharmacological methods [16].

Biomechanical studies of the ascending aortic wall were performed by Xuan et al. [30]. The authors conducted the mechanical properties analysis of the normal human aortic root and ascending aorta. The authors have shown influence of the region of the ascending aorta and its roots on the biomechanical properties, especially failure stress, failure stretch and elastic modulus. Pasta et al. [19] conducted tests on the human thoracic nonaneurysmal aorta and nondissected ascending thoracic aortic aneurysm (ATAA). A comparative analysis of the ATAA was made with bicuspid aortic valve (BAV) and with tricuspid aortic valve (TAV). They found that the delamination strength was lower in BAV-ATAA compared to TAV-ATAA. Vorp et al. [28] measured the biomechanical properties of ascending aorta aneurysm wall and compared them with those obtained for healthy ascending aorta. The results indicated that the wall of ascending aorta aneurysm was approximately by 30% weaker and appreciably stiffer than nonaneurysmal ascending thoracic aorta wall. Duphrey et al. [4] measured the elastic modulus of the ascending thoracic aortic aneurysm from tissues from two different locations, greater curvature (GC) and lesser curvature (LC). The authors indicated that the ascending thoracic aortic wall was anisotropic with the circumferentially oriented specimens being the stiffest. Furtheromore, the aorta wall was stiffer longitudinally in the GC than in the LC. In 2016, Duphrey et al. [5] also performed the biomechanical analysis of the ascending aorta aneurysm. Their results highlighted the relationship between failure properties and the age of patients, showing that patients below 55 years display significantly higher strength. The authors also showed the correlation between the extensibility of the tissue and the physiological elastic modulus, independently of the age. Okamoto et al. [17] obtained the age dependence of elastic properties of dilated ascending aorta aneurysm. The authors showed increases in mean circumferential stress with blood pressure and diameter and declining wall strength with age that may increase the risk of rupture or dissection. Yamada et al. [31] performed the mechanical studies of aorta obtained from patients with ascending aorta dissection. The authors revealed age-related distensibility and aortic wall content in elderly patients. They obtained the increase in collagen content as a consequence of vascular wall remodeling with aging likely suppressing the increasing rate of distensibility for acute aortic dissection candidate patients. Babu et al. [1] conducted the biaxial mechanical studies of human ascending aorta wall with type A of dissection. The author did not find any differences in material properties of aorta dissected wall specimens, regardless of patient's age. Furthermore, they did not find the correlations between wall thickness and diameter with the patient age. Sokolis et al. [23] performed the mechanical tests of the ascending thoracic aneurysm

wall layers. The results of their research indicate that the deformational response for pressures 100 mmHg was attributable to the media. The adventitia resisted distention at high loads, and the intima contributed the least to the tensile strength of intact wall. Furthermore, they showed that the load-bearing structural elements were organized more circumferentially than longitudinally, especially in the medial layer. Deveja et al. [3] quantified the degree of layer contribution to the failure properties of intact ascending thoracic aorta aneurysm wall and nonaneurysmal ascending aortic wall. The authors reported that ascending thoracic aorta aneurysm is not associated with wall weakening, only with stiffening and reduced extensibility. They obtained non-significant differences in intimal and adventitial failure stress and reduced failure stretch of all layers in ATAA with tricuspid aortic valve than in nonaneurysmal aorta.

Over the past few years, the problem of characterization of the biomechanical response of the ascending aorta and its aneurysm has been an issue. Most of current knowledge pertains to the ascending aorta, the ascending aorta aneurysm and the layer-specific mechanical properties of the ascending aortic aneurysms that may precede dissection. There are a few studies examining the biomechanical properties of the aortic dissection and there is few information about the layer-specific failure properties of the dissected ascending aorta aneurysm wall. Advanced mechanical analyses of the aorta dissection mechanisms based on mechanical experiments may deeply improve current knowledge of these life-threatening events and improve clinical decision criteria. This is why the aim of this study was to determine and evaluate the mechanical strength and biomechanical properties of the individual layers (adventitia, media, intima) of dissecting wall of the ascending aortic aneurysm. The aim was achieved by performing biomechanical tests, followed by tensile tests on the individual layers. The biomechanical assay was complemented by histological analysis. Our results provide data on layer-specific mechanical properties that underlie the deformation characteristics of the aorta wall under pressure. This information cannot be detected using clinical imaging of the aorta. We assume that these results in the future may serve as inputs in finite element models aimed at assessing the dissection potential of the ascending aorta aneurysm wall. Further, such studies may also be useful in mathematical models to assess the growth and remodeling of tissues and in evaluating the contributions of individual aorta wall layers to the overall arterial biomechanics.

2. Materials and methods

2.1. Research material

The research material consisted of the walls of dissecting ascending aortic aneurysms (DAAA) ($n = 12$, Fig. 1a), which were collected from patients during cardiac surgeries performed at the Department of Cardiac Surgery at the Wroclaw Medical University. The procedure involved replacing a segment of the vessel due to dissection. Detailed information about patient population, i.e., hypertension, connective tissue syndromes, diameter of the aneurysm and type of aortic valve are given in Table 1.

