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A comprehensive morphometric analysis of the internal thoracic artery with emphasis on age, gender and left-to-right specific differences

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Summary. Aims of the study. The aim of this analysis was the morphometric description of the internal thoracic artery (ITA) with an emphasis on age, gender and left-to-right specific differences, as well as on age and atherosclerosis related changes of the elastic skeleton. Methods. Forty eight arteries were obtained during forensic autopsies from 32 persons who had died of non-vascular causes. The following morphometric parameters were analyzed: thickness of the intima, the medial layer and the wall, the intima-to-media ratio and the elastic skeleton parameters.

Results. The intima thickness increases significantly with aging (ANOVA F=34.061, p<0.001), as does the intima-to-media ratio (ANOVA F=10.831, p<0.001). With aging, there is a significant increase in the thickness of the media (F=56.519; p<0.001) and of the wall (F=34.094; p<0,001). There is a significant increase in the media thickness during the development of atherosclerosis in the ITA (ANOVA F=11.848, p<0.001). No significant difference was found when these data were analyzed based on the left-to-right principle or depending on gender of the patients. However, the analysis of the elastic skeleton parameters indicated that the combined effects of aging, atherosclerosis and male gender lead to the degeneration of the elastic skeleton of the ITA.

Conclusion. The grade of atherosclerosis gradually increases with aging as shown by morphometric analysis. The increase in the medial layer thickness suggests the potential for positive remodeling of the ITA during aging and atherosclerosis. The left/right position has no influence on morphometric parameters of the ITA, while male gender affects parameters of the elastic skeleton.

Key words: Internal thoracic artery, Morphometry, Histology, Aging, CABG

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Introduction

The internal thoracic artery (ITA) was the first arterial graft introduced into surgical practice with an excellent patency rate. This valuable characteristic, as well as years of intensive surgical experience yielding excellent results, initiated numerous basic research projects of the ITA. However, we are still not able to answer why the ITA has low grade atherosclerosis. We do know several ITA features that made it superior to other arterial and venous grafts, but we still need more answers. We have to keep in mind that for most of our patients, coronary artery disease is still the major

Abbreviations: ITA, internal thoracic artery; CABG, coronary artery by-pass grafting

problem, and its conservative management, including CABG surgery, is the major solution.

Our initial research confirmed that the ITA is the artery of the transitional (mixed) type and that the elastic skeleton structure of the ITA is strongly affected by aging (Labudović Borović et al., 2010). We further hypothesized that the high adaptability of the ITA originates from its transitional (mixed) histological structure that united the characteristics of elastic and muscular arteries.

Arteries of the transitional type and specialized arteries are defined by the particular organization of their medial layer (Fawcett, 1986). The *tunica media* of the ITA is made up of two sublayers: the internal layer and the external layer (Fig. 1A,B). The internal medial (muscular) layer is similar to muscular arteries, with circularly oriented sheets of smooth muscle cells (Fig. 1A,B), while the external layer resembles elastic arteries with spirally oriented elastic lamellae and sheets of smooth muscle cells (Fig. 1A,B) (Labudović Borović et al., 2010). The number of elastic lamellae of the external medial layer gradually decreases throughout the length of the artery.

Also, similarly to muscular arteries and as opposed to elastic arteries, the amount of subendothelial connective tissue, in unaltered arteries, is small (Fig. 1A,B). The thickness of the adventitia is proportionally higher and equals the thickness of the medial layer, this feature also being characteristic of muscular arteries but absent in elastic arteries.

In the present study, the focus of our investigation was shifted towards several other points, namely: the thickness of the intima, the thickness of the medial layer, the thickness of the wall and the intima-to-media ratio and their gradual changes during aging and during the development of atherosclerosis. These parameters were compared between males and females, as well as between the left and right arteries. The combined influence of aging and atherosclerosis on the elastic skeleton of the ITA was also investigated, thus becoming an extension to our previously published data (Labudović Borović et al., 2010).

The objective in this phase of the research was to form a broad and reliable base for further, similar comparative investigations of arterial and venous grafts and for the comparisons of the ITA with other potential grafts as well as with coronary arteries. This investigation is particularly important in the era of total arterial revascularization (Suma et al., 1989; Sato et al., 2000) and coronary artery reconstruction with the ITA (Khalifa et al., 2011). Morphological and morphometric studies of the ITA have proved to be important in the evaluation of translation of new technologies into cardiac surgery (Winkler et al., 2011; Winkler and Grapow, 2012).

The idea to investigate the potential morphometric differences between the left and right arteries came from the frequent usage of both mammary arteries for revascularization. This mode of revascularization is by some authors considered to be superior to combined revascularization with the ITA and the saphenous vein (Ascione et al., 2001) and is still an area of intensive clinical research (Taggart et al., 2006), while there is, at the same time, a continual effort to improve the technique of bilateral internal mammary grafting (Zeitani et al., 2005). Finally, one of the questions considered was whether there was any difference

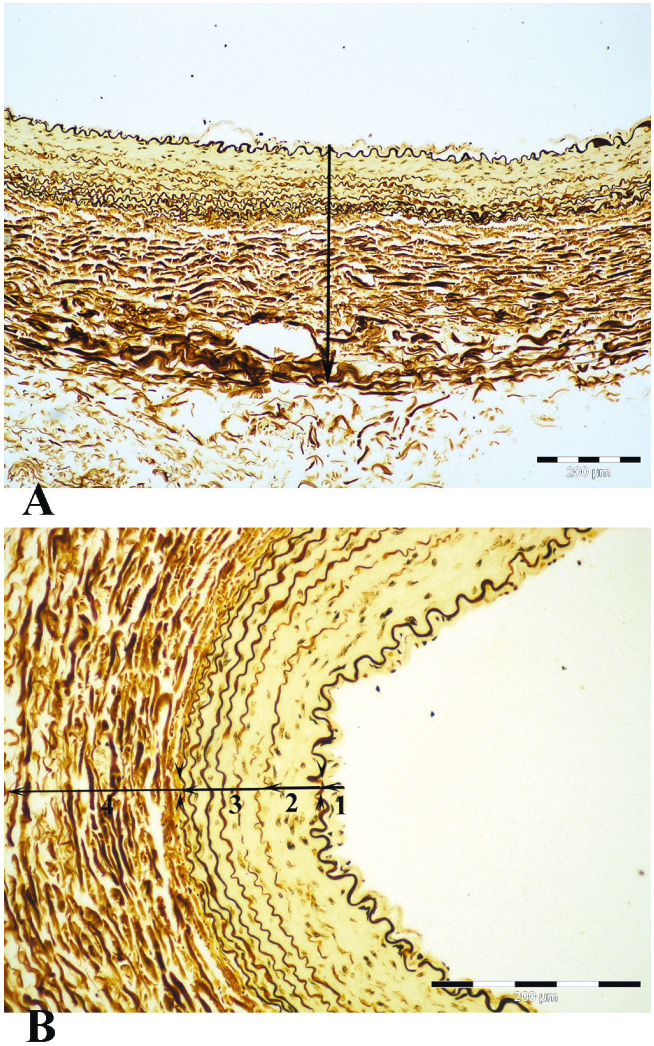


Fig. 1. A. Internal thoracic artery (Silver methenamine staining). Artery of the transitional type, arrow indicates the orientation of the axis of the ocular micrometer during morphometric analysis; it also indicates the full thickness of the arterial wall. **B.** Internal thoracic artery (Silver methenamine staining). 1: Tunica intima (endothelium and a small amount of subendothelial connective tissue - feature of muscular arteries); 2: Tunica media - internal, muscular, layer; 3: Tunica media - external medial layer with well-formed elastic lamellae (feature of elastic arteries); 4: Tunica adventitia - thickness of the adventitia is equal to or even larger than the thickness of the medial layer - feature of muscular arteries; arrowheads indicate the internal elastic membrane and the external elastic membrane. Bar: 200 μm.

