Heliyon 10 (2024) e23538

Contents lists available at ScienceDirect

Heliyon

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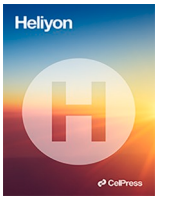

Insights into ascending aortic aneurysm: Interactions between biomechanical properties of the aortic wall and tissue biomarkers

Svetlana I. Sazonova [a,*], Viktor V. Saushkin [a], Dmitriy S. Panfilov [a], Ivan V. Stepanov [a], Anna M. Gusakova [a], Anatoliy B. Skosyrsky [b], Alexander V. Vrublevsky [a], Ayas O. Uvanchikov [a], Boris N. Kozlov [a]

[a] *Cardiology Research Institute, Branch of the Federal State Budgetary Scientific Institution «Tomsk National Research Medical Center of the Russian Academy of Sciences» (Cardiology Research Institute, Tomsk NRMC), 634012, Kievskaya 111 a, Tomsk, Russian Federation*
[b] *National Research Tomsk State University (TSU), Tomsk, Russian Federatio, 634050, Lenin Ave. 36, Tomsk, Russian Federation*

ARTICLE INFO

Keywords:
Ascending thoracic aortic aneurysm
Mechanical strength
Strain
Metalloproteinases
Interleukins
Cytokines

ABSTRACT

Background: It remains difficult to understand the association between the local mechanical properties of ascending thoracic aorta aneurysm (asTAA), its tissue, and its cellular and molecular changes. The purpose of our study was to investigate the relationship between biomechanical properties, histopathological findings, and tissue biomarkers of asTAA.
Methods: Intraoperative asTAA samples from 30 patients were studied. All samples were examined histologically and underwent a tensile test. We determined the tensile strength ($\sigma_в$, MPa), the strain (ε, mm/mm*%), and the area under the strength-strain curve (S) along with the concentrations of tissue matrix metalloproteinases (MMP-1 et al.) and their inhibitors, their interleukins (IL) -6 -10, and their tumor necrosis factor (TNF) -α.
Results: It was found that 43.3 % of asTAA patients had atherosclerosis, 3.3 % had aortitis, and 53.3 % of patients had connective tissue dysplasia. Differences in the studied parameters between these subgroups were not found. Age correlated with ε ($r = -0.49$) and S ($r = -0.54$). ε was also associated with media fibrosis degree ($r = -0.5$), collagen/elastin ratio ($r = -0.61$), and IL-10 ($r = 0.52$). IL-10 correlated with collagen/elastin ratio ($r = -0.58$), TNF-α ($r = 0.77$), and MMP-1 ($r = 0.71$).
Conclusion: Tissue IL-10 has a protective effect on the elastic structures of the aortic wall and is positively associated with the activity of MMP-1 and pro-inflammatory cytokines. IL-6 is associated with media fibrosis degree, and negatively affects strength-strain parameters of asTAA samples.

1. Introduction

Ascending thoracic aortic aneurysm (asTAA) is a potentially life-threatening pathology with a high risk of aortic-related deaths if left untreated. About 22 % patients with acute aortic syndrome die at home before they receive medical care. For people who reach a hospital alive, the in-hospital mortality is up to 34 % [1]. To date, surgical management of asTAA is the only way to prevent aortic-related events. According to the 2022 ACC/AHA Guideline for the Diagnosis and Management of Aortic Disease surgery is

* Corresponding author.
E-mail address: sazonova_si@mail.ru (S.I. Sazonova).

https://doi.org/10.1016/j.heliyon.2023.e23538
Received 20 July 2023; Received in revised form 6 December 2023; Accepted 6 December 2023
Available online 10 December 2023

indicated in asymptomatic patients with aneurysms of the aortic root or ascending aorta who have a maximum diameter of ≥5.5 cm, but this threshold may be reduced in several clinical situations [2]. For patients who do not meet the criteria for surgical correction the cardiovascular risk reduction, antihypertensive therapy, and the use of statins and beta-blockers are currently recommended [3]. However, there has been conflicting evidence about the efficacy of medical treatment of TAAs. [3], and there is still a need for more therapeutic options due to the heterogeneous and unpredictable progression of the disease. This situation is partly due to the fact that the asTAA pathogenesis is not clear enough, and molecular targets for the treatment of the disease have not been found yet. Hypothetically, several processes, including cystic medial necrosis, inflammation, matrix degradation, and remodeling, are involved in the development of aortic aneurysms of any localization [4,5]. All of these contributors are still being investigated [5,6]. It is thought that ineffective cell-matrix interactions make the vessel more vulnerable to increased hemodynamic load, and that asTAA rupture occurs because of complex biological reactions that are triggered in response to a local mechanical stress [7].

The pathogenesis of asTAA differs from aneurysms of descending localizations, presumably due to the peculiarities of its embryogenesis [4]. In particular, vascular smooth muscle cells (VSMCs) of the ascending aorta arise from neural crest cells, whereas the descending aortic VSMCs arise from the paraxial mesoderm [8]. Currently, there is no generally accepted unified concept of asTAA development, and asTAA is considered a complex multifactorial disease [3]. At the same time, there are several points that are not in doubt. In particular, it is known that asTAA is associated with genetic abnormalities and mutations. The involvement of fibrillin 1 in asTAA development has also been proven, but the mechanisms through which it acts are still unclear [6]. Presumably, the mutations in fibrillin 1 are functioning through dysregulation of transforming growth factor-β signaling [6]. The involvement of matrix metalloproteinases (MMP) in the asTAA development is also evident. They play an important role in both the degradation and the stabilization of the extracellular matrix [6]. The role of circulating miRNAs in the regulation TGF-β signaling pathway in the pathological ascending aorta was shown recently [9]. Other cellular mechanisms of asTAA formation, including mutations in the contractile proteins of VSMCs, genetic variants of the lysyloxidase, are discussed [6]. The mechano-biological theory of asTAA development is another important direction of research activity [7].

The direct ex vivo evaluation of the biomechanical properties of aortic specimens is another way to investigate asTAA pathogenesis, and it may prove the usefulness of new parameters with potential predictive value. Studies devoted to this issue have been actively performed in recent years. In paticular, Duprey A. et al., using biaxial loading conditions, showed the correlation between the extensibility of the tissue and the physiological elastic modulus, independently of the age, asTAA diameter or the aortic valve phenotype [10]. In the study of Martin et al., an elevated blood pressure and aortic tissue stiffening were found to be risk factors for asTAA rupture [11]. Nightingale M et al., suggested application of novel biomechanical testing parameters (low-strain tangential modulus and low-stress onset transition zone), which showed promise as measures of aortic function and tissue properties under physiologic loading conditions [12]. Both parameters correlated with elastin fragmentation, collagen alteration, tensile strength, and common supraphysiologic mechanical parameters in patients with asTAA [12].