Table 1. Patients characteristics (BAV – biscupid valve)

No.	Aneurysm diameter [mm]	BAV	Other connective tissue syndroms	Hypertension
1	49	–	–	+
2	43	–	–	–
3	55	+	–	–
4	50	+	–	–
5	48	+	–	+
6	45	–	–	+
7	47	+	–	+
8	50	–	–	+
9	58	–	–	+
10	45	–	–	+
11	52	–	–	+
12	51	+	–	+

Aneurysm diameter as evaluated from pre-operative computed tomography.

In all the cases, there were Stanford type A dissections. The material was collected with the consent of the Bioethics Committee (approval no. KB-14/2019). All aortas were collected from men (mean age: 48 ± 12 years).

Immediately after excision, the material was placed in normal saline (0.9% NaCl), frozen at –4 °C and sent to the Department of Mechanics, Materials and Biomedical Engineering at the Wrocław University of Science and Technology for testing of mechanical properties. Within 48 hours of collection the ATAA samples, the recovered tissue was equilibrated to room temperature (23 °C) by immersion in fresh phosphate buffered saline and tested as described below.

Preparation of test specimens

Prior to testing, the flat cuboidal specimens ($n = 32$) were punched out (Fig. 1b). The specimens were

punched out in two directions: longitudinal/axial (A) (n = 10) and circumferential (C) (n = 22) relatively to the long axis of the vessel. The number of specimens cut out from a single vessel fragment depended on its size. Each of the excised specimens was then manually divided (using the so-called "blunt" method) into three layers, i.e., the adventitia, media, and intima. This way, 32 specimens of each layer were obtained, which gave a total of 96 specimens. The geometric dimensions of the specimens depended on the shape of the punch and were 5 mm in width and 30 mm in length.
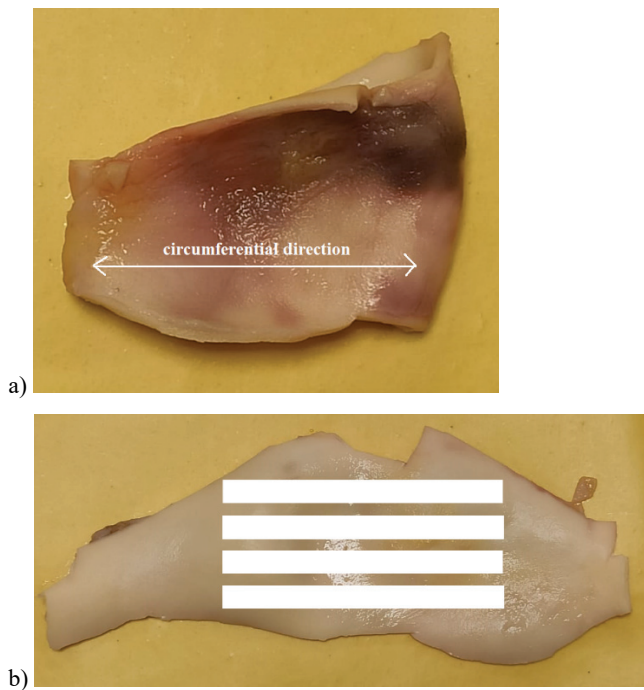

a)

b)

Fig. 1. Photographs of the dissected (a) tubular
and (b) cut-open ascending aortic tissue

The method of cutting out the circumferential specimens was marked.

The specimens prepared in this way were divided into six measurement groups: axial adventitia (AA), axial media (AM), axial intima (AI), circumferential adventitia (CA), circumferential media (CM), and circumferential intima (CI).

Until the measurements were taken, the specimens prepared in this way were stored in normal saline (0.9% NaCl).

2.2. Research method

Tests of mechanical properties

The mechanical tests were performed at the Department of Mechanics, Materials and Biomedical Engineering of the Wrocław University of Science and Technology. Each of the obtained specimens was subjected to a quasi-static uniaxial tensile test. This test was performed using an MTS Synergie 100 testing machine. After clamping each specimen with the grips of the testing machine (the length of the measuring section was 20 mm), the thickness of the specimen was measured using a video extensometer (ME 46-350, Messphysik®). The measurement was carried out only in one plane and only before the start of testing. Then, the above-mentioned test started and consisted of two phases. The first phase was prestretching, which involved loading and unloading the specimen four times by 2 mm, i.e., 10% of the measuring section length. At the end of prestretching, the uniaxial tensile test began automatically and continued until the specimen ruptured. The loading speed in both phases was the same and amounted to 2 mm/min. The applied research methods has been used before by other authors [26], [27].