between genders in the basic morphometric parameters and, therefore, should a different outcome be expected in elaborate and new procedures of coronary artery by-pass surgery between female and male patients.

Materials and methods

Arterial samples

The analysis included autopsy material consisting of 48 arteries obtained from 32 patients who had died a sudden death excluding causes of vascular origin. The study was approved by and conducted in accordance with the ethical principles approved by the collegium of forensic experts. According to available medical records, nineteen patients had definite previously established medical conditions (Table 1).

Harvesting of the arteries

The arteries were harvested by an experienced surgeon and an expert histologist in keeping with the technical principles of surgical graft harvesting. The exact procedure of arterial harvesting for the purpose of the analysis has been previously described (Labudović Borović et al., 2010).

Examined groups

Forty eight arterial samples were divided into three examined groups. The characteristics of the examined groups are presented in Table 1.

The preparation of tissue for light microscopy and histomorphometry

The tissue was prepared for morphological and morphometric analysis according to the procedure described in the previous study (Labudović Borović et al., 2010). In brief, the morphological analysis included the whole length of the harvested arteries. All arteries were cut into 1 cm long segments and prepared for light microscopy. Blocks of tissue were serially sectioned into 5 micrometers-thick sections, which were sampled at different levels. Afterwards, sections were stained with the application of selective techniques for elastic fibers: the Weigert van Gieson technique with resorcin fuchsin, the Verhoef van Gieson method or Pincus' staining with acid orcein. Randomly sampled sections from each group were stained with hematoxylin and eosin, by applying Masson's trichrome technique, Van Gieson collagen staining or methenamine silver staining.

For the purpose of the immunohistochemical analysis, the following antibodies and dilution ratios were used: vimentin (DAKO M7020, 1:100), desmin (DAKO M0724; 1:50), alpha smooth muscle actin (alpha SMA) (DAKO M0851; 1: 50), myosin heavy chains (DAKO M3558; 1:50), CD68 (DAKO M0876; 1:50), CD45 (DAKO M0701, 1:50), CD45Ro (DAKO M0742; 1:50), CD3 (DAKO A0452; 1:50) and CD43 (DAKO M0786; 1:50). The visualization kits were LSAB+/HRP (DAKO K0679), EnVision/HRP (DAKO K4005) or UltraVision/HRP (Thermo Scientific LabVision TL-060-HD) with DAB (diaminobenzidine) or AEC (3-amino-9-ethylcarbazole) as chromogens.

Histomorphometry was performed by the use of the ocular micrometer. The systematic field sampling method was applied and each section was inspected in 10 microscopic fields uniformly distributed over the circumference of the arterial cross-section. The ocular micrometer was oriented normally to the wall of the vessel. Only the cross sections were analyzed. The terminal parts of each block, oblique sections and branching points, as well as sections with major technical flaws were excluded from the analysis.

The thickness of the intima, the thickness of the medial layer, and the thickness of the wall were measured at x 100 magnification of the standard Olympus CH microscope. The thickness of the wall included the intima, the medial layer and the adventitia,

Table 1. Characteristics of patients and arteries in examined groups.

Age - related groups (years)	Number of patients	Male patients	Female patients	Risk factors		Number of arteries	Mean age of patients (years)	Mean length of arteries (cm)
				Medical Condition	Number of patients			
Group (1) (20-40)	9	5	4	Mitral valve prolapse	1	15	27.25±2.96	13.18±3.32
Group (2) (41-60)	15	9	6	Ischemic coronary disease and arterial hypertension	6	22	50.79±5.62	13.02±2.98
				Rheumatoid arthritis and anemia	1			
				Alcoholism and kahexia	1			
Group (3) (≥61)	8	5	3	Ischemic coronary disease, arterial hypertension and/or premature ventricular contractions	8	11	68.56±6.02	15.14±1.77
				Obesity	2			
Total	32	19	13	Total	19	48	48.94±16.92	13.59±2.90

and did not include the periadventitial connective tissue (Fig. 1A,B). The intima-to-media ratio was calculated. Two morphometric parameters, the thickness of the intima and the intima-to-media ratio, were used to assess the grade of atherosclerosis. Also, all analyzed slides were classified according to the classification of the American Heart Association Committee on Vascular Lesions of the Council of Atherosclerosis (1995) (Figs. 1-3), except for grade 1 that included sections with intimal hyperplasia and sections where solitary macrophage-derived foam cells were present (Fig. 3). Between the defined atherosclerotic grades, significant differences were confirmed as to the mean values of the intima thickness and the intima-to-media ratio (Table 3). We compared the methodology and developed it in accordance with similar studies (Ferro et al., 1991; Van Son et al., 1993; Kaufer et al., 1997; Ruengsakulrach et al., 1999).

Since the ITA is the artery of the transitional (mixed) type, we analyzed two other separate problems. Firstly, the thickness of the internal medial layer and its changes during aging and the development of atherosclerosis were studied. Secondly, Multivariate and Multivariable Linear Regression Analysis was used to assess changes of the elastic lamellae during the same processes. This analysis was the extension of previously described results obtained in our previous study related to age and atherosclerosis induced changes of the elastic skeleton (Labudović Borović et al., 2010). The internal medial layer as well as the mode of the elastic skeleton analysis has been defined in a previous publication (Labudović Borović et al., 2010). The internal medial layer is presented in Fig. 1A,B.

All slides were analyzed using the Olympus CH microscope or the Olympus BX 41 microscope and photo documented by the Carl Zeiss Jenaval microscope and the Pentacon camera or the Olympus C5060-ADU wide zoom camera and the Olympus DP-soft Image Analyzer program.

The described method was used to obtain the results for the parameters for each serial centimeter of the arterial length separately, and at the same time, the mean values of the parameters for these same segments of the arteries were used as units for the statistical analysis.

Table 2. Mean values of the morphometric parameters for the internal thoracic artery - all groups.

