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Natural history of the aortic wall changes in adults with the degenerative tricuspid aortic valve stenosis: the morphometric proofs and implications for echocardiography

Promene aortnog zida kod odraslih osoba sa degenerativnom stenozom trikuspidnog aortnog zaliska: morfometrijski dokazi i implikacije za ehokardiografiju

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Abstract

Background/Aim. So far, no study has been focused exclusively on the tricuspid aortic valve stenosis (TAV) in the aorta without severe dilatation and none has aimed at correlating the high mycroscopy findings with the echocardiographic parameters. This research was conducted on the postulate that detecting the histopathological changes of different severity in the aortic wall could tailor decision about an aortic surgery. The aim of this study was to grade the histopathological changes in the wall of the nonseverely dilated ascending aorta in patients with the severe, calcific TAV stenosis and to correlate them with the echocardiographic parameters in order to analyze when the ascending aorta should be replaced simultaneously with the aortic valve replacement (AVR). **Methods.** The samples from 37 patients subjected to the AVR and the samples from the control group were analyzed morphologically. The echocardiographic parameters obtained in the TAV stenosis patients were preoperatively correlated with the morphological data, age and gender, diameters of the ventriculo-aortic junction (AA), the sinus Valsalvae (SV) and sinotubular junction (STJ), the largest diameter of the visualized ascending aorta (AscA), the sinus Valsalvae index (SVI) and AscA/AA index. **Results.** We confirmed morphometrically the exact region of the hemodynamic stress influence with the mathematical distinction in comparison to the controls. In this region, the gradual elastic lamellae disruption was proved by a statistically significant difference through the 3 grades. The elastic skeleton alterations were potentiated with aging and in females. The morphometric parameters of the ascending aorta wall statistically significantly correlated with the echocardiographic parameters: AA, SV, AscA and SVI. The echocardiographic parameters tended to be higher in the most severe grade 3, in the patients younger than 65 years of age. The AscAof more than 4.5 cm was associated with the irreversible morphological defects in these patients. **Conclusion.** The hemodynamic stress induced by the TAV stenosis leads to the ascending aorta elastic lamellae disruption that could be histopathologically graded and correlated with the echocardiographic parameters of the ascending aorta providing a potential tool for decision-making process in cases when the ascending aorta replacement is considered simultaneously with the AVR.

Key words:
aorta; aging; aortic valve stenosis; echocardiography; heart valve prosthesis; histological techniques; tissues.

Apstrakt

Uvod/Cilj. Istraživanja usmerena isključivo na stenozu trikuspidalnog aortnog zaliska aorte bez teške dilatacije nisu rađena, kao što nijedna studija nije imala za cilj korelaciju nalaza svetlosne mikroskopije sa ehokardiografskim parametrima. Ovo istraživanje je sprovedeno na osnovu pretpostavke da nalaženje patohistoloških promena u zidu aorte, različitog stepena težine, može uticati na kreiranje strategije operisanja aorte. Cilj rada je bio da se kod bolesnika sa

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teškom degenerativnom stenozom trikuspidnog aortnog zaliska stepenuju patohistološke promene zida ascendentne aorte, koja nije bila značajno dilatirana i korelišu sa ehokardiografskim parametrima, u cilju procene pogodnog vremena simultane zamene ascedentne aorte i aortnog zaliska. **Metode.** Morfološkom analizom je bilo obuhvaćeno 37 uzoraka, uzetih od bolesnika kod kojih je vršena operacija zamene aortne valvule i od kontrolne grupe ispitanika. Ehokardiografski parametri [dijametri aorte na nivou ventrikuloaortnog spoja (AA), sinusa Valsalve (SV) i sinotubularnog spoja (STJ), najveći dijametar ascendentne aorte (AscA), indeks sinus Valsalve (SVI) i AscA/AA indeks], izmereni kod bolesnika sa stenozom aortne valvule, bili su korelisani sa morfološkim nalazom, starošću i polom ispitanika. **Rezultati.** Morfometrijskom analizom potvrđeno je da su reprezentativni uzorci prikupljeni iz regiona najvećeg hemodinamskog stresa. Potvrđeno je progresivno oštećenje elastičnih lamela u tri različita gradusa. Potvrđeno je veće oštećenje elastičnih lamela kod starijih osoba i kod žena. Morfometrijski parametri zida ascendentne aorte korelisali su značajno sa ehokardiografskim parametrima: AA, SV, AscA i SVI. Vrednosti izmerenih ehokardiografskih parametara bile su veće kod bolesnika sa najtežim gradusom morfometrijskih promena (gradus 3), mlađih od 65 godina. Vrednost AscA veća od 4,5 cm bila je udružena sa ireverzibilnim morfološkim promenama. **Zaključak.** Hemodinamski stres uzrokovan stenozom aortnog zaliska dovodi do oštećenja elastičnih lamela zida aorte koja se mogu gradirati patohistološki i korelisati sa ehokardiografskim parametrima ascendentne aorte što obezbeđuje dodatni kriterijum za odlučivanje kod onih bolesnika kod kojih se razmatra zamena aortnog zaliska istovremeno sa zamenom asendentne aorte.

Ključne reči:
aorta; starenje; zalistak, aortni, stenoza; ehokardiografija; zalisci srca, veštački; histološke tehnike; tkiva.

Introduction

The influence of a stenotic jet and the severity of the tricuspid aortic valve (TAV) stenosis to the aortic wall changes and to the aortic root and ascending aorta parameters have not been explicitly and unambiguously determined [1]. Only few studies investigated the histopathological defects of the elastic skeleton in patients with the aortic valve dysfunction with a limited number of the elastic skeleton parameters [2–5]. However, none was focused exclusively on the TAV stenosis in the aortas without severe dilatation, and none aimed at correlating the light microscopy findings with the echocardiographic parameters.

In the present study, we investigated the spectrum of structural changes in the ascending aorta walls of the patients with the severe degenerative, calcific aortic stenosis of the TAV. Recognition of the evolution of the aortic wall changes due to the aortic stenosis is essential for tailoring the surgical guidelines for the degenerative aortic stenosis treatment. The main goal of our study was to evaluate whether and when the replacement of the ascending aorta is warranted simultaneously with the aortic valve replacement (AVR).

A design of the study was led by several questions. First, whether there were some changes in the basic structure of the ascending thoracic aorta due to the severe stenosis of the TAV? Second, whether there was a gradual progression of those changes, caused by the hemodynamic disturbances in the setting of the aortic stenosis? Third, whether we could apply a grading system for the aortic wall changes? And more importantly, whether we could find the irreversible changes in the ascending aorta wall in the patients with the severe aortic stenosis, but with no dilated aorta? Finally, whether we could correlate a histological grade with the echocardiographic parameters in order to obtain a reliable insight into the aortic wall structure by means of the noninvasive diagnostics?

Apart from the hemodynamic derangement that the aortic stenosis causes, aging has a potential for the degenerative changes of the ascending aorta. The next question was whether we could distinguish the aging-induced changes from the aortic stenosis-induced changes. Eventually, we analyzed if there were some gender-related differences in the remodeling process.

We focused on the severe TAV stenosis and its influence on the ascending aorta wall. The chosen method to assess the aortic wall changes in this study was the analysis of the elastic skeleton parameters. We included a wide range of parameters comprising the internal elastic membrane parameters, elastic lamellae parameters and interlamellar fibers parameters.

Methods

Overall patients data

The wall segments of the ascending aorta of 30 patients who were undergoing the AVR, because of the severe, symptomatic TAV stenosis, were analyzed at the Dedinje Cardiovascular Institute, Belgrade, Serbia from August 2005 to April 2009. There were 14 (46.7%) male and 16 (53.3%) female patients. The mean age of the patients was 64.1 ± 7.8 years. The diameter of the ascending aorta was < 5 cm in all patients, with the mean value of 3.49 ± 0.63 cm. The minimal diameter was 2.6 cm and the maximal diameter 4.7 cm. We aimed at investigating the ascending aortas that were < 5.5 cm in diameter being the cut-off value for the indication for the aortic root surgery in the patients with the TAV stenosis [1]. Our classification of the nonseverely dilated ascending aorta, stressed out that the patients in our group had much smaller diameter than the patients indicated for a surgery according to the existing guidelines.

The exclusion criteria from this study group were the patients with the moderate or severe aortic regurgitation, aortic stenosis and acute or chronic aortic dissection, the patients who previously had a cardiac operation and the ones who had the aortic stenosis combined with a connective tissue disorder, bicuspid or congenitally malformed the aortic valve.

The aortic wall samples in the control group were obtained during the autopsies at the Institute of Pathology, Faculty of Medicine, University of Belgrade from the patients who died of noncardiac and nonvascular causes from April 2009 to May 2009. In the control group, there were 7 patients, 3 (42.9%) males and 4 (57.1%) females. The mean age of the patients in the control group was 68.9 ± 14.2 years. Exclusion criteria were the same as for the study group.

The study was approved by the Ethics Committee of the Dedinje Cardiovascular Institute. Informed consent was obtained from all patients.