During the tests, changes in force and displacement were recorded. Next, assuming the incompressibility of the blood vessel wall and taking into account the geometric dimensions of the specimens, including thickness (A, 201.7 μm ± 95.5 μm; M, 702.2 μm ± 280.6 μm; I, 272.5 μm ± 140.4 μm), the normal components of the Green strain tensor and Cauchy stress tensor were calculated. This way, the stress–strain curves were obtained, based on which the following were determined: mechanical tensile strength (σ_{max}), defined as the maximum stress; maximum strain (ε_{max}) obtained at the point corresponding to the strength; and maximum tangent Young's modulus (E). The maximum elastic modulus was taken from each curve as the maximum slope prior to failure [4].

2.3. Microscopic analysis

After mechanical testing, the specimens were sent to the Department of Pathomorphology and Oncological Cytology at the Wroclaw Medical University for microscopic evaluation. The analysis was carried out to (i) validate the separation of the layers and (ii) analyze the structural changes of each layer. The analysis concerned the main structural components responsible for the transfer of mechanical loads, i.e., elastin and collagen fibers and smooth muscle cells as well as the presence of proteoglycans in the vessel wall.

For microscopic analysis, the specimens were fixed in 10% buffered formalin, processed in an automated tissue processor, and embedded in paraffin. Paraffin

blocks were used to prepare 4-µm-thick sections that were subsequently stained according to standard histochemical protocols. A number of staining methods were used to highlight different structures of the aortic layers: hematoxylin and eosin (BioOptica, Milan, Italy), Mallory's trichrome stain (BioOptica), and Azan stain (BioOptica). For evaluation, all slides were digitalized with an automatic Pannoramic MIDI scanner (3D Histech, Budapest, Hungary). The scanned slides were reviewed by a surgical pathologist with CaseViewer software (3D Histech, Budapest, Hungary). Boundaries between intima/media and media/adventitia were defined as the innermost and the outermost of the medial elastic lamellae, respectively.

2.4. Statistical analysis

The study involved a statistical analysis consisting of two steps. The first step was to verify compliance of the distribution of the determined parameters with normal distribution. This step was performed using the Shapiro–Wilk test ($\alpha = 0.05$). In step two, the statistical significance of the differences between the study groups was tested. The differences obtained between the layers in a given direction were verified using the Friedman test ($\alpha = 0.05$). On the other hand, the differences between the directions were verified using the Wilcoxon test ($\alpha = 0.05$). The test results of the mechanical properties were presented as the median (Me). Statistical analysis of the experimental data was performed using Statistica 13.1 (StatSoft) and Prism 7 (GraphPad) software.