Dependent Variable/ The age-related groups	N	Mean	SD	SE	95% C.I. Lower Bound	95% C.I. Upper Bound	MIN.	MAX.	The Bonferroni Multiple Comparison Group (2)	The Bonferroni Multiple Comparison Group (3)
Thickness of the tunica intima (micrometers)				ANOVA F=34.061, p<0.001(*)						
Group (1)	103	9.12	10.74	1.06	7.02	11.22	0.70	63.40	p<0.001(*)	p<0.001(*)
Group (2)	112	20.93	25.09	2.37	16.23	25.63	1.00	116.70		p=0.001(*)
Group (3)	65	33.11	41.47	5.14	22.84	43.39	4.00	232.20		
Total	280	19.41	27.77	1.66	16.15	22.68	0.70	232.20		
Thickness of the tunica media (micrometers)				ANOVA F=56.519, p<0.001(*)						
Group (1)	105	159.42	40.29	3.93	151.62	167.21	63.00	250.00	p<0.001(*)	p<0.001(*)
Group (2)	114	188.89	49.29	4.62	179.75	198.04	78.00	327.00		p<0.001(*)
Group (3)	59	245.20	63.41	8.26	228.67	261.72	120.00	390.00		
Total	278	189.71	58.71	3.52	182.78	196.64	63.00	390.00		
Thickness of the wall (micrometers)				ANOVA F=34.094, p<0.001(*)						
Group (1)	104	241.48	66.10	6.48	228.63	254.34	82.00	393.00	p<0.001(*)	p<0.001(*)
Group (2)	110	281.19	81.11	7.73	265.86	296.51	140.00	593.00		p<0.001(*)
Group (3)	58	359.91	109.36	14.36	331.16	388.67	181.00	669.00		
Total	272	282.79	93.54	5.67	271.63	293.96	82.00	669.00		
Intima-to-media ratio				ANOVA F=10.831, p<0.001(*)						
Group (1)	103	0.0554	0.0530	0.0052	0.0451	0.0658	0.0051	0.3200	p=0.009(*)	p<0.001(*)
Group (2)	109	0.1070	0.1182	0.0113	0.0846	0.1295	0.0033	0.5070		p=0.037(*)
Group (3)	58	0.1463	0.1993	0.0026	0.0939	0.1987	0.0047	1.2142		
Total	270	0.0956	0.1081	0.0078	0.0805	0.1111	0.0033	1.2142		
Thickness of the internal medial (muscular) layer (micrometers)				ANOVA F=29.766; p<0.001(*)						
Group (1)	90	47.18	14.80	1.56	44.08	50.28	21.00	97.00	p<0.001(*)	p<0.001(*)
Group (2)	79	66.31	21.80	2.45	61.43	71.19	28.00	163.00		p=0.406
Group (3)	49	60.48	17.20	2.46	55.54	65.42	34.00	115.00		
Total	218	57.10	20.00	1.36	54.43	59.77	21.00	163.00		

* The mean difference is significant at the 0.05 level. The Bonferroni Multiple Comparison tested the difference between the groups. MIN: minimal value for the parameter; MAX: maximal value for the parameter. Notice the statistically significant difference between different groups in the intima thickness and the intima-to-media ratio. The thickness of the tunica media and the thickness of the wall increase statistically significant during aging. Besides, the thickness of the internal medial (muscular) layer increases during aging, along with degenerative changes of the elastic lamellae. The increase is greatest until the forties, consistent with the greatest decrease in the number of elastic lamellae in this period.