Intraoperative aortic wall sampling

The diagnosis of a severe TAV stenosis was established by preoperative echocardiography [5]. Transverse aortotomy was made approximately 1 cm above the take-off of the right coronary artery, slightly above the level of the sinotubular junction. The aortic wall specimens were taken from the distal lip of the incision at the convexity of the ascending aorta, 2 to 4 cm above the level of the aortic valve annulus [6]. The samples of the aortic wall with the minimal dimensions of 1 mm × 9 mm and maximal dimensions of 3 mm × 12 mm were excised, immediately fixed in 4% neutral buffered formaldehyde by the immersion procedure, and subsequently processed for the morphological and morphometric analysis.

During the autopsies, the matched control samples were obtained from the same region. For the control group, we chose the samples with intimal hyperplasia or the atherosclerotic lesion types I to III in the selected region.

The echocardiographic parameters

The echocardiographic parameters of the aortic root and the ascending aorta were determined preoperatively from the parasternal longitudinal section according to the standard 2D procedure. The diameters at the level of ventriculo-aortic junction (AA), sinus Valsalvae (SV), sinotubular junction (STJ) and the largest diameter of the visualized ascending aorta (AscA) were measured.

The index of the sinus Valsalvae (SVI) was calculated as the ratio between measured and predicted diameter (pSV) at the level of the sinus of Valsalva. The predicted diameter at the level of the sinus Valsalvae (pSV) was calculated according to the regression formula pSV (cm) = 1.92 + 0.74 × BSA (m^2), where the BSA stands for body surface area [5].

Preparation of the arterial samples for the analysis

The tissue was prepared for the morphological and morphometric analysis according to the procedure described in the previously published studies of our group [6, 7]. Out of 30 serial sections per patient, 3 sections were chosen for the morphometric analysis with respect to the following several rules: the oblique sections were excluded from the analysis as well as the sections with major technical flaws. Also, a minimal distance between chosen sections had to be at least 100 μm.

The sections were stained applying some selective techniques for the elastic fibers: the Weigert van Gieson technique with resorcin fuchsine, the Verhoeff van Gieson method, or the Pincus' staining with acid orcein.

All sections were graded according to the principle described by Schlatmann and Becker [8] for gradation of the aortic wall changes during aging and Niwa et al. [9] for gradation of the congenital aortic stenosis. In both analyses we confirmed the statistically significant differences among the grades. However, we decided to proceed with the Schlatmann and Becker [8] gradation system since the criteria for grading were more precise, hence the reproducibility of the results was also higher with this system.

The grades were established according to the most severe changes on the magnification ×200 of the Olympus BX41 microscope.

Grade 1 is present if there are fewer than 5 foci of the elastic lamellae fragmentation in 1 microscopic field. Focus of the elastic lamellae fragmentation comprises 2 to 4 neighboring elastic lamellae. The orientation of the smooth muscle cells is preserved (Figures 1 A–B).

Grade 2 means that 5 to 10 foci of the elastic lamellae fragmentation in 1 microscopic field and foci are confluent or scattered throughout the media of the aorta. Each focus comprises 2 to 4 neighboring elastic lamellae. The orientation of the smooth muscle cells is preserved (Figures 1 C–D).

Grade 3 is present if there are foci with the elastic fragmentation in 5 or more neighboring elastic lamellae, irrespective of the number of foci per microscopic field. The smooth muscle cells of the media show alterations in orientation (Figures 1 E–F).

A pathologist, who performed the analysis, was blind for the patient's data. The slides were reexamined twice to obtain the final data as advised in similar studies [10].

Morphometric analysis

Two sets of the morphometric parameters were measured.

The following morphometric parameters were measured to analyze the lesions induced by the hemodynamic influence of the severe TAV stenosis and to confirm the remodeling pattern of the lesions: the tunica intima thickness, the maximal tunica intima thickness, the tunica media thickness, the tunica adventitia thickness and the aortic wall thickness.

The following parameters of the elastic skeleton were measured to confirm the morphometric differences among the grades: the maximal thickness of the internal elastic lamina (IEL), the number of the IEL fenestrations per 50 μm, the size of the IEL fenestrations, the number of the elastic lamellae per 50 μm, the maximal thickness of the elastic lamellae, the number of the elastic lamellae fenestrations per 50 μm, the size of the elastic lamellae fenestrations, the number of the interlamellar elastic fibers per 50 μm of the length of the interlamellar unit, the maximal thickness of interlamellar fibers, the number of fenestrations per interlamellar fiber and the size of fenestrations of interlamellar fibers

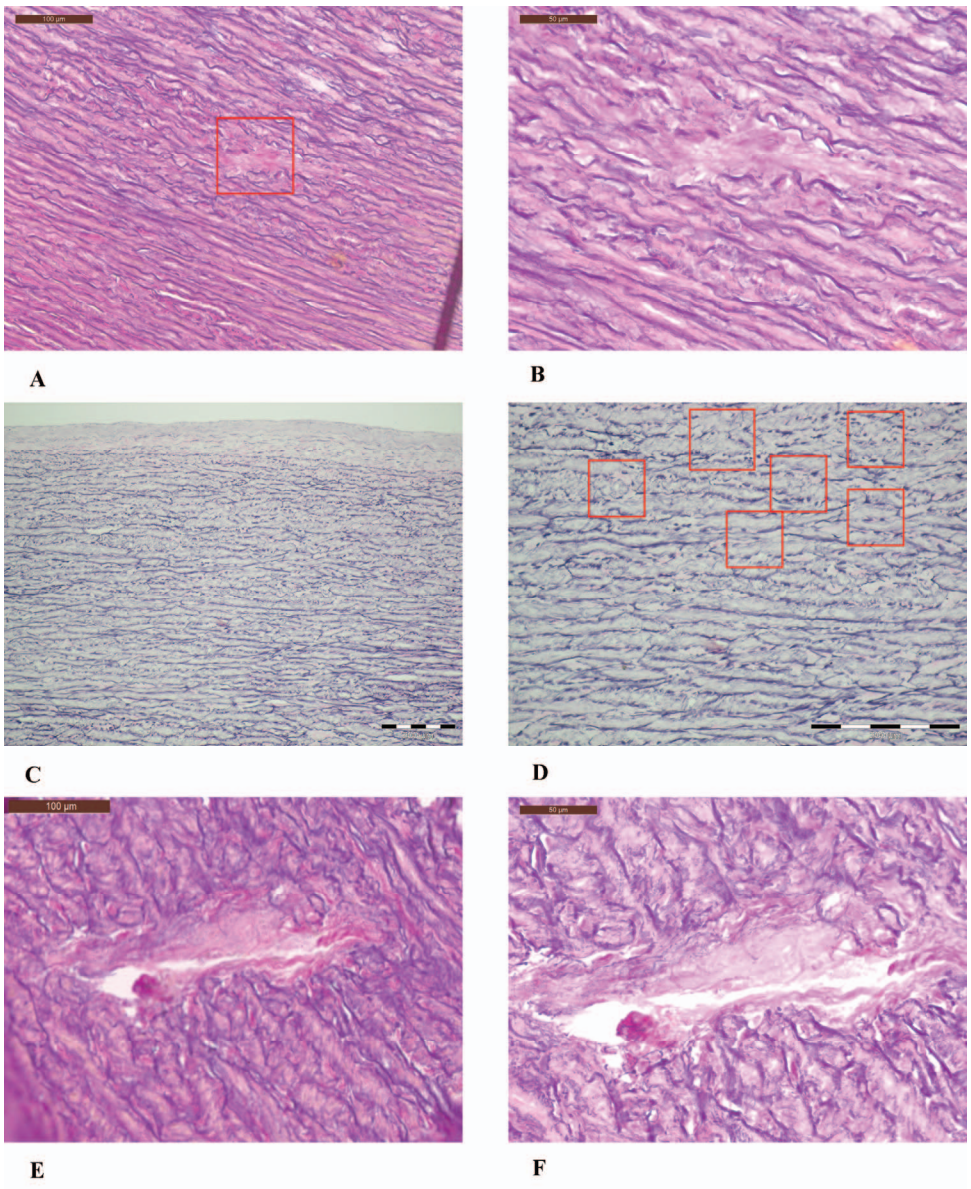

Fig. 1 – The ascending aorta in the patients with the degenerative tricuspid aortic valve stenosis – A) and E) Weigert van Gieson staining, ×200, bar = 100 µm; B) and F) Weigert van Gieson staining, ×400, bar = 50 µm; C) PTAH staining, ×100, bar = 200 µm; D) PTAH staining, ×200, bar = 200 µm; A)–B) grade 1; one focus of the elastic lamellae fragmentation in 1 microscopic field of the Olympus BX41 microscope, ×200; a focus of the elastic lamellae fragmentation comprises 2 to 4 neighboring elastic lamellae; orientation of the smooth muscles is preserved; C)–D) grade 2; 6 foci of the elastic lamellae fragmentation in 1 microscopic field of the Olympus BX41 microscope, ×200; the confluent or scattered foci throughout the media of the aorta; orientation of the smooth muscle cells is preserved; E)–F) the presence of foci with the elastic fragmentation in 5 or more neighboring elastic lamellae with a disruption of the smooth muscle cells.

The optical microscope Olympus BX41 with the Olympus C5060-ADU wide zoom camera and the Olympus DP-soft Image Analyzer program was used for the morphometric analysis.