3. Results

3.1. Microscopic analysis

Microscopic examination of separated aortic layers revealed an imperfect dissection of the intima. In addition to the extremely thin and delicate intima, the specimens contained a significant amount of the media (Figs. 2A, B). Separation of the media resulted in the best quality, i.e., the analyzed specimens did not contain fragments of the other aortic layers (Fig. 2C). Precision of dissection varied in the case of the adventitia and ranged from complete and clean separation to suboptimal separation with adjoining portions of the media (Figs. 2D, E). Additionally, pathological examination of the specimens demonstrated early degenerative changes within the media, i.e., accumulation of proteoglycans and fragmentation of elastic fibers, but no significant fibrosis (Figs. 2F and G). Several small fissures (up to 2 mm) were present in sections from the media; although they most likely represented force-related changes that had occurred during failure testing, areas of pre-existing degeneration adjacent to these micro-tears suggested reduced structural strength and increased mechanical vulnerability of the affected sites (Fig. 2H).
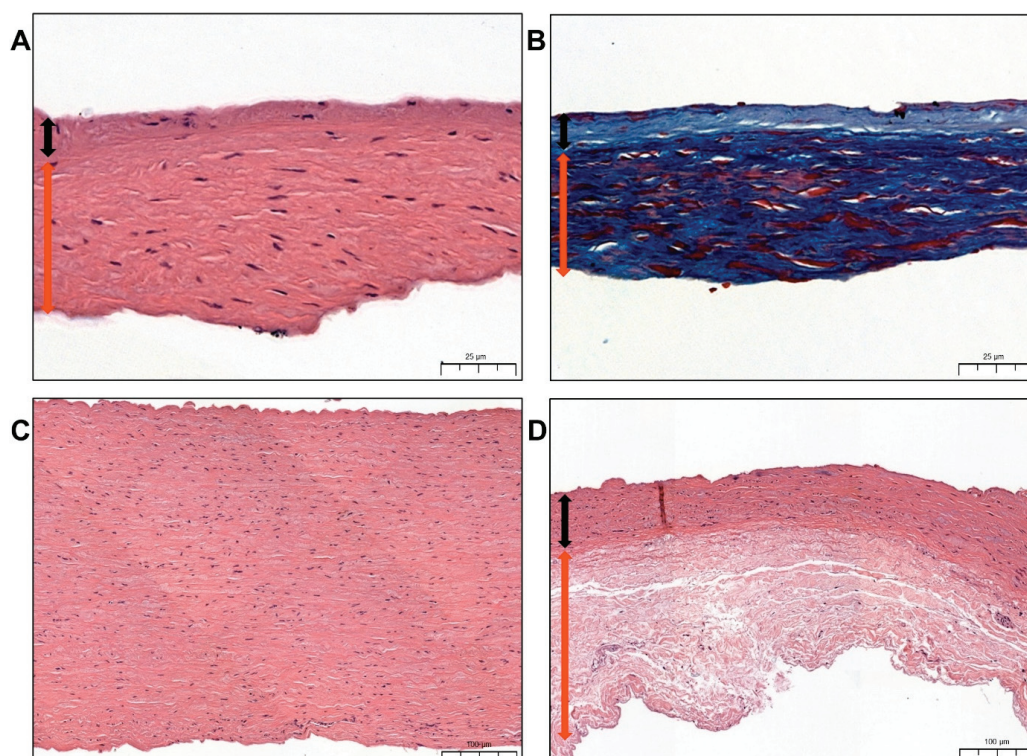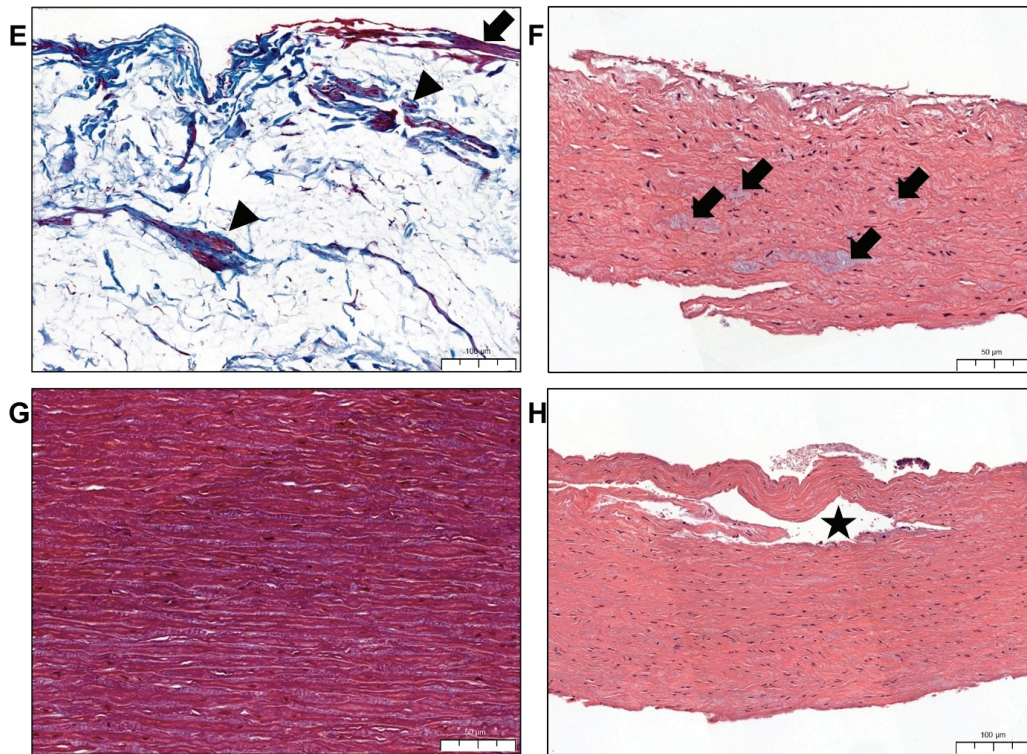
Fig. 2. Microscopic details of aortic wall specimens in circumferential sections.
Thin and delicate intimal lining of the aorta (black arrow) was separated together with part of the media (red arrow)
(A, H&E stain, ×400). The Azan stain highlights a structural difference between the intima (black arrow) and media (red arrow):
numerous smooth muscle cells stained dark red are present in the latter layer (B, Azan stain, ×400).
The aortic media shows optimal separation, i.e., no structures of the intima or adventitia are present (C, H&E stain, ×100).
This adventitial specimen shows suboptimal separation, i.e., adventitial connective tissue (red arrow)
was separated together with the outermost portion of the media (black arrow) (D, H&E stain, ×100).
Another sample of adventitia showing near perfect dissection: the trichrome stain highlights in red only minimal traces
of medial musculature (arrow) in addition to the vasa vasorum (arrowheads) (E, Mallory's trichrome stain, ×100).
Foci of bluish discoloration (arrows) within the media represent myxoid degeneration associated with accumulation
of proteoglycans (F, H&E stain, ×200). Narrow bands of collagen (stained blue in the trichrome stain)
in the media indicate lack of significant fibrosis (G, Mallory's trichrome stain, ×200).
The aortic media has a small fissure (asterisk); pools of accumulated proteoglycans are evident throughout the specimen,
including direct vicinity of the dissection (H, H&E stain, ×100)

3.2. Mechanical findings

Before analyzing the results of the mechanical properties, specimens were discarded where microscopic analysis showed incorrect separation of the layers. These specimens were not considered in further assessment. Finally, the 22 intima specimens, 27 adventitia specimens and the 30 adventitia specimens subjected to the analysis. The evaluation of the mechanical parameters was carried out in two stages. Initially, a comparison was made of mechanical properties obtained between the directions for a given layer, followed by a comparison of mechanical properties obtained for the layers in a given direction (Table 2).