At the same time, the association between local mechanical properties of asTAA and its changes at tissue, cellular, and molecular levels are still not fully understood. Meanwhile, the identification of such relationships can contribute to a more complete understanding of the pathogenesis of the disease, improve the risk stratification of asTAA patients, and reveal new therapeutic targets to recover mechano-sensitive remodeling of the matrix. Thus, the purpose of our study was to investigate associations between

Table 1
Baseline characteristics of enrolled patients (n = 30).

Parameter	Value
Age, years (Me) [Q1; Q3]	59 [29; 74]
Males (n (%))	16 (53.3)
Diabetes mellitus (n (%))	2 (6.7)
Arterial hypertension n (%)	19 (63.3)
Chronic heart failure (NYHA (I, II, III) (n (%))	10 (33.3)
Dyslipidemia (n (%))	10(33.3)
Bicuspid aortic valve	8 (26.7)
Tricuspid aortic valve	22 (73.3)
Diameter of ascending aorta (CT)	50,8 [45; 67,6]
LV EF, % Me [Q1; Q3]	65[29; 76]
LV EDV, ml Me [Q1; Q3]	108[12; 310]
LV ESV, ml Me [Q1; Q3]	36[19; 149]
Therapy (n=30)	
STATINs n (%)	9 (30.0)
ACEIs n (%)	8 (27.0)
ARBs n (%)	3 (10.0)
none	10 (33.0)
Indications for ascending aortic replacement (n=30)	
Maximum diameter of asTAA ≥5.0 cm, n (%)	17 (56.7)
Repair or replacement of an aortic valve + asTAA with a maximum diameter of ≥4.5 cm, n (%)	8 (26.7)
Growth rate of asTAA ≥0.3 cm/y in 2 consecutive years, or ≥0.5 cm in 1 year, n (%)	5 (16.6)

LV—Left ventricular; EF—ejection fraction; EDV—end diastolic volume; ESV—end systolic volume; CT—computed tomography angiography. ACEIs - angiotensin-converting-enzyme inhibitors; ARBs - *angiotensin receptor blockers;* asTAA - ascending thoracic aortic aneurysm.

biomechanical properties, histopathological findings, and tissue biomarkers of asTAA.

2. Methods and materials

2.1. Patients

From October 2021 to November 2022, asTAA intraoperative samples from 30 patients (16 men, 14 women, median age 59 [29; 74] years, with maximum diastolic diameter of the ascending aorta, according to computed tomography angiography (CT-angiography), 50.8 [45.0; 67.6] mm) were obtained and tested.

Inclusion criteria were: age 45–70 years, left ventricle ejection fraction >55 %, non-syndromic aortic diseases (idiopathic, familial), and indications for ascending aortic replacement [2], in particular.

1. Asymptomatic patients with aneurysms of the aortic root or ascending aorta who have a maximum diameter of ≥5.0 cm.
2. Patients undergoing repair or replacement of an aortic valve who have a concomitant aneurysm of the ascending aorta with a maximum diameter of ≥4.5 cm.
3. Patients with an aneurysm of the aortic root or ascending aorta of <5.0 cm, whose growth rate confirmed by tomograpic imaging is ≥ 0.3 cm/y in 2 consecutive years, or ≥0.5 cm in 1 year.

Exclusion criteria were: previous heart attack, previous stroke, cardiac arrhythmias, previous cardiac and aortic surgery, arterial hypertension (resistant to drug therapy), congenital heart diseases, and syndromic heritable thoracic aortic diseases (Turner, Marfan, Ehlers-Danlos, Loyes-Dietz syndromes, etc.). Baseline characteristics of the enrolled patients are presented in Table 1.

2.2. Sample preparation

Ascending aortic replacement was performed in all patients. Immediately after aneurysm resection, the excised aortic wall (asTAA sample, Fig. 1a) was delivered to the pathomorphological laboratory, where it was divided into three parts. One part was placed in a cooled ($+4C^0$) Krebs–Henseleit solution. In 2 h, the samples were prepared and subjected to uniaxial biomechanical testing to failure. The second part was sampled and frozen at −70° for subsequent biomarker testing. The third part was prepared for histological evaluation by the standard method, using a Thermo Scientific Excelsior AS histological wiring machine (Thermo Fisher Scientific, Charleston, SC, USA) and embedded in paraffin by the Tissue-Tek® TEC™ 6 embedding console system (Sakura, Japan).