The thickness of the tunica intima, the tunica media, the tunica adventitia and that of the wall were measured as described in our previous publications [11]. The intima-media ratio was calculated for the purpose of the correlation with the echocardiographic parameters.

The systematic field sampling method was used [3, 7]. Agrid with thirty-five 50 µm × 50 µm fields was embedded into each microscopic field. For each patient, the morphometric parameters of the elastic lamellae and interlamellar fibers were measured on 10 microscopic fields, that is, three hundred and fifty 50 µm × 50 µm fields per section. The thickness of the elastic lamellae was measured in an axis perpendicular to the elastic lamellae. Each lamella was measured in 3 to 6 different positions and only the maximal thickness was used for further statistical processing. We choose only ideal cross-sections with the perfect contours of the elastic lamellae. The fields with the membrane reduplication were excluded from the analysis. Afterwards, the number of fenestrations per 50 µm and their size were meas-

ured. The size of fenestrations was measured as the shortest distance between the two fragments of lamellae. Major defects of the grade 3 were not considered as fenestrations, neither were they measured. These gaps were used for the gradation purposes only (Figures 1E–F), while defined fenestrations in the preserved lamellae were included into the morphometric analysis.

After that, the maximal thickness of the interlamellar fibers was measured as well as the number of fenestrations and their size.

The elastic lamellae and interlamellar fibers parameters were measured at the magnification ×400 of the Olympus BX41 microscope.

Statistical analysis

Descriptive statistics included the mean values and the median, the standard deviation (SD), the standard error (SE) and a 95% confidence interval (95% CI).

The tests were performed with the SPSS version 10.0 for Windows. The following tests were used where appropriate: one-sample Kolmogorov-Smirnov test, χ^2 test, Mood's median test, ANOVA, Mann-Whitney test, Kruskal-Wallis test, post-hoc multiple comparisons LSD test and Bonferroni multiple comparison test, Pearson correlation and Spearman's Rho. The value of $p < 0.05$ was considered statistically significant.

The data are presented as the means ± SD or the means ± SE.

The data distribution pattern was analyzed with the one-sample Kolmogorov-Smirnov test. The values of the tunica media, tunica adventitia and wall thickness were distributed according to the normal distribution and analyzed with the parametric tests. The values of the thickness of the tunica intima, maximal thickness of the tunica intima and the intima-to-media ratio were not distributed according to the normal distribution. The values of these parameters were transformed and the ln values of the tunica intima thickness, maximal tunica intima thickness and the intima-to-media were analyzed for the normality of their distribution (Table 1). The transformed values of the tunica intima thickness and the maximal tunica intima thickness had a normal distribution, they were analyzed by the parametric tests and the presented results apply to these values. The intima-to-media ratio values had no normal distribution and they were analyzed by the non-parametric tests.

The data distribution pattern was analyzed by the One-Sample Kolmogorov-Smirnov test. The values of the elastic lamellae parameters did not conform the normal distribution, hence the non-parametric tests were used in the analysis.

When the data were grouped into the age-related groups (< 65 years and ≥ 65 years), the normal distribution was confirmed for the number of the elastic lamellae and for the thickness of the elastic lamellae and interlaminar fibers. These data were analyzed using the ANOVA test.

When the data were grouped according to the gender, the normal distribution was confirmed for the number of the elastic lamellae and for the thickness of elastic lamellae. These data were analyzed using the ANOVA test.

A normal distribution of the values of the echocardiographic parameters was confirmed by the one-sample Kolmogorov-Smirnov test, hence they were analyzed with the parametric tests.

Results

Remodeling in the degenerative aortic stenosis is associated with a significant thinning of the ascending aorta wall

Remodeling pattern

A significant thinning of the ascending aorta walls and all of its tunics is characteristic of the remodeling pattern in the degenerative aortic stenosis as confirmed by the morphometric analysis (Figures 2 and 3A and Table 1). The thickness of all parameters, except the thickness of the tunica adventitia were statistically significantly smaller than in the age and atherosclerosis matched controls as confirmed by the ANOVA or Mann-Whitney tests (Figure 2 and Table 1).

These results confirmed that the jet produces changes of the aortic wall, distinctive both morphologically and mathematically. Consequently, it was used to prove that the samples were taken from the representative area of the aortic wall, the finding truly important since the geometry of the stenotic aortic orifice is complex, its influence on the jet direction is unpredictable and the aortic circumference is not equally affected in all of its parts. When the post-hoc Bonferroni multiple comparison test, or the Kruskal-Wallis test were applied, the differences between the controls and the aortic stenosis group were confirmed (Figure 2).

The morphometric parameters follow the grading pattern in the aortic stenosis

The morphometric parameters were significantly different among the grades of the aortic stenosis group as confirmed by the ANOVA, or Mann-Whitney test (Figure 2 and Table 1). The gradual increase in the thickness of the aortic wall and its layers from the grade 1 to grade 3 was observed, consistent with the gradual decay of the elastic skeleton which was proved in the further analysis. Nevertheless, the post-hoc Bonferroni multiple comparison test did not confirm differences among the grades for all parameters (Figure 2), so the linear correlation tests were used for further analysis.

Additional testing with the Pearson's correlation coefficient or Spearman's Rho confirmed trending obtained with the ANOVA, or Mann-Whitney tests. The following parameters correlated positively and were statistically significant to the grades: the thickness of the tunica intima ($p < 0.001$), the maximal thickness of the tunica intima ($p < 0.001$), the wall thickness ($p = 0.023$) and the tunica adventitia thickness ($p = 0.029$) as well as the intima-media ratio ($p < 0.001$). The tunica media thickness did not correlate significantly with the grades ($p = 0.382$).

Table 1

Values of the tunica media, tunica adventitia and the aortic wall thickness of the aortic stenosis and control groups

Parameter	Means ± SD	95% CI	Min–Max
The thickness of the tunica intima (μm)			
control group	226.51 ± 127.18	108.88–344.13	55.31–479.05
aortic stenosis group	33.02 ± 41.06	16.08–49.97	3.89–169.18
		ANOVA = 17.451, $p < 0.001$**‖	
GR 1	14.09 ± 9.97	6.96–21.22	3.89–35.31
GR 2	20.97 ± 9.00	14.53–27.40	9.09–35.81
GR 3	95.01 ± 60.39	20.03–169.99	37.49–169.18
The maximal thickness of the tunica intima (μm)			
control group	371.14 ± 191.54	193.99–548.28	82.35–618.86
aortic stenosis group	74.19 ± 90.60	36.80–111.59	8.77–329.43
		ANOVA = 11.686, $p < 0.001$**‖	
GR 1	30.84 ± 29.82	9.50–52.17	8.77–103.53
GR 2	47.58 ± 17.27	35.22–59.93	29.67–75.43
GR 3	214.14 ± 124.98	58.97–369.32	68.40–329.43
The thickness of the tunica media (μm)			
control group	1,380.37 ± 201.34	1,194.17–1,566.58	1,069.13–1,628.03
aortic stenosis group	1,041.46 ± 199.08	959.28–1,123.64	593.04–1,382.86
		ANOVA = 5.757, $p = 0.0382$**	
GR 1	975.45 ± 241.08	803.00–1,147.91	593.04–1,245.25
GR 2	1,106.76 ± 148.77	1,000.33–1,213.18	939.07–1,382.86
GR 3	1,042.87 ± 188.88	808.34–1,277.40	728.88–1,211.31
The thickness of the wall (μm)			
control group	2,028.21 ± 307.63	1,743.70–2,312.72	1,658.59–2,534.36
aortic stenosis group	1,279.96 ± 264.50	1,168.28–1,391.65	666.1–1,862.40
		ANOVA = 13.697, $p < 0.023$**	
GR 1	1,122.77 ± 325.31	872.72–1,372.82	666.51–1,670.16
GR 2	1,349.11 ± 117.59	1,264.99–1,433.23	1,208.75–1,529.03
GR 3	1,424.63 ± 256.50	1,106.14–1,743.11	1,193.96–1,862.40
The thickness of the tunica adventitia (μm)			
control group	422.55 ± 187.75	70.96–248.91	266.39–753.13
aortic stenosis group	216.28 ± 163.69	147.16–285.40	61.25–812.84
		ANOVA = 2.822, $p = 0.029$	
GR 1	130.94 ± 103.64	51.27–210.60	61.25–390.84
GR 2	242.02 ± 64.85	195.63–288.41	180.09–349.74
GR 3	318.43 ± 303.38	58.27–695.13	61.98–812.84
The intima to media ratio			
control group	0.173 ± 0.104	0.077–0.269	0.040–0.370
aortic stenosis group	0.032 ± 0.036	0.018–0.047	0.010–0.150
		Mann-Whitney test = 11.000, $p < 0.001$**‡	
GR 1	0.021 ± 0.012	0.014–0.028	0.010–0.040
GR 2	0.020 ± 0.007	0.011–0.029	0.010–0.030
GR 3	0.070 ± 0.059	0.034–0.092	0.010–0.037

GR – grade; SD – standard deviation; CI – confidence interval; Min – minimum; Max – maximum.
‖ANOVA test is applied to ln (thickness of the tunica intima) or ln (maximal thickness of the tunica intima);
****statistically significant difference; ‡non-parametric tests applied.**