Comparison of mechanical properties between the test directions

In the case of the adventitia, higher values of failure Cauchy stress, failure force, failure strain, and failure displacement were found for longitudinal specimens. The greatest differences between the test directions were found for failure displacement (40%) and failure Cauchy stress (38%). The smallest differences were found for failure force (1.3%) and failure displacement (4%). Higher values of Young's modulus and stiffness coefficient were obtained in the circumferential direction. In this case, the differences between the test directions were 21% and 24%, respectively. The intima was also characterized by higher values of the

Table 2. Comparison of median mechanical properties obtained for circumferential and axial specimens
(AC – circumferential adventitia, AA – axial adventitia, MC – circumferential media, MA – axial media,
IC – circumferential intima, IA – axial intima)

	AC	AA	*p*-value	MC	MA	*p*-value	IC	IA	*p*-value
Failure Cauchy stress [MPa]	1.402	2.264	0.0020	0.232	0.121	0.0313	0.212	0.196	0.0156
Failure force [N]	4.050	4.105	0.0020	1.480	0.810	0.0020	0.760	0.780	0.0039
Failure strain [–]	0.090	0.149	0.0078	0.1740	0.102	0.0078	0.081	0.089	0.0039
Failure displacement [mm]	8.530	8.875	0.0020	10.540	9.300	0.0020	8.145	8.270	0.0039
Young's modulus [MPa]	26.110	20.690	0.0020	2.261	1.856	0.0020	3.192	5.285	0.0039
Stiffness coefficient [N/mm]	1.060	0.804	0.0020	0.349	0.244	0.0020	0.200	0.282	0.0039

mechanical parameters in the longitudinal direction. The greatest differences between the test directions were found in the case of Young's modulus (40%) and stiffness coefficient (29%). The only parameter whose values were higher in the circumferential direction was failure Cauchy stress; however, in this case the differences between the directions were only 7.5%. In the case of the media, higher values of all analyzed mechanical parameters were obtained in the circumferential direction. The largest differences between the test directions were found for failure force (45%) and failure displacement (41%), while the lowest were found for failure Cauchy stress (7.6%) and failure displacement (12%). For each layer, the differences between the circumferential and longitudinal directions were statistically significant ($p < 0.05$).

Comparison of mechanical properties obtained for individual layers

For each of the examined layers, the stress–strain curves were typical of soft tissues (Fig. 3) and showed three characteristic regions, namely elastic, damage and failure. Most of the obtained curves were non-linear, however, for each layer there were specimens whose curves were linear (Fig. 3). Similar curves were obtained for specimens of entire walls of dissecting aneurysms, excised from the same vessel fragments as the specimens in this article. The results of that research were presented in a study by Kozuń [13].

The highest median values of failure Cauchy stress, failure force, Young's modulus, and stiffness coefficient were found in the adventitia. The values of these para-
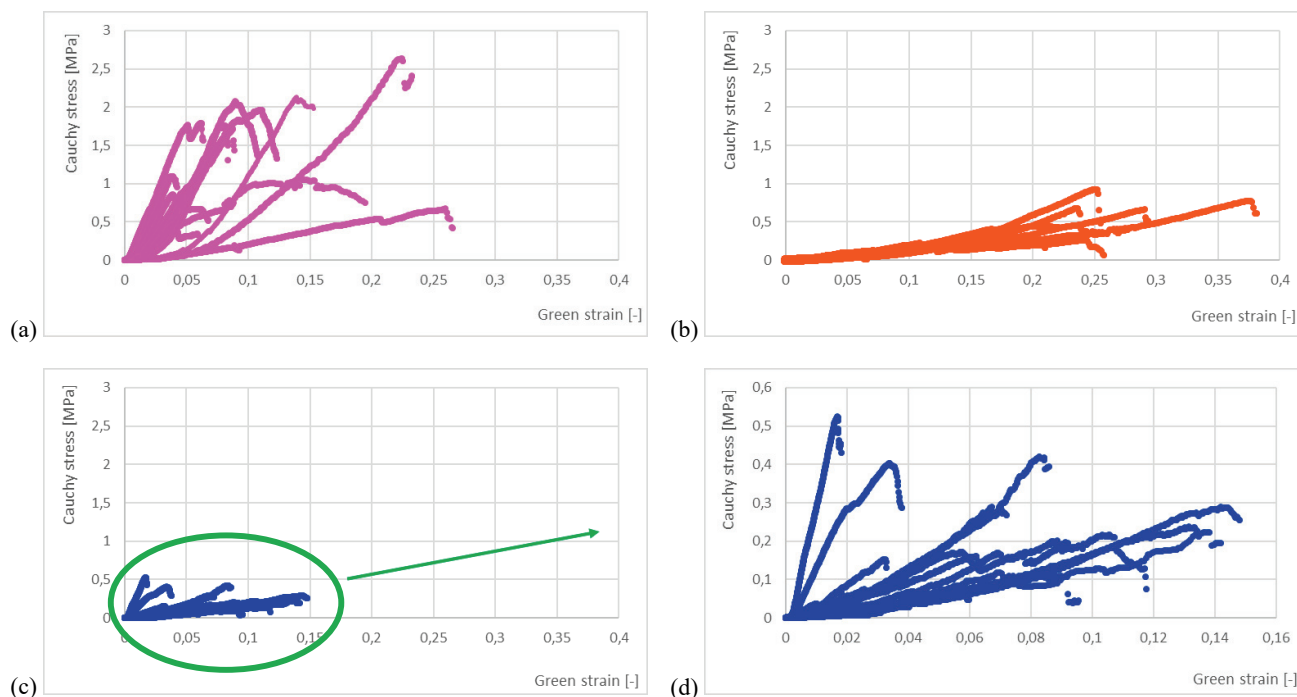