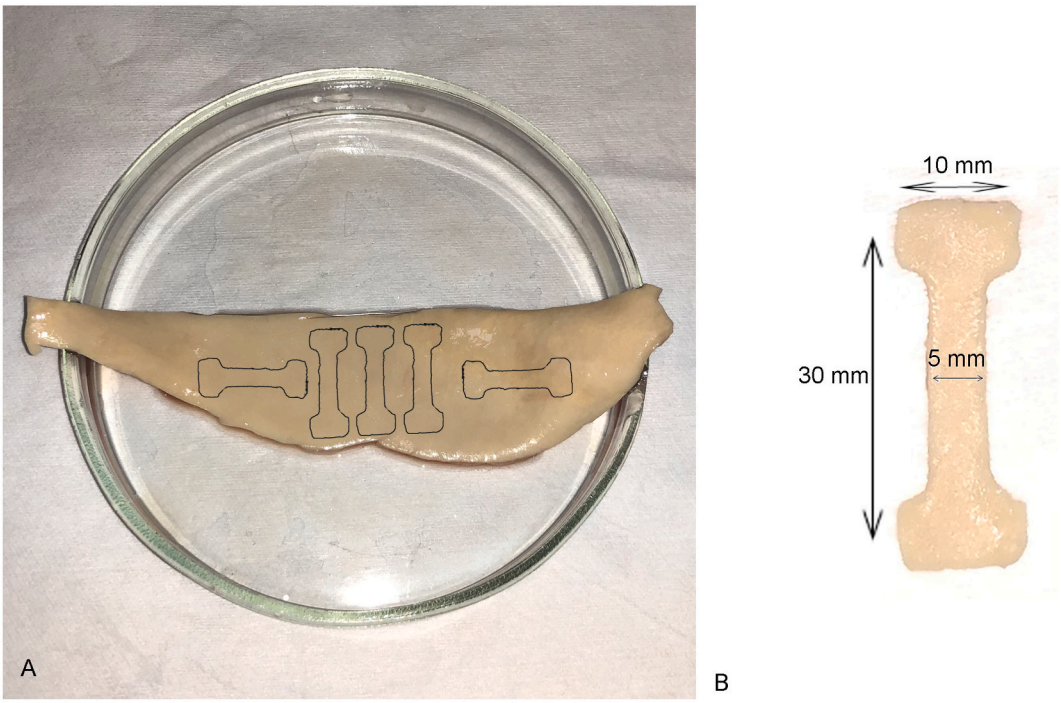

Fig. 1. (a, b). Preparation of ascending aorta samples for uniaxial tensile testing. A—Whole intraoperative sample of ascending aorta with a scheme for cutting out circumferential and longitudinal specimens. B—Excised ascending aortic specimen ready for tensile testing.

2.3. Mechanical properties

Biomechanical testing of ascending aorta specimens was performed as follows: a vessel fragment was cut along the lesser curvature, and a total of 3 specimens were then cut along the longitudinal and 2 were cut in circumferential directions, using a 3 × 1 cm stamp (Fig. 1b). The size of ascending aorta samples was defined in accordance with recommendations of previously published works [7]. The thickness of the samples was determined in mm using a binocular microscope microscope MC-5-ZOOM (Russia).

Uniaxial tensile testing of asTAA samples was performed on Instron 3343 testing machine (Great Britain). Each sample was fixed with atraumatic vascular clamps (Fig. 2a), which were securely fixed to the standard grips of the testing machine (Fig. 2b). The samples were stretched to break at a rate of 1 mm/min. The self-centering of the samples with respect to the load axis was provided by the upper standard grip with two degrees of freedom. Testing parameters are presented in Table 2.

For each sample, we determined the tensile strength (σ_B, MPa) as the maximum value on the Y axis in the graph (Fig. 3 a, b) and relative strain (ε, mm/mm ˣ %) as a percentage change in the length of the sample (along the X axis) during stretching until failure. Moreover, the area under the strength-strain curve (S) was determined automatically using Origin software (v 9.8, Origin Lab) (Fig. 3 a, b). The arithmetic means of all indicators were calculated both for longitudinal and circumferential samples of each patient.

2.4. Biomarkers assessment

First, the aortic samples were homogenized in a lysis buffer (Invitrogen) with the addition of a protease inhibitor cocktail and 1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma-Aldrich). After homogenization, the lysates were centrifuged for 10 min at a speed of 5000 g and stored at −40 °C in order to determine the concentration of tissue matrix metalloproteinases (MMP) types 1, 2, and 7

Fig. 2. (a, b). The mechanometric testing of aortic tissue sample. A—Tissue sample of the ascending aorta, fixed in atraumatic clamps. B—Instron 3343 testing machine with a sample of aortic tissue.

Table 2
Testing parameters.

Parameter	Measuring range	Value determination accuracy
Tension, N	0–50	±0,5 %
Traverse travel speed, mm/min	0,005-500	±0,2 %
Linear resizing, mm:	15–1000	0,05 %
Stretching	0–1000	
Contraction		

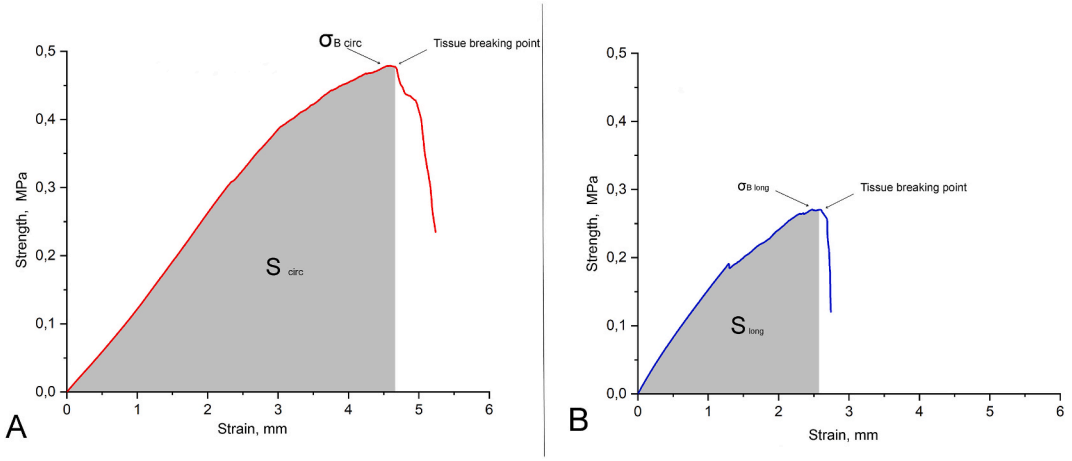

Fig. 3. (a, b). Graphs (loading diagrams) showing the change in the length of ascending aorta samples depending on the tensile stress (MPa) generated by the Instron 3343 testing machine. The arrows indicate the peaks of the curves corresponding to the tensile strength of the samples (σ_B) and break points. A—Loading diagram for circumferential sample; B—Loading diagram for longitudinal sample. $\sigma_{B\ circ}$—Tensile strength for circumferential sample. S_{circ} p—Area under the strength-strain curve for circumferential sample. $\sigma_{B\ long}$—Tensile strength for longitudinal sample. S_{long}—Area under the strength-strain curve for longitudinal sample.

(MMP-1, MMP-2, MMP-7), their inhibitors (TIMP) of types 1 and 2 (TIMP-1 and TIMP-2), interleukin 1β (IL-1β), interleukin 6 (IL-6), interleukin 10 (IL-10), and tumor necrosis factor alpha (TNF-α).

The concentration of MMP and their inhibitors in thawed aortic lysates was determined by enzyme-linked immunosorbent assay (ELISA) using commercial test systems from CUSABIO Biotech (for MMP-2, MMP-7, TIMP-2), RayBioTech (for MMP-1, TIMP-1, IL-6, IL-10, TNF-α) and QayeeBio-Technology (for IL-1β) according to the methods described in the instructions. The coefficient of variation (CV) was less than 10 %. The measurement of optical densities, calibration plotting, and the evaluation of the results were performed using an Infinite F50 microplate reader and Magellan Tracker software.