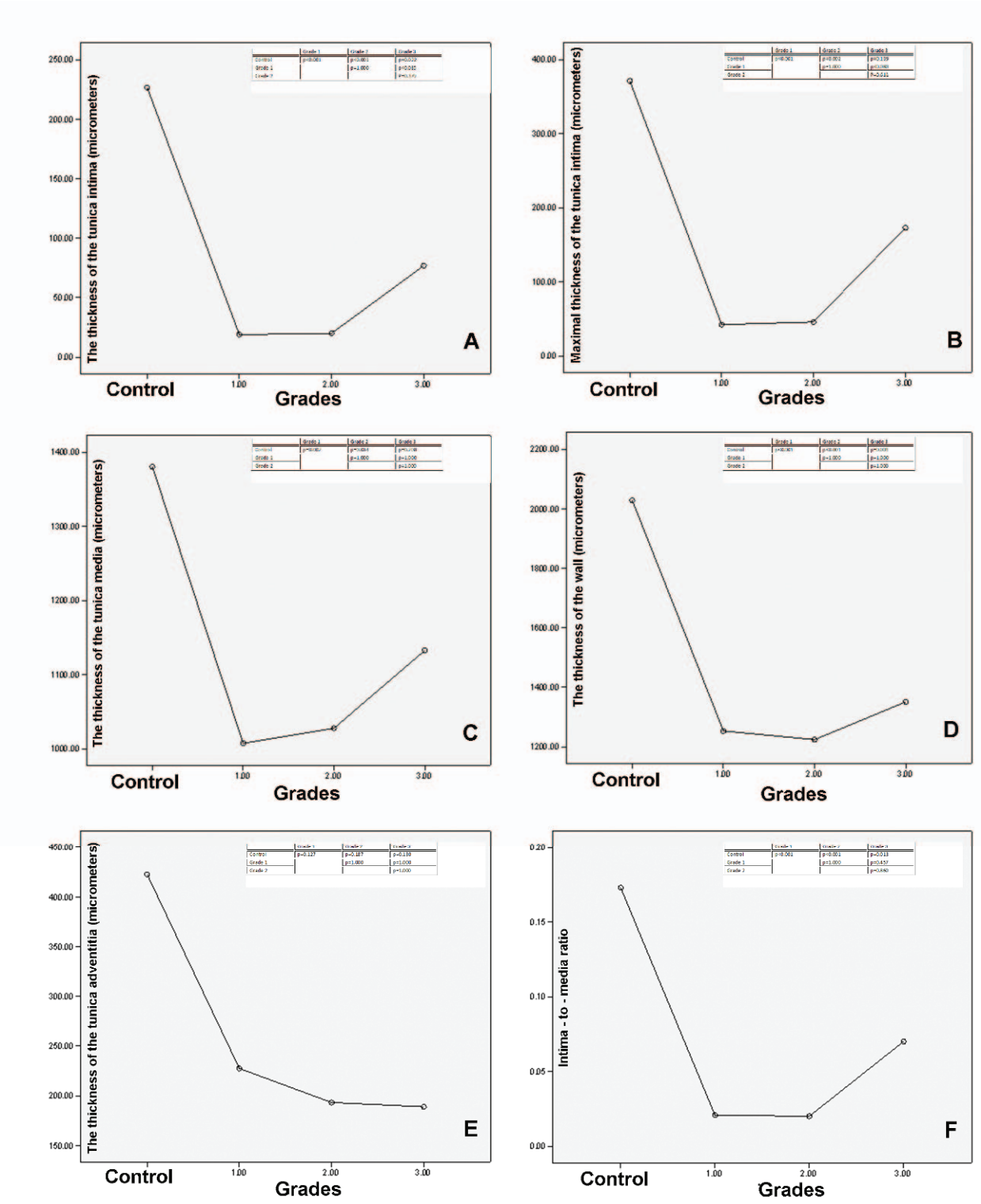

Fig. 2 – Post-hoc Bonferroni multiple comparison analysis – the aortic stenosis group vs. controls and the grades of the aortic stenosis group: A) the thickness of the tunica intima – a statistically significant difference between the controls and aortic stenosis group; a statistically significant difference between the grades 1 and 3; B) the maximal thickness of the tunica intima – a statistically significant difference between the controls and grades 1 and 2; C) the thickness of the tunica media – a statistically significant difference between the controls and the grades 1 and 2; D) the thickness of the wall – a statistically significant difference between the controls and the aortic stenosis group; E) the thickness of the tunica adventitia – no statistically significant difference; F) the intima-to-media ratio – a statistically significant difference between the controls and aortic stenosis group.

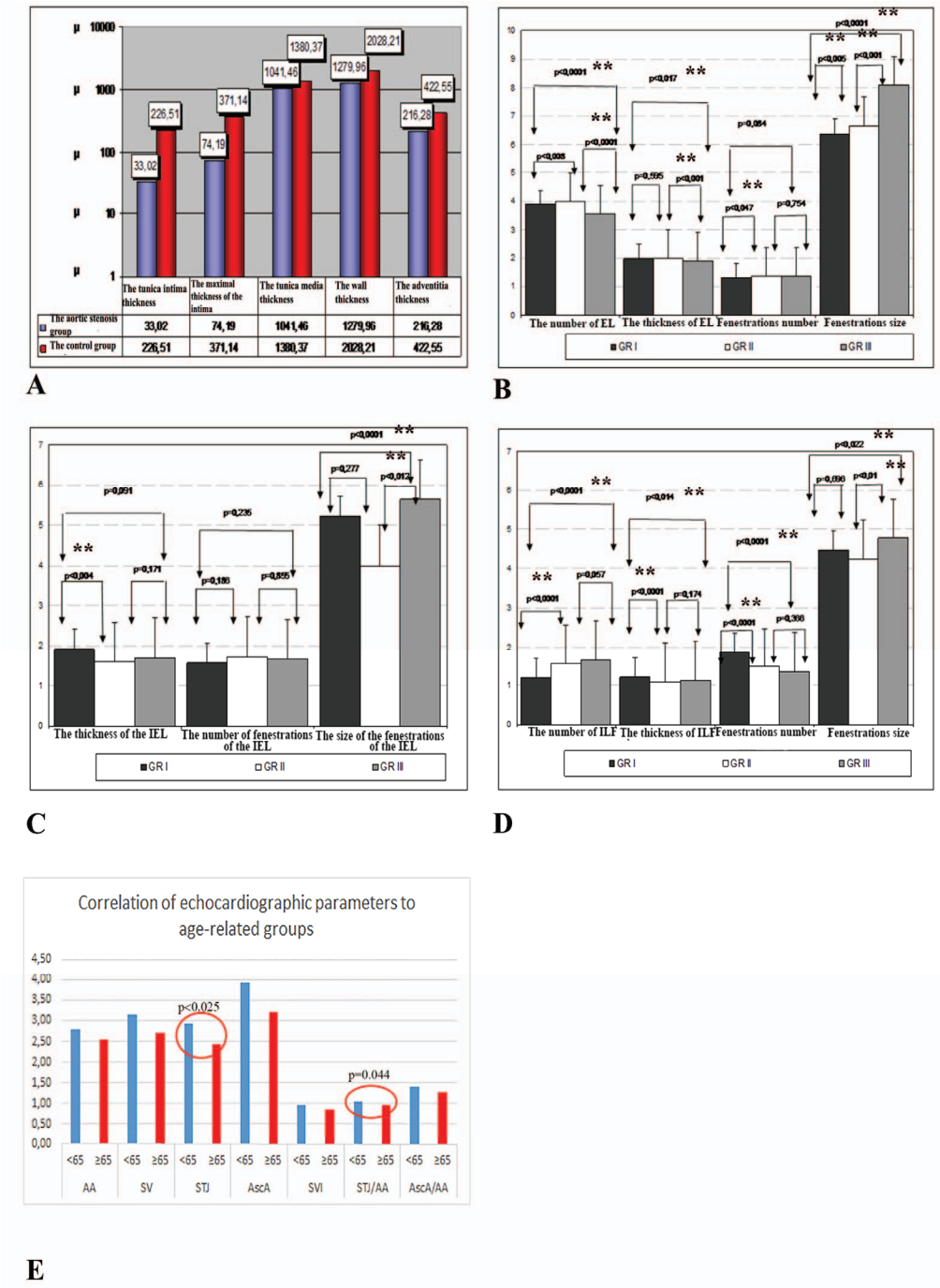

Fig. 3 – A) Comparison between the aortic stenosis group and the control group according to the thickness of the aortic wall and its layers – notice a significant thinning of the wall and all tunics; B) comparison of the different grades (GR): the morphometric parameters of the elastic lamellae – a statistically significant decrease in the thickness and the number of the elastic lamellae between the GR1 and GR3 and a statistically significant increase in the size of the GRs (GR1 vs. GR2, GR1 vs. GR2, GR2 vs GR3) and the number of fenestrations of the elastic lamellae among (GR1 vs. GR2); C) comparison of different GRs: the morphometric parameters of the IEL – a statistically significant decrease in the thickness of the IEL (GR 1 vs. GR2) and a statistically significant increase in the size of fenestrations of the IEL (GR1 vs. GR 3; GR2 vs. GR3); D) comparison of different GRS: the morphometric parameters of the interlamellar fibers – a statistically significant decrease in the interlamellar fibers thickness (GR1 vs. GR2; GR1 vs. GR3) and the number of interlamellar fibers fenestrations (GR1 vs. GR2; GR1 vs. GR3) and a statistically significant increase in the number of the interlamellar fibers (GR1 vs. GR2; GR1 vs. GR3) and the size of interlamellar fibers fenestrations (GR1 vs. GR3; GR2 vs. GR3); E) the diameter at the sinotubular junction (STJ) and the STJ/AA index correlated statistically significantly with the age-related groups.