Fig. 3. Sample Cauchy stress/Green strain curves obtained for the adventitia (a), media (b) and intima (c, d)
of the human ascending aorta in the circumferential direction

meters were, respectively, 1.40 MPa, 4.05 N, 26.11 MPa, and 1.06 N/mm. The differences obtained between the adventitia and the other layers were statistically significant ($\alpha = 0.05$). The media and intima were characterized by lower mechanical values, but it should be stressed that for all the above mechanical parameters, the differences between the media and intima were not statistically significant ($\alpha = 0.05$). The failure Cauchy stresses obtained for the media and intima were comparable and amounted to 0.23 and 0.21 MPa, respectively. The difference between these values was 9%. The differences between the media and intima were 48% for failure force, 43% for stiffness coefficient and 29% for Young's modulus; in the case of Young's modulus, a higher value was obtained for the intima (3.19 MPa).

The presented analysis concerns the circumferential direction, however, in the case of the longitudinal direction, the obtained relationships between the layers were similar for the respective parameters. The values of the mechanical parameters obtained for the longitudinal direction were higher compared to the circumferential direction, as discussed in the section comparing the mechanical properties between the test directions and presented in box plot (a) (Fig. 4).

4. Discussion

This study determined the mechanical properties of individual layers of the dissected wall of the human ascending aortic aneurysm. Based on it, assessing the contribution of each layer to the transfer of mechanical loads in the dissecting vessel was performed. It is worth noting that Stanford type A of aorta dissection was present for every vessel sampled. The biomechanical assay was complemented by histological analysis. The mechanical parameters of each layer were determined in a uniaxial tensile test, which continued until the specimen ruptured. Based on literature, in carrying out the research, it was assumed that all three layers (adventitia, media and intima) are incompressible materials. Therefore, when describing their behavior under the mechanical loads, the research used Cauchy stress and Green strain. The mechanical parameters were analyzed separately in the two directions in which the specimens were stretched, i.e., circumferential and longitudinal.