2.5. Histological evaluation

From the paraffin blocks, 3–4 μm thick microtome sections were made using a Thermo Scientific HM355S (USA) rotary microtome. Each section was stained with hematoxylin and eosin on a Leica ST5010 AXL automatic histostainer (Germany). Routine microscopy

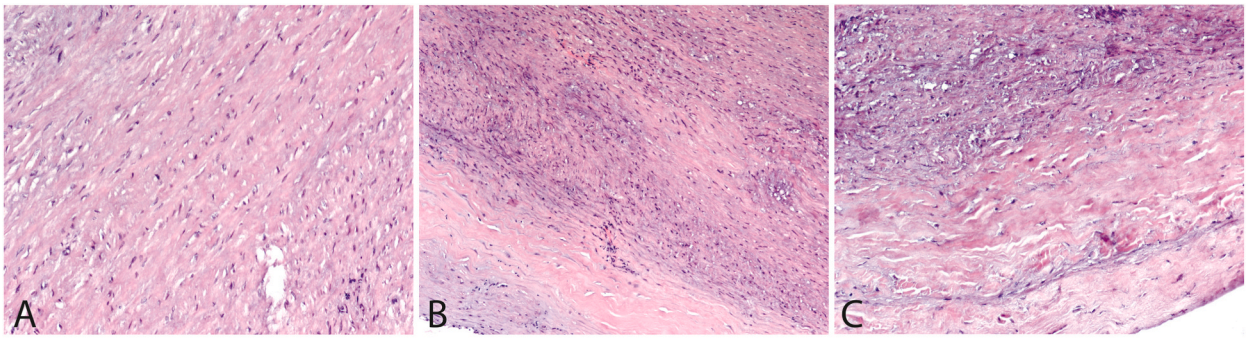

Fig. 4. (a, b, c). Various degrees of fibrosis in the ascending aorta wall. Hematoxylin and eosin staining; magnification 200×. A—Weak fibrosis; B—moderate fibrosis; C—severe fibrosis.

was performed on a Zeiss AxioImager M2 microscope (Germany) in a bright field. Digital scanning of histological specimens was performed on a Leica Aperio AT2 histoscanner (Germany) located in the Core Facility « Medical genomics», Tomsk NRMC. The presence or absence of atherosclerotic lesions in the studied aortic aneurysm fragments were assessed and classified according to the American Heart Association classification [13]. Furthermore, the presence of inflammation was estimated, and inflammatory cells (lymphocytes, macrophages) were counted in 10 fields of view (using 40× objective lens) in a bright field. Depending on the proportion of the connective tissue in the aortic sample, the severity of fibrosis was classified as weak (connective tissue less than 1/6 of an asTAA sample thickness), moderate (connective tissue from 1/6-1/2 of an asTAA sample thickness), or severe (connective tissue more than ½ of an asTAA sample) (Fig. 4 a,b,c). For differential staining of collagen and elastin, the Picro-Mallory method (Ergo Production, Russia) was used. Collagen fibers were stained in dark blue and elastic fibers were stained with a stain varying from pink to orange color (Fig. 5 a,b,c). The spreading and staining intensity of both connective tissue components was assessed visually on a scale from 1 to 3 and the collagen/elastin ratio was calculated.

Considering the histological data, we divided samples into 2 subgroups—samples with morphological signs of atherosclerosis (Subgroup 1) and samples without morphological signs of atherosclerosis (Subgroup 2). We then compared biomechanical parameters and levels of tissue biomarkers in these subgroups.

2.6. Statistical analysis

Statistical data processing was performed using Statistica 10 software (StatSoft, Inc., USA). The Shapiro–Wilk test was used for testing the normality of the distribution of the variables. Since the normal distribution was not confirmed for all the quantitative data, the results were presented as a median (Me) and quartiles (Q1; Q3). Statistical significance of quantitative data in Subgroup 1 (samples with morphological signs of atherosclerosis) vs Subgroup 2 (samples without morphological signs of atherosclerosis) was determined using the non-parametric U–test (Mann–Whitney). Since each patient's asTAA specimen was subjected to tensile testing, biomarker ELISA testing, and histopathological examination, the search for relationships between these parameters was performed with Spearman correlation. Differences were considered significant at $p < 0.05$.

3. Results

According to the results of a histological evaluation, atherosclerotic lesions of the ascending aortic wall were detected in 13 of 30 cases (43.3 %), aortitis was detected in 1 patient (3.3 %).

Considering the etiopathogenetic heterogeneity of the study group, the patient with aortitis was excluded from the research. In addition, we compared, using the Mann–Whitney *U* test, biomechanical parameters and tissue biomarkers in the patients with atherosclerosis of the aorta (Subgroup 1) and without atherosclerosis of the aorta (Subgroup 2). Since no significant differences were found (Table 3), the subsequent correlation analysis was performed on combined data of both subgroups (Table 4).

The medians of the biomechanical parameters of asTAA specimens, concentrations of tissue cytokines, metalloproteinases, and their inhibitors are all presented in Table 3. The levels of aortic tissue biomarkers defined in the current study are consistent with our data published previously [14].

None of the studied characteristics correlated with the ascending aortic diameter. At the same time, we found a moderate negative correlation between ε_{circ} and $S_{circ,}$ with age (Fig. 6(a–f)). Additionally, ε_{circ} was negatively associated with media fibrosis degree ($r = 0.-050$) and collagen/elastin ratio ($r = 0.-0.61$), and it was positively correlated with Il-10 tissue concentration ($r = 0.52$) (Fig. 6(a–f)). At the same time, IL-10 showed a strong negative correlation with collagen/elastin ratio ($r = 0.-0.58$), and a strong positive correlation with TNF-α ($r = 0.77$) and MMP-1 ($r = 0.71$), which has collagenolytic activity (Fig. 7(a–e)).

Parameter S_{circ} demonstrated a moderate negative correlation ($r = 0.-0.55$) with tissue Il-6 (Fig. 6), which also strongly positively correlated with media fibrosis degree ($r = 0.63$) and moderately correlated with MMP-1 ($r = 0.46$) (Fig. 7a-e).