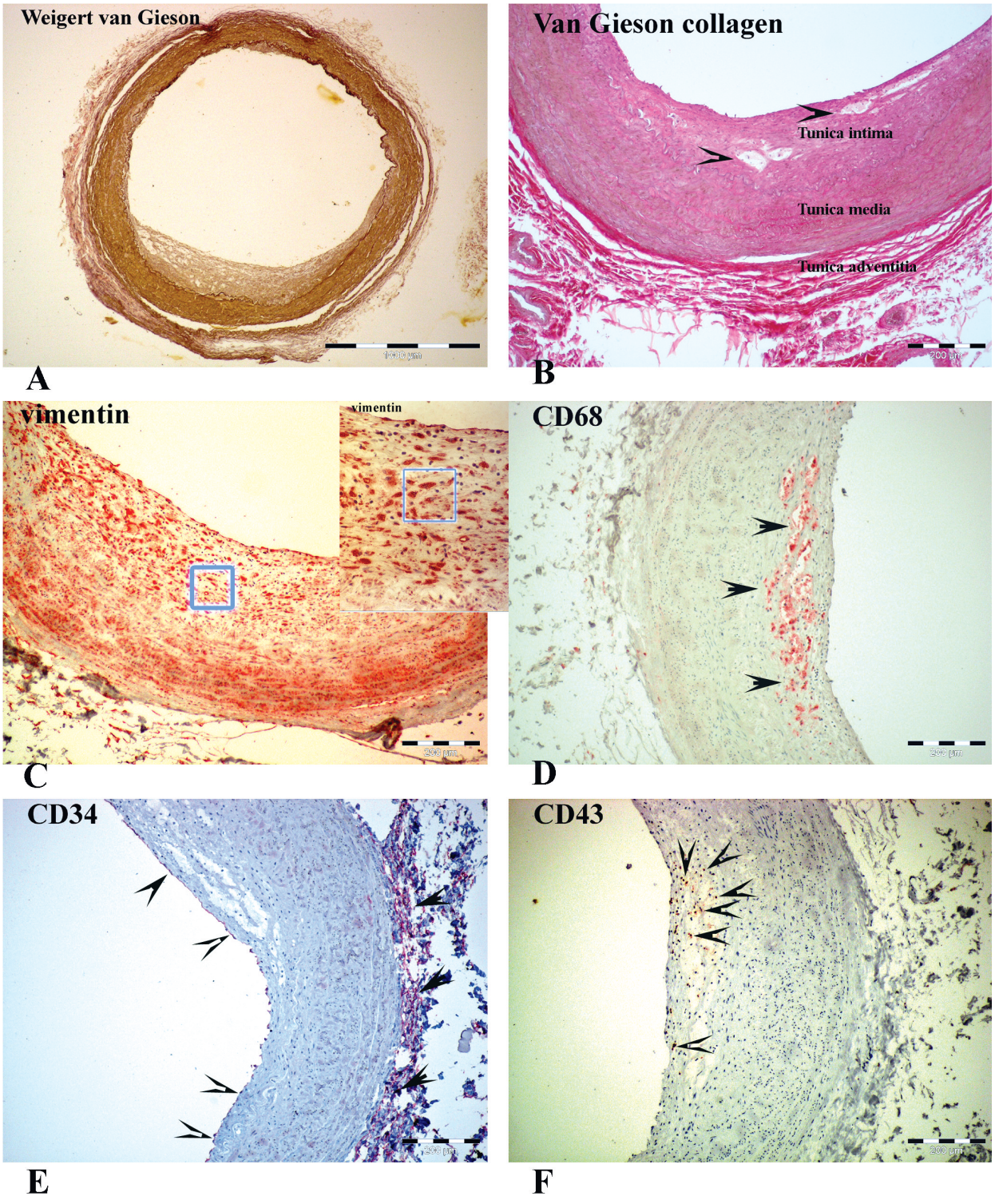


Fig. 2. A. Internal thoracic artery (Weigert van Gieson technique with resorcin fuchsine). Atherosclerosis grade 3, numerous foam cells derived from macrophages and smooth muscle cells, extracellular lipids and extensive extracellular matrix. The following microphotographs on Fig. 2B-2F are provided from serial sections of the same block of tissue. **B,** Internal thoracic artery - detail of the section in figure 2A (Van Gieson collagen technique). Extensive extracellular matrix deposition and groups of foam cells (arrowheads). The extracellular matrix of the intima is formed of a mixture of collagen type I fibers and elastic fibers. **C.** Internal thoracic artery - detail of the section in figure 2A (vimentin; EnVision+/HRP, AEC). Foam cells derived from smooth muscle cells; these cells are vimentin positive, characteristic of vascular smooth muscle cells transformed from contractile into highly active synthetic phenotype; preserved vimentin+ endothelium at luminal surface; inside the blue rectangle is a detail shown in the upper right corner of Figure 2C; notice the obvious morphology of foam cells in a blue rectangle. **D.** Internal thoracic artery - detail of the section in figure 2A (CD68; EnVision+/HRP, AEC). CD68+ foam cells derived from macrophages (arrows). **E.** Internal thoracic artery - detail of the section in figure 2A (CD34; EnVision+/HRP, AEC). CD34+ preserved endothelium (arrowheads) and numerous CD34+ adventitial cells (arrows). **F.** Internal thoracic artery - detail of the section in figure 2A (CD43; EnVision+/HRP, AEC). obscure lymphocyte infiltrate; CD43 is a transmembrane protein sialophorin, syaloglicoprotein expressed at the surface of T lymphocytes. Bars: A, 1 mm; B-F, 200 μm; inset in C, x 265.

Statistical analysis

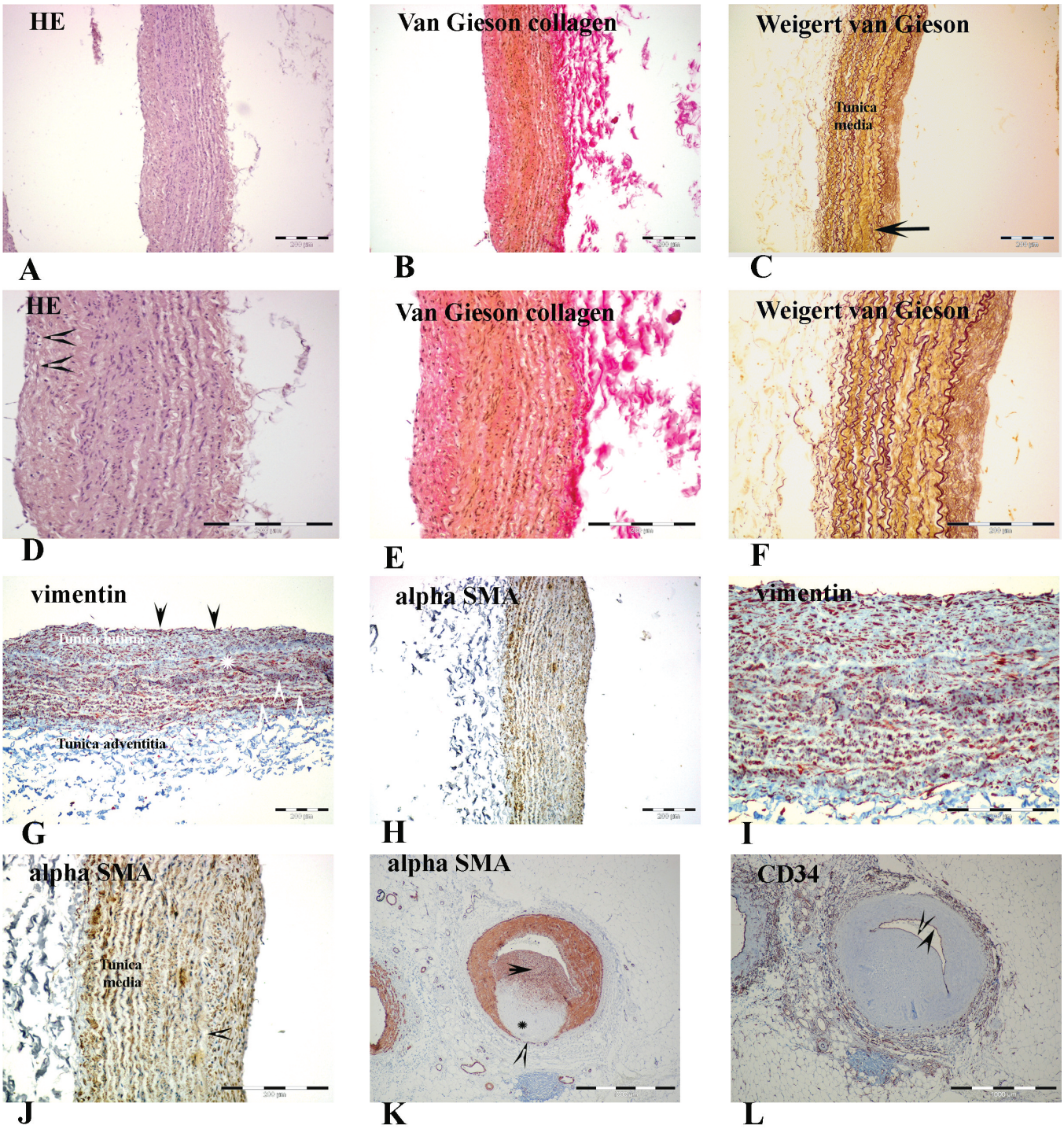
The morphometric values were expressed as the mean ± SD. In addition to the mean values and the SD, the standard error (SE) and the 95% confidence interval (95% C.I.) were calculated. The following statistical methods were used for testing the statistical significance: the One-way ANOVA test with the Bonferroni as the Multiple Comparison Test, Student t-test together with Levene's Test for Equality of Variances, Chi-square test, Pearson Correlation Coefficient, Spearman Rho Correlation Coefficient and Multivariate and Multivariable Linear Regression Analysis. The value of $p<0.05$ was considered statistically significant. The tests were performed with the SPSS version 10.0 for Windows.

Prior to the analysis, the data were tested with the Kolmogorov-Smirnov Test for the normality of their distribution. Data not distributed according to the normal distribution were transformed and expressed as normalized values of variables and the tests were performed with normalized values. The statistical significance for both normalized and non-normalized values were confirmed with applied tests. The data on statistical significance presented in tables are calculated with the normalized values.