The analysis of the mechanical parameters obtained for each of the layers showed that, regardless of the stretching direction, the adventitia had the highest
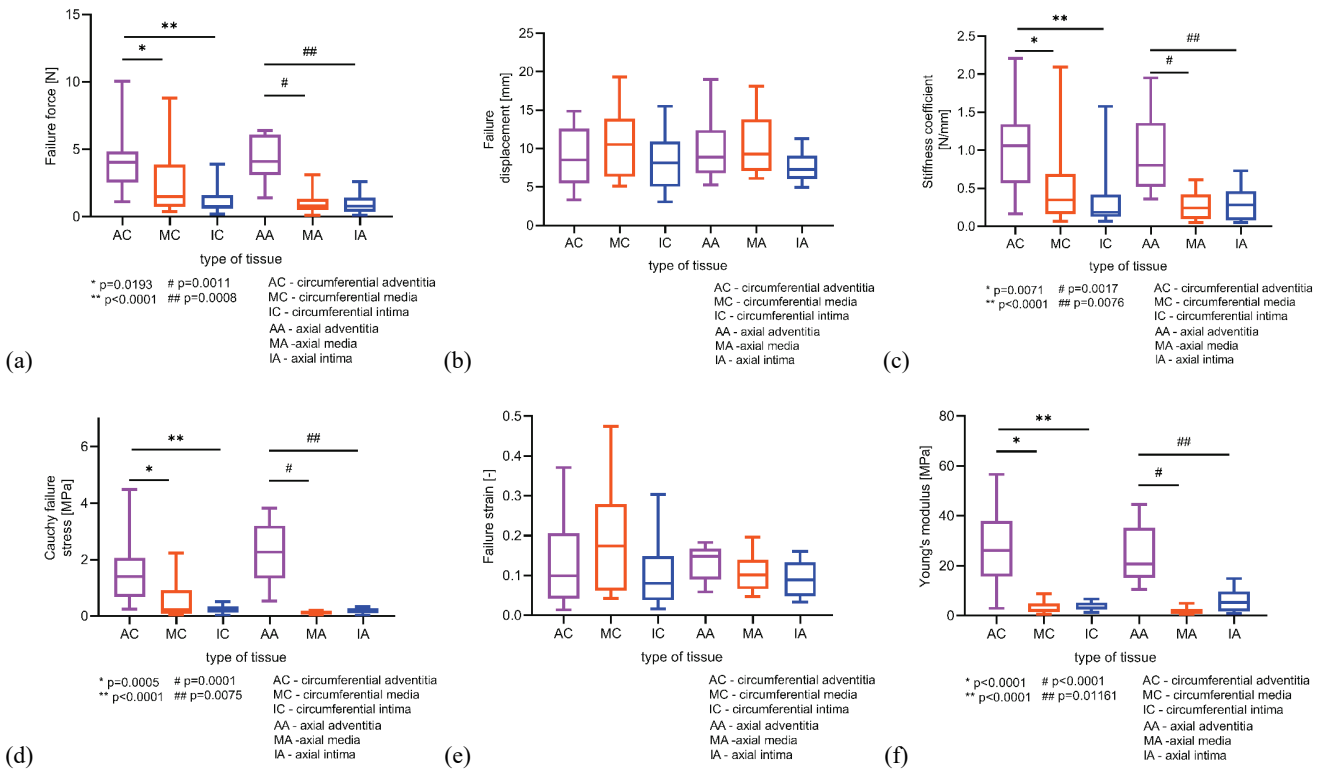

Fig. 4. Mechanical parameters obtained from tensile tests of the specimens of circumferential adventitia (AC),
circumferential media (MC), circumferential intima (IC), axial adventitia (AA), axial media (MA), axial intima (IA).
The results were presented as a median and the first (Q1) and third (Q3) quartiles

mechanical strength, maximum force and stiffness. The values of these parameters obtained for the remaining layers were lower, and the differences obtained between the adventitia and the other two layers were statistically significant ($p < 0.05$). Similar results of mechanical strength were obtained by Sokolis et al. [23] for the layers of nondissected ascending thoracic aneurysm. The failure strain values in the circumferential direction were highest for the media (0.174) and in the longitudinal direction they were highest for the adventitia (0.149). The failure strain values obtained for the intima in the longitudinal and circumferential directions (0.081 and 0.089, respectively) were similar to the values obtained for the adventitia in the circumferential direction (0.090). It should be noted, however, that differences in the values of this mechanical parameter obtained for each layer were not statistically significant ($\alpha = 0.05$). Results obtained by Sokolis et al. [23] and Manopoulos [15] were similar, i.e., failure stretches of the nondissected ascending aneurysm tissue did not show significant differences between layers. It should be stressed, however, that in contrast to the results obtained in this study, Manopoulos [15] and Sokolis [23] did not obtain statistically significant differences even in the case of the adventitia and the other layers.

The highest strength obtained for the adventitia may indicate that in the case of a dissecting aneurysmal wall, as in healthy tissue the adventitia protects the vessel against destruction. Histological analysis of the research material showed no quantitative or qualitative changes of collagen fibers in the adventitia, thus demonstrating that the mechanical function of this layer remained unchanged. It should be noted that this layer is also characterized by the highest value of stiffness coefficient. The increase in stiffness coefficient of this layer has not been discussed in the literature. Histological analysis of the media indicates that rupture of the media during mechanical loading occurred in the area where there was an accumulation of proteoglycans (Fig. 2F). This confirms the theory presented by Roccabianca [21], according to which the presence of proteoglycan pools leads to stress concentrations and contributes to the reduction of mechanical parameters of the tissue and, consequently, its dissection. According to Sherifova [22], stress distribution in the vessel wall is the main factor initiating dissection. The results of mechanical properties in this study do not indicate that the presence of proteoglycans contributed to the change in mechanical parameters of the media. The differences in the obtained values of failure Cauchy stress between the media and intima were not statistically significant. It

may suggest that the intima of the dissected aorta aneurysm is included in the transfer of mechanical loads. Inclusion of the intima into the process of mechanical loads transfer is at issue. It is assumed that healthy intima does not transfer mechanical loads. The intima is incorporated into the load-bearing process as result of structural remodeling taking place during aging or development of atherosclerosis [11]. The contribution of the intima of non-dissected or dissected aorta aneurysm into the process of mechanical loads has not been reported yet.