Other tissue biomarkers, such as Il-1β, MMP-2, MMP-7, TIMP-1, and TIMP-2, showed no correlations nor with parameters of

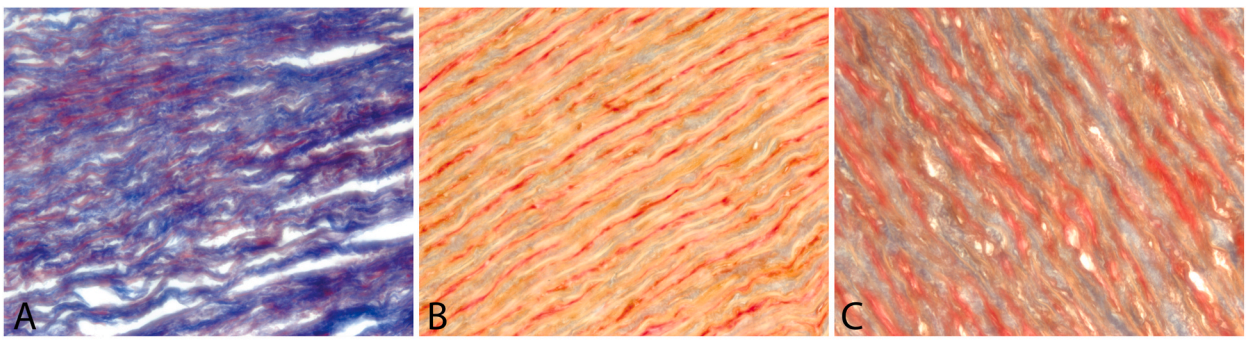

Fig. 5. (a, b, c). Different ratios of collagen and elastin in the thoracic aorta wall. Picro-Mallory staining, magnification 400×. A—Predominance of collagen fibers in the aortic wall (blue color), collagen/elastin 3:1; B—predominance of elastic fibers (yellow-orange color), collagen/elastin 1:3; C—the same ratio of collagen and elastin in the thoracic aorta wall, collagen/elastin 1:1.

Table 3
Tensile strength of intraoperative samples of the ascending aorta, as well as concentrations of cytokines, matrix metalloproteinases, and their inhibitors in the studied samples.

Parameter	Whole group, n = 29 Me (Q1; Q3)	Subgroup 1, n = 13 Me (Q1; Q3)	Subgroup 2 n = 16 Me (Q1; Q3)	P-value
$\sigma_{в\ long}$, MPa	0.64 (0.48; 0.77)	0.60 (0.45; 0.70)	0.69 (0.5; 0.82)	0.52
$\sigma_{в\ circ}$, MPa	0.81 (0.55; 1.27)	0.99 (0.8; 1.13)	0.70 (0.50; 1.53)	0.57
ε_{long}, mm/mm * %	0.45 (0.34; 0.51)	0.44 (0.33; 0.57)	0.45 (0.40; 0.49)	1.0
ε_{circ}, mm/mm * %	0.43 (0.37; 0.62)	0.43 (0.37; 0.67)	0.46 (0.38; 0.62)	0.97
S_{long}	15.53 (6.84; 20.89)	15.60 (6.17; 20.89)	15.53 (6.84; 21.82)	0.93
S_{circ}	22.08 (14.83; 35.56)	24.90 (16.87; 33.39)	17.85 (10.48; 43.35)	0.42
Il-1β, pg/ml	81.45 (78.86; 83.85)	81.16 (77.17; 85.56)	82.22 (79.68; 83.51)	0.41
Il-6, ng/ml	1.26 (1.14; 1.31)	1.31 (1.17; 1.36)	1.23 (1.13; 1.28)	0.31
Il-10, pg/ml	5.67 (4.19; 6.24)	4.89 (3.75; 6.10)	5.77 (4.88; 6.41)	0.34
TNF-α, ng/ml	2.33 (1.81; 2.67)	1.93 (1.44; 2.67)	2.43 (2.02; 2.64)	0.53
MMP-1, pg/ml	4.81 (2.66; 10.17)	3.63 (1.69; 10.31)	6.55 (3.33; 10.1)	0.34
MMP-7, ng/ml	1.63 (0.98; 3.82)	1.45 (0.68; 2.78)	2.00 (1.14; 3.88)	0.34
MMP-2, ng/ml	82.9 (58.1; 149.4)	61.51 (57.1; 111.3)	64.4 (58.58; 111.29)	0.23
TIMP-1, ng/ml	207.2 (167.54; 279.59)	266.86 (201.0; 279.59)	0.60 (0.45; 0.70)	0.09
TIMP-2, ng/ml	10.96 (5.19; 14.86)	10.96 (6.85; 14.07)	10.54 (4.92; 16.2)	0.82

$\sigma_{в\ long}$—Ultimate tensile stress at failure for longitudinal aorta specimens; $\sigma_{в\ circ}$—ultimate tensile stress x at failure for circumferential aorta specimens; ε_{long}—ultimate strain at failure for longitudinal aorta specimens; ε_{circ} —ultimate strain at failure for circumferential aorta specimens; S_{long}—area under the stress-strain curve for longitudinal aorta specimens; S_{circ}—area under the stress-strain curve for the circumferential aorta specimens; Il—interleukin; TNF-α—tumor necrosis factor α; MMP—matrix metalloproteinase; TIMP—tissue inhibitor of metalloproteinases; P—value is shown for difference between Subgroup 1 and Subgroup 2.

Table 4
Correlations between the tensile strength, tissue biological markers, diameter of ascending aorta and patient's age (n = 29).

Parameter	Age (years)	*AA diameter (mm)*	*Media fibrosis degree*	*Collagen/ elastin*	*Il-1β, pg/ml*	*Il-6, ng/ml*	*Il-10, pg/ml*	MMP-2, pg/ml	MMP-7, ng/ml	TIMP-1, ng/ml	TIMP-2, ng/ml
$\sigma_{в\ long}$	–	–	–	–	–	–	–	–	–	–	–
$\sigma_{в\ circ}$	–	–	–	–	–	–	–	–	–	–	–
ε_{long}	–	–	–	–	–	–	–	–	–	–	–
ε_{circ}	r = −0.49	–	r = −0.5	r = −0.61	–	–	r = 0.52	–	–	–	–
S_{long}	r = −0.50	–	–	–	–	–	–	–	–	–	–
S_{circ}	r = −0.54	–	–	–	–	r = −0.55	–	–	–	–	–

AA - ascending aorta; «- » - no statistical significance; r - the Spearman correlation coefficient at p-level <0.05. $\sigma_{в\ long}$ – ultimate tensile stress index at failure for longitudinal aorta specimens; $\sigma_{в\ circ}$ - ultimate tensile stress index at failure for circumferential aorta specimens; ε_{long} – ultimate strain index at failure for longitudinal aorta specimens; ε_{circ} – ultimate strain index at failure for circumferential aorta specimens; S_{long} - area under the curve « stress-strain » for longitudinal aorta specimens; S_{circ} - area under the curve « stress-strain » for circumferential aorta specimens; Il – interleukin; TNF-α – tumor necrosis factor α; MMP - matrix metalloproteinase; TIMP - tissue inhibitor of metalloproteinases.

mechanometric testing, nor with histopathological findings (Table 4).