Table 3. Differences in the mean values of all parameters for different atherosclerotic grades.

Dependant Variable/ Grades of atherosclerosis	N	Mean	SD	SE	95% C. I. Lower bound	95% C. I. Upper bound	MIN.	MAX.	The Bonferroni Multiple Comparison Grade 1	Grade 2	Grade 3	Grade 4
The thickness of the tunica intima					ANOVA F=142.23, p<0.001 (*)							
Grade 0	155	5.49	6.74	0.54	5.42	7.56	0.70	65.10	p<0.001(*)	p<0.001(*)	p<0.001(*)	p<0.001(*)
Grade 1	49	14.78	7.18	1.03	12.71	16.84	1.50	52.70		p=0.013(*)	p<0.001(*)	p<0.001(*)
Grade 2	26	25.39	11.90	2.33	20.59	30.20	1.90	65.60			p=0.001(*)	p<0.001(*)
Grade 3	32	50.12	35.66	6.30	37.27	62.98	14.40	219.00				p=0.105
Grade 4	18	80.11	47.47	11.19	56.50	103.71	30.80	232.20				
Total	280	19.41	27.78	1.66	16.15	22.68	0.70	232.20				
The thickness of the tunica media					ANOVA F=11.848, p<0.001 (*)							
Grade 0	158	171.35	49.33	3.92	163.60	179.10	63.00	387.80	p=0.001(*)	p=0.570	p<0.001(*)	p=0.027 (*)
Grade 1	47	206.48	66.82	9.75	186.86	226.10	78.00	390.00		p=1.000	p=0.287	p=1.000
Grade 2	25	204.06	60.01	12.00	179.29	228.83	99.00	328.60			p=0.403	p=1.000
Grade 3	30	234.53	49.89	9.11	215.90	253.15	122.50	342.50				p=1.000
Grade 4	18	212.47	62.99	14.85	181.14	243.79	81.70	310.00				
Total	278	189.71	58.71	3.52	182.78	196.64	63.00	390.00				
The thickness of the wall					ANOVA F=17.511, p<0.001 (*)							
Grade 0	154	251.47	79.01	6.37	238.89	264.05	82.00	595.00	p=0.023 (*)	p=0.032 (*)	p<0.001(*)	p<0.001(*)
Grade 1	47	291.01	83.77	12.22	266.41	315.60	142.00	492.00		p=1.000	p=0.008 (*)	p=0.009(*)
Grade 2	25	297.54	69.13	13.83	269.01	326.08	171.00	418.00			p=0.135	p=0.101
Grade 3	28	362.68	84.99	16.06	329.73	395.64	181.00	669.00				p=1.000
Grade 4	18	384.58	123.39	29.08	323.22	445.95	192.00	638.00				
Total	272	282.79	93.54	5.67	271.63	293.96	82.00	669.00				
The intima-to-media ratio					ANOVA F=80.301, p<0.001 (*)							
Grade 0	151	0.0392	0.0346	0.0028	0.0337	0.0448	0.0033	0.2999	p<0.001(*)	p<0.001(*)	p<0.001(*)	p<0.001(*)
Grade 1	47	0.0779	0.0484	0.0071	0.0637	0.0921	0.0047	0.2319		p=0.027(*)	p<0.001(*)	p<0.001(*)
Grade 2	25	0.1329	0.0707	0.0141	0.1037	0.1621	0.0071	0.3080			p=0.102	p<0.001(*)
Grade 3	30	0.2162	0.1532	0.0278	0.1590	0.2734	0.0488	0.9080				p=0.078
Grade 4	17	0.3804	0.2447	0.0594	0.2545	0.5062	0.1490	1.2142				
Total	270	0.0958	0.1278	0.0078	0.0805	0.1111	0.0033	1.2142				
The thickness of the internal medial (muscular) layer					ANOVA F=7.883, p<0.001 (*)							
Grade 0	120	51.56	15.77	1.44	48.71	54.41	21.00	97.00	p=0.988	p=0.005 (*)	p=0.005 (*)	p=0.001(*)
Grade 1	37	57.71	23.62	3.88	49.84	65.59	21.00	163.00		p=0.410	p=0.894	p=0.144
Grade 2	17	66.86	13.52	3.28	59.92	73.81	46.00	91.00			p=1.000	p=1.000
Grade 3	29	65.88	24.47	4.54	56.57	75.18	39.00	115.00				p=1.000
Grade 4	15	71.89	21.76	5.62	59.84	83.94	39.00	113.00				
Total	218	57.10	20.00	1.36	54.43	59.77	21.00	163.00				

* The mean difference is significant at the 0.05 level. The Bonferroni Multiple Comparison tested the difference between the groups. MIN: minimal value for the parameter; MAX: maximal value for the parameter. Notice the statistically significant difference between different atherosclerotic types in the intima thickness and the intima-to-media ratio, except between intermediate lesions (grade 3) and atherosclerotic plaques (grade 4). The thickness of the tunica media increases during the initial development of atherosclerosis. Wall thickness increases statistically significantly with development of atherosclerosis, especially between unaltered segments and those with "fatty dots" and any other type of atherosclerotic lesion. Also, the thickness of the internal medial (muscular) layer increases significantly.



Figs. 3. A, D. Internal thoracic artery (hematoxylin and eosin staining). Intimal hyperplasia with grade 1 atherosclerosis; notice foam cells derived from macrophages (arrowheads on **D**). The microphotographs. **A-J.** Are provided from serial sections of the same block of tissue. **B, E.** Internal thoracic artery (Van Gieson collagen technique). Deposition of collagen fibers within the intima. **C, G.** Internal thoracic artery (Weigert van Gieson technique with resorcin fuchsine (elastica van Gieson)). Numerous elastic lamellae and a visible inner, medial, muscular layer marked by an arrow. **G, I.** Internal thoracic artery (vimentin; EnVision+/HRP, AEC). Abundant vimentin immunoreactive smooth muscle cells in the intima - the intimal hyperplasia; well preserved endothelium (arrows); notice circular orientation of the smooth muscle cells in the inner muscular medial layer (white star) and spiral orientation at different angles in the external medial layer (white arrowheads). **H, J.** Internal thoracic artery (smooth muscle actin; UltraVision/HRP, DAB). Intimal hyperplasia - smooth muscle cells; arrowhead indicates the internal elastic lamina. **K.** Internal thoracic artery (smooth muscle actin; EnVision+/HRP, AEC). Atherosclerosis, grade 4; occlusion of the lumen, thinning of the medial layer (arrowhead), numerous smooth muscle cells (arrow) and lipid core (star). The microphotographs on **K-L** are provided from serial sections of the same block of tissue. **L.** Internal thoracic artery (CD34; EnVision+/HRP, DAB). Atherosclerosis, grade 4 occlusion of the lumen, thinning of the medial layer, numerous smooth muscle cells in the intima and lipid core, but preserved endothelium. Bars: A-J, 200 μm; K, L, 1 mm.