AA – diameters at the level of the ventriculo-aortic junction, SV – diameters of the sinus of Valsalva, STJ – diameters at the level of the sinotubular junction, AscA – the largest diameter of the visualized ascending aorta, SVI – the sinus of Valsalva index.

The elastic lamellae changes follow the progression and natural history of the aortic stenosis

The analysis of the elastic lamellae aimed at proving that there was a significant difference in the elastic skeleton parameters among the different grades and consequently grades following the progression of the degenerative changes of the aortic wall caused by the aortic stenosis. Furthermore, morphometry of the elastic lamellae aimed at proving that the established grades were accurate and specific enough to detect the real histopathological changes caused by the aortic stenosis and not by aging. Finally, morphometry of the elastic lamellae aimed at identifying the irreversible changes. It was important to confirm the difference between each grade and the control group in that respect.

The remodeling pattern – the elastic lamellae changes follow the grading pattern

Firstly, the elastic lamellae parameters and their differences in the aortic stenosis group and the control group were compared. It was proved by the Mood's median test and non-parametric Z test as the post-hoc test that the remodeling of the elastic lamellae in the aortic stenosis was associated with augmentation of the elastic lamellae as compared to the control group (Table 2). The elastic lamellae were significantly thicker and more numerous, while the size of their fenestrations was reduced. The aortic stenosis obviously induced upgrading of the elastic lamellae, in comparison to the control group with a statistical significance ($p < 0.001$).

Secondly, the elastic lamellae changes among different grades of the aortic stenosis group were studied. Among the different grades in the aortic stenosis group, a significant degradation of the elastic lamellae was confirmed by applying the Mood's median test ($p < 0.001$) and the non-parametric Z test as the post-hoc test. The number of the elastic lamellae and their thickness decreased significantly while the number of elastic lamellae fenestrations and their size increased significantly (Table 2 and Figure 3B).

Later regularity was also observed when the parameters of the IEL were analyzed (Figure 3C).

Remodeling pattern – evolution of the interlamellar fibers changes follows the grading pattern

The reorganization of the interlamellar fibers was also detected in comparison to the aortic stenosis group and the control group. The number of the interlamellar fibers and the size of interlamellar fibers fenestrations was significantly higher in the control group when compared to the aortic stenosis group (Table 3). The interlamellar fibers thickness was significantly lower in the control group in comparison to the aortic stenosis group while the number of interlamellar fibers fenestrations did not differ between the control group and the aortic stenosis group (Table 3).

Table 2

Morphometric parameters of the elastic lamellae – comparison between the control group and the grades of the aortic stenosis group

Parameters of the elastic lamellae	N	Mean ± SD	Min–Max	*p*
The number				
control	504	3.33 ± 0.96	3.25–3.42	
GR 1	1,311	4.46 ± 1.56	4.37–4.54	< 0.001**
GR 2	1,831	3.54 ± 1.31	3.48–3.59	
GR 3	453	3.27 ± 1.18	3.16–3.37	
The thickness (μm)				
control	305	1.56 ± 0.52	1.50–1.62	
GR 1	973	2.20 ± 0.65	2.16–2.24	< 0.001**
GR 2	1,400	1.84 ± 0.61	1.81–1.87	
GR 3	306	1.85 ± 0.62	1.79–1.92	
The number of fenestrations				
control	432	1.20 ± 0.79	1.13–1.28	
GR 1	1,195	1.32 ± 0.87	1.27–1.37	< 0.001**
GR 2	1,392	1.35 ± 0.94	1.31–1.40	
GR 3	326	1.46 ± 0.90	1.37–1.55	
The size of fenestrations (μm)				
control	526	10.59 ± 6.83	10.01–11.18	
GR 1	1,352	6.33 ± 4.22	6.11–6.56	< 0.001**
GR 2	1,699	7.17 ± 4.73	6.98–7.37	
GR 3	433	7.68 ± 5.28	7.23–8.13	

A statistically significant increase in the thickness of elastic lamellae between the control group and the aortic stenosis group; there is a statistically significant difference among different grades in the elastic lamellae thickness; the size of fenestrations decreased significantly between the control group and the aortic stenosis group; the size of elastic lamellae fenestrations increased significantly among the grades; the number of the elastic lamellae increased significantly between the aortic stenosis group and the control group; there is a significant decrease in the elastic lamellae number among the grades; the number of the elastic lamellae fenestrations increased significantly between the control group and the grade 3.

GR – grade; SD – standard deviation;***p* **– statistically significant difference.**

Table 3

Morphometric parameters of the interlamellar fibers – comparison between the control group and the grades of the aortic stenosis group

Parameter of interlamellar fibers	N	Mean ± SD	95% CI	*p*
The number				
control	252	1.58 ± 0.68	1.50–1.67	
GR 1	479	1.20 ± 0.84	1.14–1.27	< 0.004**
GR 2	725	1.56 ± 0.77	1.49–1.64	
GR 3	236	1.66 ± 0.82	1.60–1.73	
The thickness (μm)				
control	361	0.85 ± 0.33	0.82–0.89	
GR 1	501	1.21 ± 0.53	1.17–1.25	< 0.001**
GR 2	790	1.09 ± 0.40	1.06–1.12	
GR 3	189	1.13 ± 0.46	1.10–1.17	
The number fenestrations				
control	61	1.45 ± 0.78	1.25–1.65	
GR 1	235	1.85 ± 1.05	1.73–1.97	= 0.122
GR 2	309	1.48 ± 0.86	1.36–1.59	
GR 3	77	1.37 ± 0.68	1.28–1.45	
The size of fenestration				
control	88	5.29 ± 2.76	4.70–5.87	
GR 1	341	4.46 ± 2.42	4.25–4.68	< 0.002**
GR 2	451	4.23 ± 1.93	4.00–4.45	
GR 3	128	4.77 ± 2.52	4.49–5.05	

For abbreviations see under Tables 1 and 2.

A statistically significant increase in the thickness of interlamellar fibers between the control group and the aortic stenosis group; the interlamellar fibers thickness decreased statistically significantly among grades GR1 and GR3; the size of fenestrations decreased significantly between the control group and the aortic stenosis group; the size of interlamellar fibers fenestrations increased significantly between grades GR1 and GR3; the number of the interlamellar fibers is proved to have no significance in the context of the multiple comparison.

In the aortic stenosis group, the number of the interlamellar fibers and their thickness increased significantly among grades, as did the number of elastic lamellae fenestrations and their size. The number of interlamellar fibers fenestrations decreased in the subsequent grades (Table 3 and Figure 3D).

Influence of aging

Out of 30 patients in the aortic stenosis group, 13 (43.3%) were younger than 65 years of age while 17 (56.7%) were older than 65 years of age. There was no statistically significant difference among the number of the patients in the group younger than 65 years of age, and group older than 65 years of age (χ^2 = 0.533, p = 0.465). Further analysis showed that the ageing affects all parameters of the elastic skeleton with the statistical significance, except the number of fenestrations of the elastic lamellae as proved by the ANOVA or Z test (Table 4).

The changes were more severe in the inner media, and there seemed to be a progression of the changes from the inwards to the outwards of the aortic media (Figures 1B and 1C).

The next question was whether we could distinguish effects of aging and the aortic stenosis, or the described grades were merely misapprehended changes induced by ageing. The ANOVA or Kruskal-Wallis tests were applied to test the differences between the control group and the aortic stenosis group of patients younger than 65 years of age and in the group of patients older than 65 years of age. It was confirmed that a statistical significance persisted (Table 5). Since the established differences were the effects of the aortic stenosis, they were potentiated with ageing, but they were not entirely dependable on ageing. The number of the elastic lamellae fenestrations changed intensively with ageing. The size of the elastic lamellae fenestrations had even greater alterations in the patients younger than 65 years of age. Certain parameters (the number of the interlamellar fibers and the interlamellar fibers fenestrations) were proved to have no significance in the setting of the age-dependent analysis (Table 5).

Influence of gender

Most of the male patients (42.9%) had the grade 3, while most of the female patients (56.3%) were in the grade 1. There was no statistically significant difference among the number of the male and female patients in different grades (χ^2 = 2.162, p = 0.339).

Nevertheless, when the multifactorial analysis was applied and influence of the gender factor was tested simultaneously with the group factor, the females seemed to be more susceptible to the elastic lamellae defects since their elastic skeleton parameters indicated thinning and a statistically significant decrease in the number of the elastic lamellae and the increased size of fenestrations as confirmed by the ANOVA or Kruskal-Wallis tests with the post-hoc multiple comparison LSD test (Table 6).