4. Discussion

The thoracic aortic aneurysm formation is a complex process involving various cell types and molecular regulatory systems. Over the past decade, significant progress has been made in understanding the molecular biology of thoracic aortic aneurysm, and it has been established that this pathology is associated with inflammation and degradation of the extracellular matrix (ECM) [6]. Atherosclerosis is not considered a leading cause of asTAA, unlike pathophysiological mechanisms underlying an abdominal aorta aneurysm. However, in the presented study, atherosclerotic changes in the asTAA tissue were detected in almost half (43.3 %) of the enrolled patients. At the same time, we did not find any significant differences between the subgroups with and without morphological signs of atherosclerosis in terms of strength and extensibility, as well as in the concentrations of tissue cytokines and metalloproteinases. These results may indicate that systemic atherosclerosis, asTAA development, and related aortic complications are independent processes. The above assumption is in line with the recent study of Grewal et al. (2023), where the majority of enrolled patients with asTAA dissections demonstrated nonprogressive intimal lesions, whereas the control group (postmortem thoracic aorta specimens) mostly demonstrated progressive intimal atherosclerotic lesions. The authors concluded that patients with ascending aortic dissection hardly exhibited any form of progressive atherosclerosis [15]. At the same time, our results, concerning MMP-2, are inconsistent with the previously published data, which showed that atherosclerosis is accompanied by an increase in MMP-2

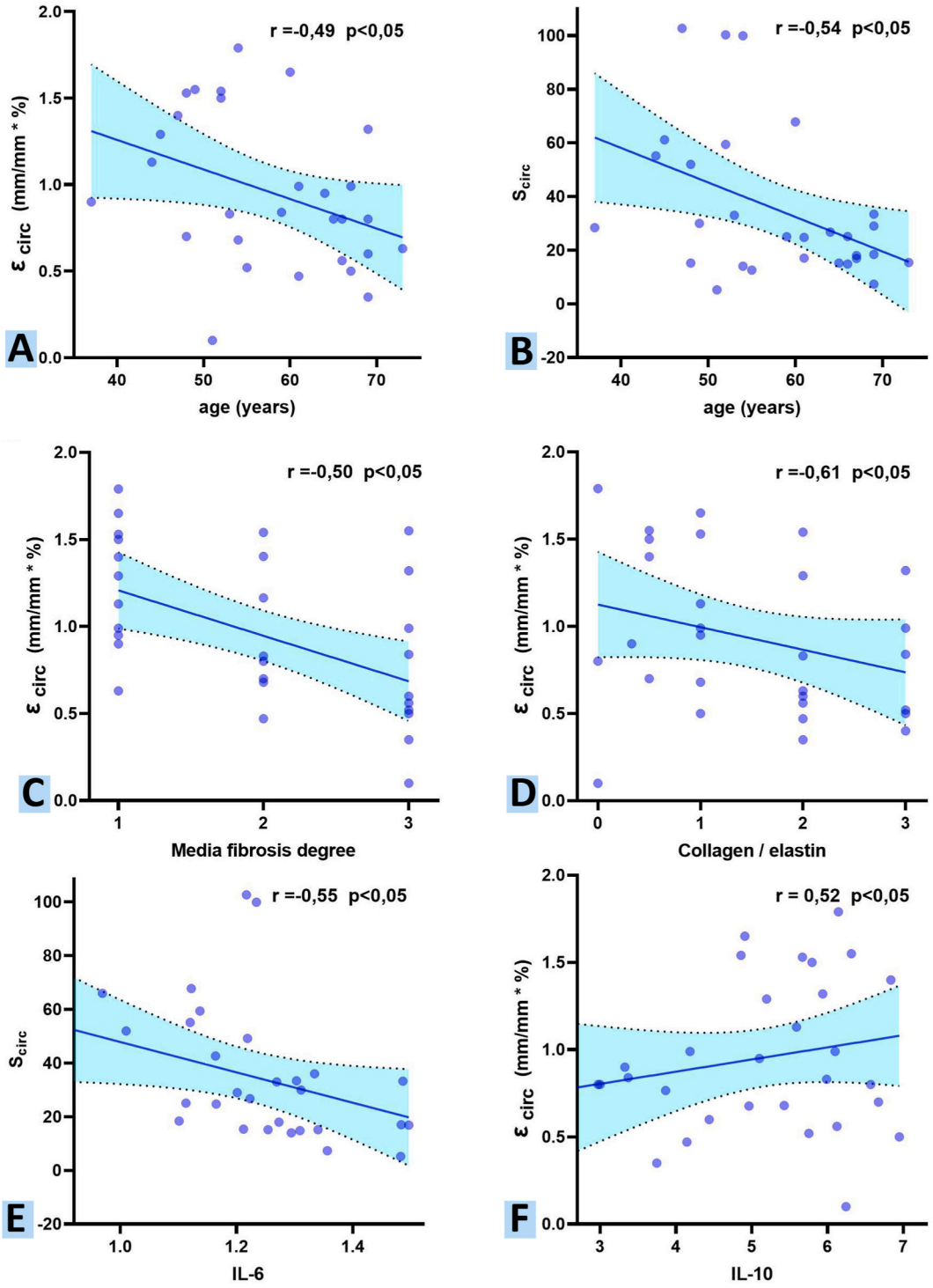

Fig. 6. (a–f). Correlations between the biomechanical parameters of asTA specimens, patient's age, tissue interleukins, and histopathological findings (n = 29). A – scatterplot of ε_{circ} vs age; B – scatterplot of S_{circ} vs age; C – Scatterplot of ε_{circ} vs media fibrosis degree; D - scatterplot of ε_{circ} vs collagen/elastin; E – scatterplot of S_{circ} vs Il-6; F - scatterplot of ε_{circ} vs Il-10. ε_{circ} —ultimate strain at failure for circumferential aorta specimens; S_{circ}—area under the stress-strain curve for the circumferential aorta specimens; Il-6 —interleukin-6; Il-10 —interleukin-10; asTA—ascending thoracic aorta.