Results

Atherosclerosis of the internal thoracic artery

The ITA is known for its lack of atherosclerosis. Our observations coincide with this belief. Out of 288 analyzed segments, 162 (56.25%) were without changes (Fig. 1A,B). Intimal hyperplasia and intimal hyperplasia with solitary foam cells were present in 49 segments (17.01%), fatty streaks in 26 segments (9.03%), while intermediate lesions and atherosclerotic plaques were observed in 32 (11.11%) and 19 (6.60%) segments. With aging, the absolute number of segments without atherosclerosis decreased and the same is true for the percentage of these segments. Namely, among the youngest patients (group 1) 79.05% of the segments were without changes, while in the oldest group (group 3) this percentage was 22.73%. At the same time, the percentage of atherosclerotic plaques was 0.95% in group 1 and 7.58% in group 3. The Chi-square test proved the statistical significance of the observed differences among the examined groups (Pearson Chi-Square=60.276, p<0.001). It is also worth mentioning that among the changed segments the most numerous were those with intimal hyperplasia and that their number increased with aging.

The composition of the atherosclerotic lesions

Most atherosclerotic lesions of the internal thoracic artery develop on the basis of previously existing intimal hyperplasia. The occurring foam cells were derived from macrophages, but also, often, from smooth muscle cells even in the early atherosclerotic lesions and among younger people (Fig. 2). The endothelium was universally preserved above the lesions, even in older people (Figs. 2, 3). However, the inflammation was of low grade with obscure lymphocyte inflammatory infiltrate (Fig. 2F).

Morphometric parameters of the internal thoracic artery: aging and development of atherosclerosis

The mean intima thickness

The mean value of the intima thickness for the whole sample was 19.41±27.77 micrometers (Table 2). The lowest value was established for the youngest group (group 1), the value gradually increased in group 2 and the highest value was in group 3, which consisted of the oldest examinees, with statistically significant differences amongst the groups (F=34.061, p<0.001) (Table 2; Fig. 4A).

The highly positive correlation between the thickness of the intima and aging was confirmed by the Pearson Correlation Coefficient (Pearson Correlation Coefficient=0.309; p<0.001). Also, we confirmed that aging was a highly predictive factor for the thickness of the intima, by applying Multivariate and Multivariable Regression Analysis (Table 6). During the development of atherosclerosis, the thickness of the intima gradually increased, as confirmed by the ANOVA analysis with the Bonferroni test (Table 3).

The mean thickness of the medial layer

The mean thickness of the medial layer was 189.71±58.71 micrometers for the whole sample (Table

Table 4. Mean values for all parameters - gender specific differences.

Dependant Variable/ Gender					Levene's Test for Equality of Variances		t-test for Equality of Means			Mean Difference	SE Difference	95% C.I. of the Difference	
	N	Mean	SD	SE	F	p	t	df	p			Lower bound	Upper bound
Thickness of tunica intima													
Males (1)	165	20.22	26.51	2.07	1.272	0.260	1.910	276	0.057	0.26	0.13	-0.079	0.520
Females (2)	112	15.74	21.53	2.04									
Thickness of the tunica media													
Males (1)	163	195.43	57.53	4.52	0.039	0.843	2.666	278	0.008(*)	18.44	6.92	4.82	32.06
Females (2)	116	176.99	54.43	5.14									
Thickness of the wall													
Males (1)	160	282.12	88.40	7.01	1.020	0.313	0.877	272	0.381	0.03	0.04	-0.042	0.110
Females (2)	113	270.26	80.75	7.74									
Intima-to-media ratio													
Males (1)	159	0.0931	0.1121	0.0089	0.514	0.474	0.878	269	0.380	0.12	0.13	-0.144	0.376
Females (2)	111	0.0877	0.1023	0.0099									
Thickness of the internal, medial (muscular) layer													
Males (1)	128	57.53	20.80	1.85	0.936	0.334	0.208	219	0.835	0.01	0.05	-0.080	0.100
Females (2)	93	56.51	19.10	2.02									

* The mean difference is significant at the 0.05 level. Notice that there was no statistical significance among morphometric parameters for different genders, except for the mean thickness of the tunica media that was larger in males.