Directional analysis of mechanical properties showed that for the adventitia and intima, higher values of all mechanical parameters except for Young's modulus (for the adventitia) and failure Cauchy stress (for the intima) were obtained in the longitudinal direction. The differences between the test directions ranged from 1% (failure force) to 40% (failure strain) for the adventitia and from 2% (failure displacement) to 40% (Young's modulus) for the intima. In the case of the media, the relationships between the directions were opposite, i.e., the values of all mechanical parameters were higher in the circumferential direction. The differences between the analyzed directions in terms of the values of the mechanical parameters obtained for the media ranged from 12% to 47%. It should also be emphasized that for each determined mechanical parameter and each layer, the differences between the analyzed directions were statistically significant ($p < 0.05$), but only in the case of the media are the mechanical properties higher in the circumferential direction, which is typical for healthy tissue. In the case of the other layers, the relationships between the directions are reversed and the values of mechanical parameters are higher in the longitudinal direction, indicating an impaired ability of the adventitia and intima to transfer mechanical loads in the circumferential direction. The reduction of the mechanical strength of the intima in the circumferential direction increases susceptibility of this layer to the effects of arterial blood pressure, which strains the vessel wall in the circumferential direction and thus may contribute to the damage of this layer, which, in turn, may be a predisposing factor for the development of dissection. The initiation of dissection within the intima was already noted in the works by Pasta [19] and Sokolis [23]. However, it should be emphasized that when dissection propagates in the aortic wall, dissection resistance of the vessel wall is also significantly determined by the mechanical properties of the interface between the layers. Kozuń [12] showed that the interface between the intima and the other layers is characterized by lowest peel strength

values and, therefore, is most susceptible to delamination, which additionally confirms the hypothesis about the initiation and development of dissection within the intima. These results are in contradiction with the results obtained by Sokolis [23] and Teng [26] according to whom the differences in the values of mechanical parameters between the directions for the adventitia and intima of the wall of ascending aortic aneurysm are not statistically significant. Discussions on this topic concern mainly entire blood vessel walls and include various factors affecting anisotropy, such as lesions [7], sites of specimen excision [8], vessel wall thickness or diseases [24]. The results obtained in this study, indicating anisotropy of mechanical properties of individual layers of the dissected aneurysmal wall, are surprising, given that earlier research by Kozuń [13] on the same test material showed no statistically significant differences between the longitudinal and circumferential directions in terms of the values of mechanical parameters of the entire vessel wall. The isotropic character of the mechanical properties was also demonstrated by the author in the case of the dissecting wall of a nonaneurysmal aorta [13].

5. Conclusions

The research conducted in this study show the dissection does not affect the mechanical strength of the adventitia. The value of this parameter of the adventitia is the highest in comparison with the media and intima what is typical for healthy tissue. It may suggest that the mechanical function of the adventitia in healthy ascending aorta and dissected ascending aorta aneurysm is the same, i.e., it protects the vessel against destruction. Histological analysis of dissected aorta wall layers shows fragmentation of elastin fibers and an accumulation of proteoglycans in the media. The intima of dissected ascending aorta aneurysm is included in the transfer of mechanical loads. Furthermore the results indicate that the mechanical properties of the dissected ascending aorta aneurysm are direction dependent. In the case of the adventitia and intima, the relationships between the directions are reversed in comparison with healthy vessel and the values of mechanical parameters are higher in the longitudinal direction, indicating an impaired ability of the adventitia and intima to transfer mechanical loads in the circumferential direction. There is an increased risk of rupture of the adventitia and intima of the dissected ascending aorta aneurysm.

Limitations

Our study is one of the few to provide information on the mechanical properties of the dissected wall of ascending aortic aneurysm. However, the layers were separated manually and histological analysis showed that the separation process was not always performed correctly. Most errors related to the intima. The specimens where the dissection process was not carried out correctly (e.g., the intima included smooth muscle cells typical of the media) were not considered in the analysis of the results. Nevertheless, it cannot be stated with absolute certainty that the layers were tested in isolation. In addition, the analysis of mechanical properties did not take into account the regions from which the specimens were excised [15].

Another limitation is that healthy tissues and non-dissected aorta aneurysm were not included in the research group. We compared the results obtained in this study with the results obtained for these tissues, presented in the literature. It should be emphasized that healthy tissues are collected post mortem. It could affect the mechanical properties of the wall. In addition, even if tissue is non-dissected, some structural changes (i.e., intimal hyperplasia) related to the aging process may be present. Moreover, the histopathological analysis was performed after the tensile tests. Therefore, some of the structural alterations (e.g., small fissures) might have artifactual nature. Nevertheless, the microscopic examination gave a general qualitative overlook on the structural elements of the specimens.

In the case of soft tissues, which include the blood vessel wall, the measurement of thickness is also a significant limitation. In the case of layers of the vessel wall, the thickness value varies by up to several dozen percent depending on the test site. This study attempted to verify the thickness of the examined layers using histological techniques. However, there are large discrepancies between the thickness results obtained by histological techniques and those obtained by a video extensometer due to the fact that prior to histological measurements, the examined tissues were formalin-fixed and paraffin-embedded, which led to their shrinkage and, consequently, differences in the thickness values obtained.

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