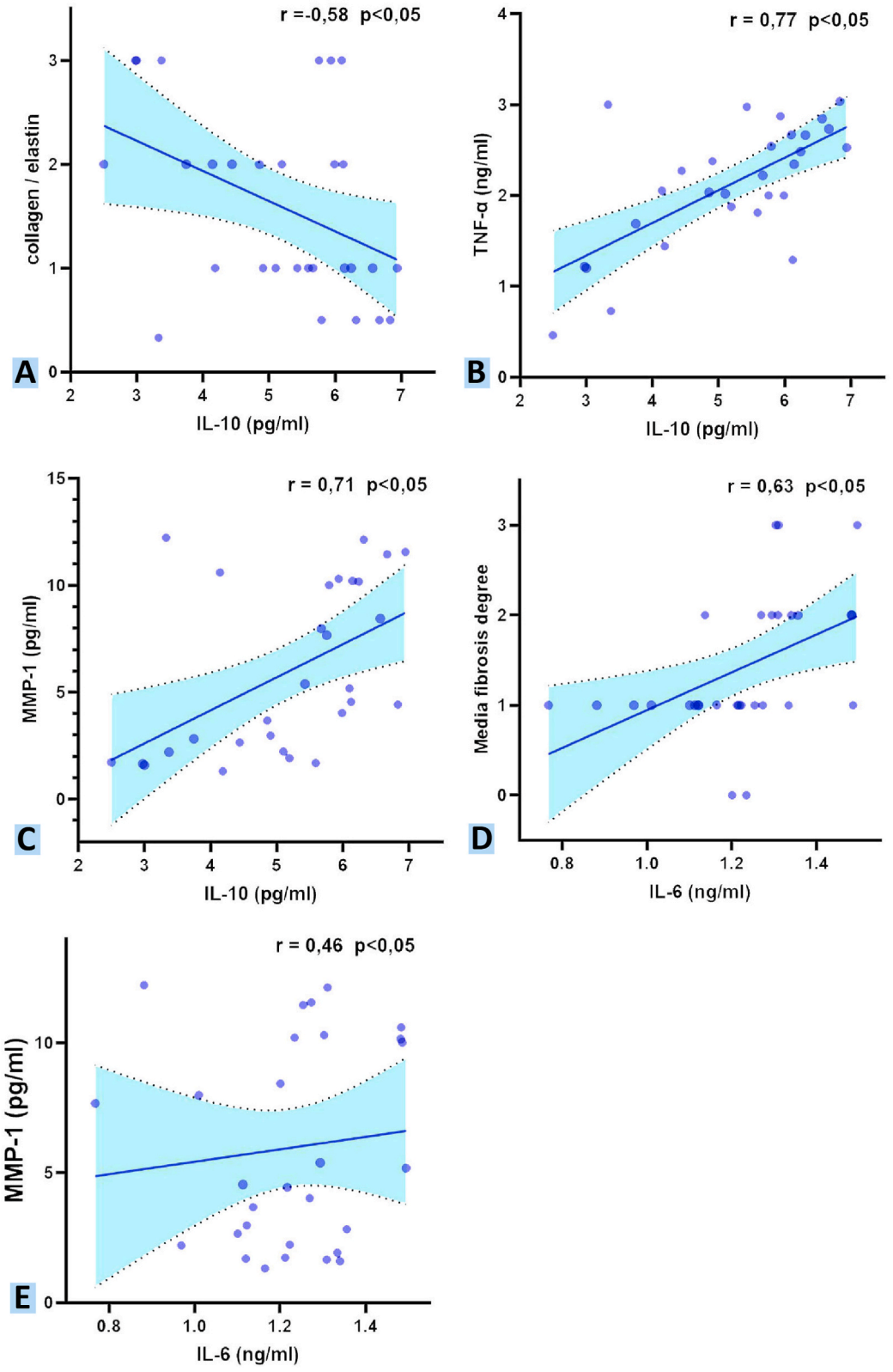

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Fig. 7. (a–e). Correlations between tissue cytokines, MMP-1, and collagen/elastin ratio of asTA specimens (n = 29). A - scatterplot of collagen/elastin vs Il-10; B - scatterplot of TNF-α vs Il-10; C - scatterplot of MMP-1 vs Il-10; D - scatterplot of media fibrosis degree vs Il-6; E – scatterplot of MMP-1 vs Il-6. Il-6 —interleukin-6; Il-10 —interleukin-10; TNF-α — tumor necrosis factor α; asTA—ascending thoracic aorta; MMP-1—matrix metalloproteinase 1.

concentration in the asTAA tissue in comparison with non-atherosclerotic aneurysm samples [16]. This issue requires further research.

Another result of the current study was the established negative correlation between parameters related to aortic strain (ε_{circ}, S_{long}, S_{circ}) and the age of patients. These results are in good agreement with the world experience concerning the study of the effect of aging on the mechanical properties of the vessel. In particular, several studies have shown that the elasticity and mechanical strength of the aorta decrease with increasing age [10,17].

In the presented study, the concentrations of some pro- and anti-inflammatory cytokines as well as different types of MMPs (collagenases, gelatinases, and stromelysins) in asTAA tissue were determined; the established levels of biomarkers were consistent with the data published earlier [14,16].

We have found a direct correlation between the strain of circumferential aortic samples (ε_{circ}) and the concentration of the anti-inflammatory cytokine IL-10, which once again confirms the hypothesis of the negative effect of inflammation on the structural and functional state of the aortic wall [18,19] and corresponds with our previous research results [14]. It should be noted that the role of IL-10 in the pathogenesis of asTAA is currently not well understood. At the same time, in experimental models of abdominal aortic aneurysm, it has been shown that IL-10 reduces inflammation in the aneurysm tissue, thereby promoting the proliferation and phenotypic transformation of smooth muscle cells and inhibiting the rupture of elastic fibers [20]. Probably, the same effects are also characteristic of the asTAA tissue. In the presented study, IL-10 also demonstrated a strong negative correlation with collagen/elastin ratio and strong positive correlation with MMP-1, which is involved in the processes of collagen degradation. This interaction is likely mediated by the effect of TNF-α on the activity of the anti-inflammatory cytokine. In particular, TNF-α was strongly associated with IL-10, MMP-1, and had a weak negative correlation with the collagen/elastin ratio.