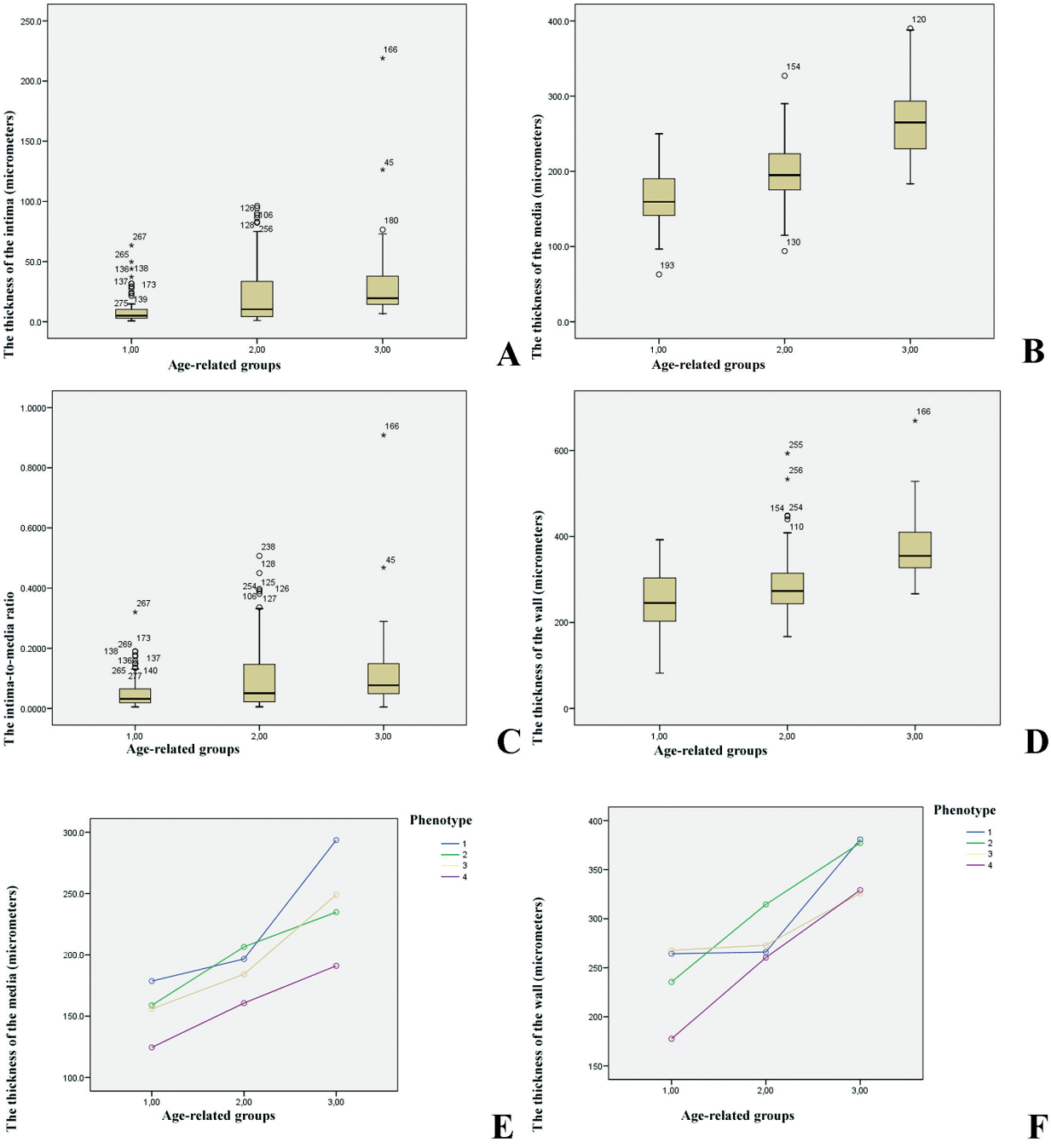


Fig. 4. Mean values of all morphometric data for all groups. Numbers above graphs represent the serial number of segments with extreme values of the given morphometric parameter. Prior to the analysis the data were tested with the Kolmogorov-Smirnov Test for the normality of their distribution. Four phenotypes of the external medial layer for graphs **E** and **F** are defined as to the number of elastic lamellae (this number does not include the internal and the external elastic laminas): 1. the elastic pattern, which contains between 7 and 15 elastic lamellae; 2. the elasto-muscular pattern, with four to six concentric elastic lamellae; 3. the musculo-elastic pattern, with one to three elastic lamellae; 4. the muscular pattern, with circularly oriented smooth muscle cells and very rare, solitary elastic fibers (Labudoviç Boroviç et al., 2010). Both the thickness of the medial layer and of the wall increase in all medial phenotypes. *The thickness of the media (**B** and **E**) = The thickness of the tunica media

2). Again, during aging, the thickness of the medial layer gradually increased and statistical significance was confirmed among age-related groups by the ANOVA with the Bonferroni as the Multiple Comparison Test (F=56.519; p<0.001) (Table 2; Fig. 4B), by Pearson Correlation Coefficient (Pearson Correlation Coefficient=0.515; p<0.001) and by Multivariate and Multivariable Regression Analysis (Table 6).

When we cross-checked the data, according to the grades of atherosclerosis, independently of aging, the thickness of the medial layer was statistically significantly larger when comparison was made between

Table 5. Left-to-right specific differences - the mean values of all parameters.

Dependant Variable/ Left/right	N	Mean	SD	SE	Levene's Test for Equality of Variances F	Levene's Test for Equality of Variances p	t-test for Equality of Means t	t-test for Equality of Means df	t-test for Equality of Means p	Mean Difference	SE Difference	95% C.I. of the Difference Lower bound	95% C.I. of the Difference Upper bound
Thickness of the tunica intima													
Right (1)	135	17.15	21.28	1.83	0.030	0.862	-0.270	272	0.787	-0.70	2.59	-5.81	4.41
Left (2)	139	17.85	21.64	1.84									
Thickness of the tunica media													
Right (1)	141	191.58	59.58	5.02	1.565	0.212	1.279	280	0.202	8.70	6.81	-4.69	22.10
Left (2)	141	182.87	54.60	4.60									
Thickness of the wall													
Right (1)	138	281.77	79.76	6.79	0.501	0.480	1.525	273	0.128	14.80	9.70	-4.31	33.91
Left (2)	137	266.98	81.16	6.93									
Intima-to-media ratio													
Right (1)	129	0.0886	0.0991	0.0087	0.333	0.564	0.106	259	0.916	0.0013	0.012	-0.022	0.025
Left (2)	132	0.0874	0.0925	0.0080									
Thickness of the internal, medial (muscular) layer													
Right (1)	114	59.10	20.94	1.96	2.354	0.126	1.750	215	0.081	4.66	2.64	-0.58	9.86
Left (2)	103	54.44	17.90	1.76									

*: The mean difference is significant at the 0.05 level. Notice that there was no statistical significance in the mean values of different morphometric parameters between the right-sided and left-sided arteries.

Table 6. Regression analysis of different predictors of the ITA morphometric parameters.

		Unstandardized Coefficients B	Unstandardized Coefficients SE	Standardized Coefficients Beta	t	p	95% Confidence Interval for B Lower Bound	95% Confidence Interval for B Upper Bound
R^2=0.098	(Constant)	4.658	4.968		0.938	0.349	-5.122	14.438
	Age-related groups	8.739	1.651	0.306	5.292	<0.001(*)	5.488	11.990
	Gender	-2.266	2.515	-0.052	-0.901	0.368	-7.218	2.686
Dependent Variable: Thickness of the tunica intima								
R^2=0.055	(Constant)	0.027	0.024		1.153	0.250	-0.019	0.073
	Age-related groups	0.030	0.008	0.235	3.868	<0.001(*)	0.015	0.046
	Gender	0.004	0.012	0.022	0.356	0.722	-0.019	0.027
Dependent Variable: Intima-to-media ratio								
R^2=0.315	(Constant)	131.495	11.700		11.239	<0.001(*)	108.461	154.530
	Age-related groups	33.369	4.191	0.431	7.962	<0.001(*)	25.117	41.620
	Gender	-9.317	5.905	-0.080	-1.578	0.116	-20.943	2.309
	Grade of atherosclerosis	9.388	2.417	0.212	3.883	<0.001(*)	4.628	14.147
Dependent Variable: Thickness of the tunica media								
R^2=0.570	(Constant)	188.248	16.935		11.116	<0.001(*)	154.902	221.594
	Age-related groups	27.472	6.024	0.249	4.560	<0.001(*)	15.611	39.333
	Gender	8.036	8.504	0.049	0.945	0.346	-8.708	24.781
	Grade of atherosclerosis	27.594	3.489	0.435	7.910	<0.001(*)	20.725	34.46
Dependent Variable: Thickness of the wall								

*: The mean difference is significant at the 0.05 level. Notice that age is highly predictive factor for the thickness of the intima and the intima-to-media ratio, while both age and atherosclerosis are predictive for the thickness of the tunica media and the thickness of the wall.

the segments without atherosclerosis and those with atherosclerosis (Table 3). However, among different grades of atherosclerosis there were no statistically significant differences in the mean thickness of the medial layer (Table 3).