Table 4

Influence of ageing (< 65 years and ≥ 65 years) to the morphometric parameters of the elastic lamellae and interlamellar fibers

Parameters	Mean ± SD	95% CI	*p*
Elastic lamellae			
the number			< 0.001†**
< 65	4.05 ± 1.33	3.99–4.10	
≥ 65	3.58 ± 1.54	3.52–3.65	
the thickness			
< 65	2.03 ± 0.68	2.00–2.06	< 0.001†**
≥ 65	1.88 ± 0.58	1.84–1.91	
the number of fenestrations			
< 65	1.37 ± 0.94	1.32–1.42	0.999
≥ 65	1.34 ± 0.88	1.30–1.38	
the size of fenestrations			
< 65	6.07 ± 3.88	5.90–6.24	< 0.001**
≥ 65	7.79 ± 5.16	7.57–8.01	
Interlamellar fibers			
the number			0.010**
< 65	1.37 ± 0.89	1.31–1.43	
≥ 65	1.51 ± 0.79	1.46–1.56	
the thickness			
< 65	1.18 ± 0.48	1.14–1.21	0.010†**
≥ 65	1.12 ± 0.47	1.10–1.15	
the number of fenestrations			
< 65	1.69 ± 0.98	1.59–1.79	0.009**
≥ 65	1.49 ± 0.84	1.40–1.58	
the size of fenestrations			
< 65	4.07 ± 2.09	3.91–4.24	< 0.001**
≥ 65	4.99 ± 2.52	4.77–5.22	

For abbreviations see under Tables 1 and 2.
The significant influence of ageing to all morphometric parameters of the elastic lamellae except for the number of elastic lamellae.
†ANOVA
**** statistically significant.**

Table 5

Morphometric parameters and their differences in the control group and the aortic stenosis group in the age-related groups

Parameters	Aortic stenosis group vs. control group					
	GR 1 vs. control		GR 2 vs. control		GR 3 vs. control	
	< 65 years	≥ 65 years	< 65 years	≥ 65 years	< 65 years	≥ 65 years
Elastic lamellae						
the number	< 0.001**	<0.001**	0.078	<0.001**	< 0.001**	<0.001**
the thickness (μm)	< 0.001**	<0.001**	0.753	<0.001**	< 0.001**	0.017**
the number of fenestrations	0.861	0.015**	1.000	0.006**	0.469	0.922
the size of fenestrations (μm)	< 0.001**	0.932	< 0.001**	0.863	< 0.001**	0.646
Interlamellar fibers	0.090	1.000	0.414	0.998	1.000	0.923
the number						
the thickness (μm)	< 0.001**	0.011**	< 0.001**	0.233	< 0.001**	0.017**
the number of fenestrations	0.818	0.272	0.916	0.546	1.000	1.000
the size (μm)	0.066	0.006**	0.622	0.991	0.003**	0.001**

For abbreviations see under Tables 1 and 2.
Aging potentiates effects of the aortic stenosis to the elastic lamellae, but the morphometric parameters of the elastic lamellae are not entirely dependable on aging. The number of the elastic lamellae fenestrations changed intensively with aging. The size of elastic lamellae fenestrations increased significantly in the patients younger than 65 years of age. The number of the interlamellar fibers and the number of interlamellar fibers fenestrations are not changed significantly.
****p* values are statistically significant.**

Table 6

Morphometric parameters in the aortic stenosis group and the control group – influence of gender

Parameter	The aortic stenosis group	The control group	*p*	
	Mean ± SD	Mean ± SD	Gender	Groups and gender††
Elastic lamellae				
the number			0.003†**	0.001**
males	4.26 ± 1.44	3.24 ±0.89		
females	3.11 ± 0.99	3.33 ± 0.89		
the thickness				
males	2.02 ± 0.63	1.30 ± 0.51	0.273†	0.056
females	1.94 ± 0.62	1.57 ± 0.41		
the number of fenestrations				
males	1.93 ± 1.10	1.24 ± 0.54	0.325	<0.001**
females	1.33 ± 0.55	1.58 ± 0.81		
the size of fenestrations				
males	5.11 ± 3.57	11.85 ± 8.47	0.669	0.005**
females	6.95 ± 4.75	9.36 ± 5.86		
Interlamellar fibers				
the number			0.707	0.578
males	1.52 ± 0.80	1.43 ± 0.60		
females	1.40 ± 0.81	1.45 ± 0.68		
the thickness				
males	1.11 ± 0.38	0.90 ± 0.32	0.143	0.085
females	1.33 ± 0.58	0.88 ± 0.38		
the number of fenestrations				
males	1.47 ± 0.79	1.67 ± 1.06	0.983	0.021**
females	1.79 ± 1.03	1.35 ± 0.58		
the size of fenestrations				
males	4.56 ± 2.55	5.42 ± 2.13	0.843	0.414
females	4.94 ± 2.39	5.18 ± 2.42		

For abbreviations see under Tables 1 and 2.
A statistically significant decrease of the number of the elastic lamellae and increased size of elastic lamellae fenestrations in the females with the aortic stenosis. However, the number of fenestrations is smaller.
†ANOVA test; †† the *post-hoc* multiple comparisons LSD, Kruskal-Wallis test; **statistically significant.

Correlation with the echocardiographic parameters

Echocardiographic parameters and grades

The values of the echocardiographic parameters are given in Tables 7 and 8.

No statistical significance was confirmed among the echocardiographic parameters of the different grades by the ANOVA test and Bonferroni multiple comparison post-hoc testing (Table 7).

However, the additional testing by the Pearson's correlation test revealed a significant association of the echocardiographic parameters and aortic wall parameters and the defined grades.

The statistically significant and positive correlations were established between the SV and SVI and the tunica intima thickness and the intima-to-media ratio (Table 8), confirming that the SV diameter and SVI increase with the advanced structural changes of the aortic wall.

The AscA correlated statistically significantly and negatively with the tunica intima thickness and statistically significantly and positively to the intima-to-media ratio (Table 8), demonstrating that dilatation of the aorta was still an indicator of the aortic degeneration. The same analogy could be driven from the significant and negative association of the AA with the wall thickness, and the significant and positive correlation of the AscA/AA pointed (Table 8).

A significant direct correlation was confirmed between the SVI and the tunica intima thickness in the grade 1 (R = 0.632, $p = 0.050$) and between the STJ and the thickness of the wall (R = 0.677, $p = 0.032$) and that of the adventitia (R = 0.634, $p = 0.049$) in the grade 2. In the grade 3, a significant direct correlation was confirmed between the STJ/AA and the wall thickness (R = 0.882, $p = 0.048$), while an indirect correlation was confirmed between the SVI and the media thickness (R = -0.895, $p = 0.040$).

Influence of ageing

Two echocardiographic parameters identified the age-related changes – STJ and STJ/AA when ANOVA was used to test the differences of the echocardiographic parameters between the age-related groups (Figure 3E). The additional multifactorial analysis obtained by the univariate analysis of variance was used to confirm the simultaneous impact of age and grade to the echocardiographic parameters (Table 7). The increase of all echocardiographic parameters with the grades was confirmed in the group of patients younger than 65 years of age (Figure 4). These differences, however, were not statistically significant, except for the AscA parameter

(Table 7). A statistically significant difference (p = 0.032) was confirmed for the means of the largest diameter of the visualized ascending aorta (AscA) when the age factor was considered. It was 3.56 ± 0.16 cm for the whole sample, but reached 4.50 ± 0.62 cm in the grade 3 in the group younger than 65 years of age as a potential cut-off point for consideration (Table 7).

Also, the only parameter that was the highest in the GR 1 instead of the GR 3 was the means of the STJ/AA index in the group younger than 65 years of age. Value of this parameter for the whole sample was 1.015 ± 0.042 and reached 1.086 ± 0.055 in the GR 1 (p = 0.407) (Table 7).

Higher values of the echocardiographic parameters in the patients younger than 65 years of age could be associated with more intensive derangement of some elastic lamellae features in these patients as concluded previously. Morphometry confirmed that the elastic lamellae in the patients with the aortic stenosis younger than 65 years of age were more fenestrated.