The role of IL-6 in the asTAA formation is understood better. It was shown earlier that asTAA human tissue exhibits increased IL-6 levels [21]. Therefore, it is considered that IL-6 is secreted by smooth muscle cells after activation of angiotensin type 1 receptor activation by angiotensin 2 [19]. In the current study, the tissue concentration of IL-6 negatively correlated with the integral biomechanical parameter of circumferential aortic wall samples (S_{circ}) while demonstrating a strong positive correlation with media fibrosis degree and MMP-1. These results illustrate the effect of the pro-inflammatory cytokine on the biomechanical properties of the aortic wall through the upregulation of the matrix metalloproteinase system and related collagen degradation.

It is known that another strong proinflammatory cytokine IL-1β plays an important role in the medial degeneration and asTAA growth modulation [19]. However, in our study, no correlations of tissue IL-1β with biomechanical parameters of ascending aorta specimens were found.

Furthermore, no correlation between a maximum diameter of the vessel and the biomechanical parameters was revealed. These results are consistent with data obtained by Martin et al., who evaluated passive elastic mechanical properties of asTAA samples using biaxial mechanical and uniaxial failure tests [11]. Currently, the main criterion for surgical intervention in patients with dilatation of the thoracic aorta is the diameter of the vessel and the rate of its increase [22]. The basis for using these parameters in assessing the individual risk of aortic complications (dissection, rupture) is Laplace's law. According to the law, the circumferential load depends on the aortic radius and the pressure exerted on its wall [9]. At the same time, this statement does not take into account the mechano-biological properties of the aortic tissue, and, therefore, a large number of cases of acute aortic syndrome occur in patients with an aortic diameter of less than 55 mm [23]. This suggests that aortic diameter is only one of many components that influence an asTAA predisposition to rupture, and our results indirectly confirm this assumption. In addition to the diameter, other criteria for the assesment of the risk of aortic rupture have been proposed by researchers. Thus, Duprey et al., defined a rupture risk based on the extensibility of the aortic tissue and showed that this rupture risk is strongly correlated with the physiological elastic modulus of the tissue independently of the age, asTAA diameter or the aortic valve phenotype of the patient [10].

The obtained basic results can be extrapolated to a clinical practice as follows. Firstly, the fact established in the study that the age negatively affects the strength of the aortic wall, additionally confirms the advisability of a replacement of asTAA with a diameter of less than 5.5 cm, without waiting for reaching the current surgical threshold. The issue of surgical threshold decreasing is now being actively investigated, since the original studies showed that significant proportion of patients with type A aortic dissection had asTAA diameters <5.5 cm [2].

Secondly, the present study established a negative effect of IL-6 on the strength and structure of a human aortic wall. Thereby, receptors for IL-6 can be considered as a potential therapeutic target in patients, who are not undergoing surgical repair. This assumption is supported by the results of a recently published experimental study in which satralizumab, an FDA-approved IL-6 receptor blocker, slowed the progression of a descending thoracic aortic aneurysm [24]. The authors suggest clinical trials of satralizumab to be considered for this purpose.

The study has several limitations, including small sample size, and no control specimens of non-asTAA. We did not use non-TAA samples as control for ethical reasons, as the excision of the aneurysm is performed with the maximum preservation of healthy tissue. Moreover, a part of the enrolled patients were on angiotensin-converting-enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) before surgery. This could also affect the result, because both ACEIs and ARBs, in addition to the systemic hypotensive effect, protect an aortic wall [3]. In particular, it was found that ACEIs may decrease SMC apoptosis [25], and ARBs may be of benefit in decreasing aneurysm expansion by antagonizing TGF-β [26,27]. The use of statins by some of enrolled patients is also a limitation of

the study, although effect of these medications on asTAA is not proved [3]. Statins have been mostly studied in the context of abdomen aortic aneurism [28].

5. Conclusion

Our findings emphasize that that IL-10 is negatively associated with collagen/elastin ratio in asTAA while having a positive correlation with asTAA strain in the circumferential direction and a strong positive correlation with tissue MMP-1 and TNF-α. These data support the hypothesis about the protective influence of IL-10 on the elastic structures of the aortic wall occurring in response to inflammation and increasing tissue concentrations of TNF-α and MMPs. The negative effect of pro-inflammatory cytokines on the biomechanical characteristics of the thoracic aortic wall in our study was confirmed by a negative correlation between tissue IL-6 and the integral strength-strain parameter of circumferential specimens S_{circ}. This cytokine also positively correlates with media fibrosis degree and tissue MMP-1, showing its participation in MMP-1 activation and processes of collagen degradation and medial necrosis.

Ethics approval and consent to participate

All included patients gave written consent to participate in the study and agreement for publishing of their internal scans. The study was approved by the Local Ethics Committee (protocol No. 213, dated May 12, 2021) and conducted in accordance with the ethical standards of the Helsinki Declaration revised in 2008.

Consent for publication

Not applicable.

Data availability statement

Data will be made available on request.

Funding

The study was supported by the Russian Science Foundation grant No. 21-15-00160, https://rscf.ru/project/21-15-00160/

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Svetlana I. Sazonova: Writing – original draft, Funding acquisition, Conceptualization. **Viktor V. Saushkin:** Validation, Investigation, Formal analysis, Data curation. **Dmitriy S. Panfilov:** Investigation, Formal analysis, Data curation, Conceptualization. **Ivan V. Stepanov:** Methodology, Investigation, Formal analysis, Data curation. **Anna M. Gusakova:** Validation, Methodology, Investigation, Formal analysis, Data curation. **Anatoliy B. Skosyrsky:** Validation, Software, Investigation, Formal analysis, Data curation. **Alexander V. Vrublevsky:** Visualization, Resources, Project administration. **Ayas O. Uvanchikov:** Resources, Data curation. **Boris N. Kozlov:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Svetlana Sazonova reports financial support was provided by Russian Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Abbreviations

asTAA	ascending thoracic aorta aneurysm
asTA	ascending thoracic aorta
IL	interleukin
TNF-α	tumor necrosis factor α

MMP	matrix metalloproteinases
TIMP	tissue inhibitor of metalloproteinases
VSMCs	vascular smooth muscle cells
CFD	computational fluid dynamics
BCs	boundary conditions
CT	computed tomography
LV	Left ventricular
EF	ejection fraction
EDV	end diastolic volume
ESV	end systolic volume

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