Table 7

Echocardiographic parameters – impact of the grades and combined age and grade influence

Parameter	GR 1 (n = 14)	GR 2 (n = 6)	GR 3 (n = 10)	Mean ± SD	*p*
Age (years), means ± SD	61 ± 8.42	62.6 ± 8.38	67.5 ± 4.37	62.88 ± 7.84	0.244
Atherosclerosis, n					
none	12	6	8		
type 2 (fatty streaks) lesions	2				
type 3 (intermediate) lesions			2		
Echocardiographic parameters					
AA (cm)	2.57 ± 0.40	2.60 ± 0.29	2.63 ± 0.25	2.59 ± 0.34	0.941
SV (cm)	2.92 ± 0.52	2.84 ± 0.39	2.76 ± 0.39	2.87 ± 0.45	0.759
STJ (cm)	2.72 ± 0.54	2.56 ± 0.63	2.74 ± 0.39	2.69 ± 0.51	0.817
AscA (cm)	3.56 ± 0.72	3.38 ± 0.45	3.41 ± 0.61	3.49 ± 0.63	0.825
SVI	0.898 ± 0.168	0.874 ± 0.139	0.842 ± 0.137	0.880 ± 0.152	0.763
STJ/AA	1.066 ± 0.170	0.984 ± 0.180	1.045 ± 0.135	1.044 ± 0.161	0.642
AcsA/AA	1.391 ± 0.231	1.304 ± 0.149	1.295 ± 0.164	1.351 ± 0.201	0.541
Echocardiographic parameters per age and grade					
AA (cm)					
< 65 years	2.61 ± 0.40	2.63 ± 0.38	3.10 ± 0.13	2.78 ± 0.14	0.567
≥ 65 years	2.50 ± 0.45	2.55 ± 0.21	2.53 ± 0.12	2.53 ± 0.11	
SV (cm)					
< 65 years	3.03 ± 0.56	2.90 ± 0.52	3.50 ± 0.20	3.14 ± 0.18	0.490
≥ 65 years	2.73 ± 0.40	2.75 ± 0.21	2.61 ± 0.17	2.70 ± 0.14	
STJ (cm)					
< 65 years	2.84 ± 0.63	2.87 ± 0.65	3.10 ± 0.26	2.93 ± 0.20	0.717
≥ 65 years	2.52 ± 0.24	2.10 ± 0.14	2.67 ± 0.38	2.43 ± 0.16	
AscA (cm)					
< 65 years	3.62 ± 0.68	3.67 ± 0.32	4.50 ± 0.38	3.93 ± 0.25	§0.032**
≥ 65 years	3.45 ± 0.86	2.95 ± 0.07	3.19 ± 0.33	3.20 ± 0.20	
SVI					
< 65 years	0.934 ± 0.179	0.873 ± 0.193	1.060 ± 0.024	0.956 ± 0.062	0.497
≥ 65 years	0.832 ± 0.139	0.875 ± 0.050	0.798 ± 0.095	0.835 ± 0.049	
STJ/AA					
< 65 years	1.086 ± 0.149	1.083 ± 0.144	1.000 ± 0.027	1.056 ± 0.66	0.407
≥ 65 years	1.030 ± 0.219	0.835 ± 0.120	1.054 ± 0.148	0.973 ± 0.052	
AscA/AA					
< 65 years	1.391 ± 0.162	1.400 ± 0.087	1.450 ± 0.043	1.414 ± 0.083	0.506
≥ 65 years	1.392 ± 0.349	1.160 ± 0.071	1.264 ± 0.163	1.272 ± 0.066	

For abbreviations see under Tables 1 and 2.
A statistically significant difference was confirmed for the means of the largest diameter of the visualized ascending aorta (AscA) when the age factor was considered.
AA – diameters at the level of ventriculo-aortic junction, SV – diameters of the sinus Valsalvae, STJ – diameters at the level of the sinotubular junction, AscA – the largest diameter of the visualized ascending aorta, SVI – the sinus Valsalvae index.
***p* values obtained by ANOVA; ¶*p* values obtained by the univariate analysis of variance; §statistical significance per age,**
****p* values are statistically significant.**

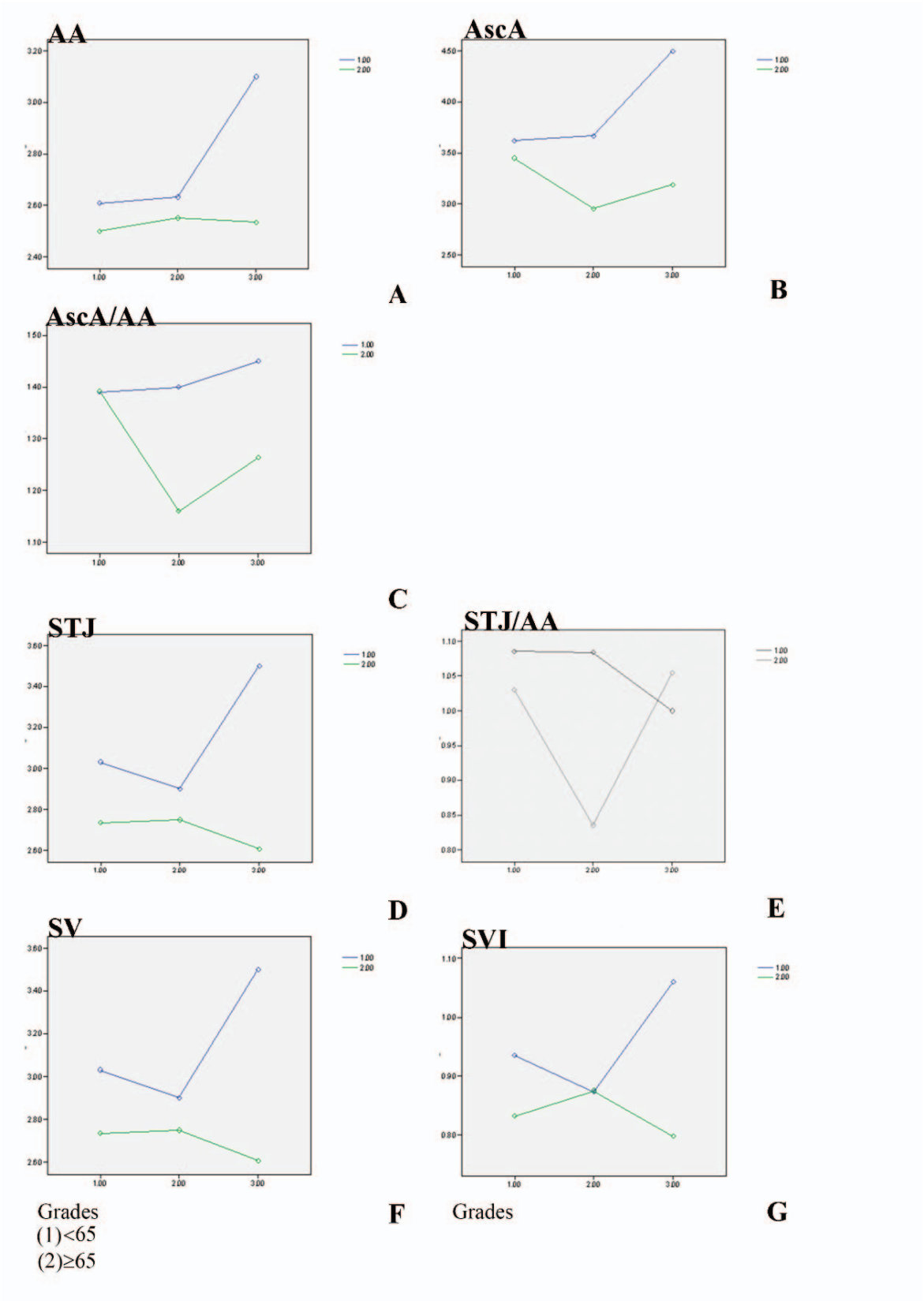

Fig. 4 – Echocardiographic parameters of the aortic stenosis group: A) AA – diameters at the level of ventriculo-aortic junction; B) AscA – the largest diameter of visualized ascending aorta; C) AscA/AA index; D) STJ – diameters at the level of the sinotubular junction; E) STJ/AA index; F) SV – diameters of the sinus of Valsalva; G) SVI – the sinus of Valsalva index.
The increase of echocardiographic parameters with grades was confirmed in a group of patients younger than 65 years (1) and in comparison to a group older than 65 years.

Table 8

Pearson's correlation between the echocardiographic parameters and morphometric parameters of the ascending aorta wall

Echocardiographic parameters	The thickness of the intima	The maximal thickness of the intima	The thickness of the media	The thickness of the wall	The thickness of the adventitia	Intima-media ratio
AA	0.030	-0.382	-0.433	-0.439	-0.475	0.148
R	0.935	0.276	0.212	0.028**	0.196	0.684
p						
SV	0.740	0.541	-0.048	-0.121	-0.265	0.799
R	0.014**	0.106	0.894	0.757	0.491	0.006**
p						
STJ	0.454	-0.121	-0.050	-0.072	-0.112	0.530
R	0.187	0.738	0.890	0.855	0.774	0.115
p						
AscA	0.501	0.147	-0.401	-0.548	-0.385	0.695
R	0.140	0.686	0.047**	0.126	0.306	0.026**
p						
SVI	0.766	0.539	0.104	0.037	-0.182	0.808
R	0.010**	0.108	0.775	0.924	0.640	0.005**
p						
STJ/AA	0.507	0.248	0.434	0.547	0.551	0.466
R	0.135	0.489	0.210	0.005**	0.004**	0.174
p						
AscA/AA	0.518	0.703	-0.081	-0.031	0.039	0.600
R	0.125	0.023**	0.825	0.937	0.922	0.067
p						

A statistically significant and positive correlations between the SV and SVI and the tunica intima thickness and the intima-to-media ratio; a statistically significant and negative correlations of the AscAwith the tunica intima thickness and a statistically significant and positive to the intima-to-media ratio; a statistically significant and negative correlation of the AA with the wall thickness; a statistically significant and positive correlation of the AscA/AA with the wall thickness.

AA – diameters at the level of ventriculo-aortic junction; SV – diameters of the sinus of Valsalva; STJ – diameters at the level of the sinotubular junction; AscA – the largest diameter of the visualized ascending aorta; SVI – the sinus of Valsalva index.

****statistically significant correlation.**

Influence of gender

Multifactorial analysis obtained by the univariate analysis of variance was used to confirm influence of the gender, age and grade to the echocardiographic parameters. A statistically significant difference was confirmed for the diameter of the SV ($p = 0.022$) and a borderline significance for the mean diameter of the STJ ($p = 0.053$) when gender factor was considered. No other statistical significance was confirmed by the multifactorial analysis of the gender, age and grade.

The mean diameter at the level of AA for the males was 2.78 ± 0.14 cm and 2.52 ± 0.096 cm for females.

The mean diameter of the SV for the males was 3.18 ± 0.12 cm and 2.70 ± 0.11 cm for the females. The mean diameter of the STJ for the males was 2.98 ± 0.19 cm and 2.50 ± 0.13 cm for the females. These parameters were proved to be gender-dependent, higher in the males and were statistically significant.

The means of the largest diameter of AscA for the males were 3.99 ± 0.21 cm and for the females 3.23 ± 0.14 cm. The means of the SVI for the males was 0.970 ± 0.057 and 0.833 ± 0.038 for the females. The means of the STJ/AA index were 1.078 ± 0.060 for the males and 1.001 ± 0.040 for the females. The means of the AscA/AA index were 1.443 ± 0.077 for the males and 1.286 ± 0.052 for the females.

Discussion

Treatment decisions for the ascending aorta replacement related to the aortic valve disease have not been clearly reported, because only a few studies have analyzed the evolution of the aortic wall changes in patients with the TAV stenosis. Majority of these studies considered a wide spectrum of disorders. Our study, however, focuses on a specific issue: the TAV stenosis and its influence to the aortic wall.

The results of the previous studies support one of two opposing views: one group provides evidence for the AVR without simultaneous replacement of the ascending aorta, while the other group of studies proves the attitude that the simultaneous AVR and the ascending aorta replacement is needed.

Girdauskas et al. [12], using magnetic resonance imaging, found that the aortic segment in a direct contact with the systolic transvalvular flow jet was located at the greater curvature in nearly all patients and the systolic transvalvular flow jet hitting the right-lateral segment of the tubular ascending aorta was the most common scenario.

Gaudino et al. [13] published the results of a follow-up study of patients submitted to the AVR only and showed moderate dilatation of the ascending aorta with the expansion rate of 0.3 ± 0.2 mm/year after 14.7 years postoperatively[11].

Similarly, Yasuda et al. [14] reported a mean ascending aorta expansion rate of 0.08 mm/m^2/year in a series of 14 patients followed for 9.7 years after surgery. Andrus et al. [15] reported results of a vast study which comprised 107 patients with an aortic diameter of ≥ 3.5 cm. They found no evidence of further dilation in the first 3 years after the isolated AVR. Botzenhardt et al. [16] even described a reduction of the aortic diameter in 10 patients with the pre-operative aortic diameter of ≥ 4 cm, 4.8 years after the isolated valve surgery. Gaudino et al. [13] and Yasuda et al. [14] supposed that correction of the aortic stenosis in these patients stabilized the hemodynamics and prevented further development of the aortic wall changes. Andrus et al. [15] concluded that in patients with the aortic valve stenosis and with the accompanying mild or moderate ascending aortic dilatation (3.5 cm to 4.9 cm) the AVR alone may be reasonable.

Ergin et al. [17] advocated more liberal indications for the AVR simultaneously with the ascending aorta replacement since it significantly improves the postoperative outcome in comparison to patients with the AVR and already dilated aorta.

The aforementioned controversies open the field for further investigations both in histological and clinical domain and for studies that correlate both aspects as our study.

Comparison of patients with the bicuspid and the TAV suggested that the presence of the bicuspid aortic valve induced the more severe aortic wall alterations than the degenerative changes of the tricuspid valve [16]. The patients with the TAV stenosis and the ascending aorta dilatation had more severe defects of the ascending aorta than the patients with the bicuspid valve and the same degree of dilatation [18].

What answers has our study offered beyond the conclusions of these previous studies and to the questions that initiated our research?

Are there any changes in the basic structure of the ascending thoracic aorta due to the severe stenosis of the TAV? We proved mathematically the significant thinning of the ascending aorta wall and all of its tunics in the patients with the aortic stenosis. Similar changes were already described in a different model of exaggerated hemodynamic forces and its influence on the arterial wall [19].

Is there a gradual progression of the aortic wall changes caused by the aortic stenosis and could we establish a grading system for these changes or apply the already existing ones? The results of our study clearly demonstrated three histopathological GRs with a mathematical distinction to the controls and among different GRs. The grading system of Schlatmann and Becker [8] was applicable. Furthermore, our supposition is that these three GRs follow the natural progression and evolution of the aortic stenosis and its hemodynamic impact to the aortic wall. The elastic lamellae and interlamellar fibers generally became thinner and fragmented. The number of the lamellae decreased statistically significantly with the GR, while the number of the interlamellar fibers significantly increased. There was a principle in spatial distribution of these changes in the aortic wall as they affected the internal media first. These observations are in line with the previous similar studies [8, 20, 21].

Could we find some irreversible changes in the wall of the ascending aorta in the patients with the severe aortic stenosis, but with no dilated aorta? We consider that the grade 3 with the destructive changes in the numerous elastic lamellae and disorganization of the smooth muscles resembled the irreversible changes.

Could we distinguish the ageing-induced changes from the aortic stenosis induced changes? This is quite a peculiar question. The morphological and morphometric characteristics of the elastic skeleton are generally changed during ageing which makes the arteries prone to different influences, including the hemodynamics. Even the "perfect" internal thoracic artery is prone to the elastic skeleton changes induced by ageing [8]. Nakashima et al. [20] proved that the number of the elastic lamellae fenestrations increased with ageing, as did the number of the interlamellar elastic fibers, their ramifications and the number of their fenestrations.

All analyzed parameters in our study changed statistically significantly with ageing. It was very important to prove that the observed GRs are not merely effects of ageing. We proved that described changes persisted in both groups of patients, the younger and older than 65 years of age, they were potentiated with ageing, but they were not entirely the effect of ageing. The elastic skeleton decay during ageing in the patients with the aortic stenosis could be explained by the synergistic effects of ageing and the aortic stenosis to the aortic wall as well as by a prolonged duration of the aortic stenosis in the group older than 65 years of age.

Female gender proved to be associated with more intensive changes in the aortic stenosis as compared to the control group. It seems that the aortic stenosis and female gender act in the same direction to the histopathological changes of the elastic lamellae and that the aorta of females is more sensitive to different hemodynamic influences as already proved for the abdominal aortic aneurysms model [11]. On the opposite, in the bicuspid aortopathy, the female gender is proved to have protective effect [22].

Finally, could we correlate the histological grades with the echocardiographic parameters? The aortic valve disease is associated with the ascending aortic dilatation because of "hemodynamic burdens caused by forceful jets" [23, 24]. Supposingly, a size of the dilatation is related to the degree of turbulence induced by the stenotic valve and the severity of stenosis [25]. However, Linhartova et al. [1] concluded that there was no independent association between the severity of aortic stenosis and the aortic diameter, indicating that factors other than the aortic stenosis itself could affect the echocardiographic parameters of the aorta. They indicated that the geometry of the aortic orifice, its influence to the flow distribution pattern and histopathological changes in the aortic wall deserved to be investigated further.

Our study proved a significant correlation of the SV and SVI parameters with the thickness of the intima and the intima-to-media ratio and indicated that the SV diameter and index increased with the structural changes of the aortic wall. The SV was also higher in the male patients.

Despite the open controversies about the ascending aorta diameter and its association with the actual structural

changes of the aorta, there was a statistically significant correlation of the AscA with the morphological parameters of the aorta wall disruption. This parameter was particularly important since it was shown that its value of 4.5 cm could be predictive for the irreversible defects especially in the patients younger than 65 years of age.

Beside that, two more parameters, the STJ and STJ/AA, correlated well with the age related changes and morphometric parameters in the GRs 2 and 3. The STJ was also higher in the male patients.

However, the grades could not be associated with simple diameters measured by echocardiography and them tested by the ANOVA analysis.

Many other authors concluded, also, that "size is not enough" [23, 24]. These studies implicate that other parameters are necessary for the estimation of the histopathological defects and the severity of the aortic stenosis.

Limitations of the study

Our study considers a relatively small number of the patients. However, due to a nature of the morphometric research, it gives a global and correct insight into the status of the elastic skeleton of the ascending aorta in patients with degenerative stenosis of the TAV.

Conclusion

Our findings strongly support the view that the aortas of patients with the TAV stenosis are submitted to the hemodynamic stress which subsequently leads to the gradual elastic lamellae disruption. The exact region of the hemodynamic influence could be confirmed morphometrically. Changes of the elastic skeleton were potentiated with ageing. Females were submitted to more intensive disruption of the elastic lamellae through the course of the aortic stenosis.

The changes of the aortic wall statistically significantly correlated with the echocardiographic parameters: AA, SV, AscA and SVI. The echocardiographic parameters tended to be higher in the grade 3 in patients younger than 65 years of age. The AscA value of more than 4.5 cm was associated with the irreversible morphological defects in these patients. The SV and STJ were higher in the male patients.

Our study is in accordance with opinions that the ascending aorta replacement should be considered in relation to the AVR, particularly in patients younger than 65 years of age with the ascending aortas of more than 4.5 cm in a diameter and the TAV stenosis.

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