These results indicate that the medial layer of the ITA gradually increases during aging and the initial development of atherosclerosis. They corroborate well with the results of the Pearson Correlation Test, which confirmed a highly positive correlation between the thickness of the medial layer and the thickness of the intima (Pearson Correlation Coefficient=0.329; p<0.001) as well as between the thickness of the medial layer and the grade of atherosclerosis (Pearson Correlation Coefficient=0.381; p<0.001). When multifactorial analysis was applied, both aging and atherosclerosis appeared to be highly predictive for the thickness of the medial layer (Table 6).

Nevertheless, we should be very careful with the interpretation of these results since Figure 3K demonstrates the decrease of the medial layer at the level of the highest intimal thickness. As shown with the Multivariate Analysis the increase in the thickness of the medial layer is characteristic for all phenotypes of the arterial medial layer during aging (F=3.309; p=0.004) (Fig. 4E).

The intima-to-media ratio

The obtained results of the mean intima-to-media ratio are in concordance with the results of the mean intima thickness, for both aging and the grade of atherosclerosis (Tables 2, 3; Fig. 4C).

The mean wall thickness

The mean wall thickness was 282.79±93.54 micrometers (Table 2). It gradually increased during aging with significant differences among age-related groups (F=34.094; p<0.001) (Table 2; Fig. 4D). The

Table 7. Regression analysis of different predictors of elastic skeleton changes in the ITA.

		Unstandardized Coefficients		Standardized Coefficients	t	p	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
R^2=0.078	(Constant)	1.760	0.278		6.325	<0.001(*)	1.213	2.307
	Age-related groups	0.596	0.170	0.172	3.513	<0.001(*)	0.262	0.929
	Intima-to-media ratio	7.070	1.814	0.237	3.897	<0.001(*)	3.503	10.636
	Grade of atherosclerosis	-0.415	0.112	-0.222	-3.689	<0.001(*)	-0.636	-0.194
Dependent Variable: Number of the internal elastic membrane fenestrations per 100 micrometers								
R^2=0.176	(Constant)	2.975	0.258		11520	<0.001(*)	2.467	3.483
	Gender	-0.555	0.182	-0.176	-3.055	0.002(*)	-0.913	-0.198
Dependent Variable: Size of the internal elastic membrane fenestrations								
R^2=0.117	(Constant)	1.789	0.081		22.024	<0.001(*)	1.629	1.949
	Age-related groups	-0.131	0.043	-0.185	-3.072	0.002(*)	0.215	-0.047
	Intima-to-media ratio	1.098	0.521	0.168	2.108	0.036(*)	0.072	2.124
	Grade of atherosclerosis	-0.111	0.027	-0.331	-4.105	<0.001(*)	-0.164	-0.058
Dependent Variable: Thickness of elastic lamellae								

*: The mean difference is significant at the 0.05 level. Aging and atherosclerosis are highly predictive factors for the number of fenestrations of the internal elastic membrane and elastic lamellae thickness, while male gender influences the size of the internal elastic membrane fenestrations.

Table 8. Summarized morphometric data from previous and present studies.

Study	Mean Age (years)	Thickness of the intima		Intima-to-media ratio		Thickness of the media		Reference No.
		Range (mm)	Mean ± SD (mm)	Range (mm)	Mean ± SD (mm)	Range (mm)	Mean ± SD (mm)	
110 discarded distal segments of the ITA	66	0.01- 0.52	0.08±0.07	0.030-2.730	0.360±0.370	0.100-0.600	0.260±0.080	Ruengsakulrach et al., 1999
27 autopsies three distinct segments	59±14.3	0.04-0.350	0.061±0.084	0.025-2.666	0.380±0.583	0.080-0.450	0.178±0.079	Ferro et al., 1991
		0.04-0.028	0.011±0.006	0.022-0.140	0.053±0.028	0.130-0.260	0.222±0.070	Ferro et al., 1991
27 autopsies/54 arteries	19-83	0.006-0.01134	0.00868±0.00103	/	/	0.07176-0.12244	0.10091±0.01070	Reddy et al., 2011
32 autopsies/48 arteries whole length	48.52±16.34	0.007-0.232	0.019±0.028	0.0033-1.214	0.096±0.108	0.063-0.390	0.190±0.059	Our present study

difference in the total wall thickness was the result of the differences present in the thickness of the intima (Pearson Correlation Coefficient=0.533; p<0.001) and the medial layer (Pearson Correlation Coefficient=0.840; p<0.001).

It also steadily increased with the development of atherosclerosis (Table 3) and correlated highly with the parameters of atherosclerosis: the thickness of the intima, the intima-to-media ratio (Pearson Correlation Coefficient=0.293; p<0.001) and the estimated level of atherosclerosis (Pearson Correlation Coefficient=0.404; p<0.001). Consistent with this, the grade of atherosclerosis, together with aging, was predictive for the thickness of the wall, as shown by the multifactorial analysis (Table 6). Multivariate analysis also showed the increase in wall thickness for medial phenotypes (F=2.607; p=0.018) (Fig. 4F).

Gender-specific differences

The mean value of the thickness of the medial layer was larger in male patients (195.43±57.53 micrometers) than in female examinees (176.99±54.43 micrometers) with a statistically significant difference (t=2.666, p=0.008). Other observed differences were not of statistical significance (Table 4).

The left-to-right-specific differences

The right-sided arteries were distinguished by somewhat larger values of the thickness of the *tunica media*, the arterial wall, the intima-to-media ratio and the thickness of the internal medial layer, but these differences were subsidiary and not statistically significant (Table 5).

Elastic skeleton changes during aging and development atherosclerosis

The morphometric parameters of the elastic skeleton of the ITA were previously analyzed (Labudović Borović et al., 2010). Since we have already concluded that changes in the elastic lamellae (including the internal elastic lamina) were age-dependent, but did not change radically with the development of atherosclerosis (Labudović Borović et al., 2010), we hypothesized that elastic skeleton changes made the ITA prone to atherosclerosis with aging. In further analysis, we were interested in how multiple factors: aging, atherosclerosis and gender influenced the parameters of the elastic skeleton of the ITA.

Firstly, we examined the correlations between the morphometric parameters of the elastic lamellae and aging or atherosclerosis. Again, our previous conclusions were confirmed. The number of the internal elastic lamina (IEL) fenestrations and their size were highly and positively correlated to aging (Spearman's rho Correlation Coefficient=0.198; p<0.001 for the number of the IEL fenestrations; Spearman's rho Correlation Coefficient=0.135; p<0.02 for the size of the IEL fenestrations), while the thickness of the elastic lamellae and the number of their fenestrations were highly and negatively correlated to aging (Spearman's rho Correlation Coefficient=-0.212; p<0.001 for the elastic lamellae thickness; Spearman's rho Correlation Coefficient=-0.205; p=0.002 for the number of fenestrations). The only parameter correlated to atherosclerosis was the thickness of the elastic lamellae (Spearman's rho Correlation Coefficient=-0.232; p<0.001).

Secondly, we matched all the descriptive parameters of atherosclerosis (the thickness of the intima, intima-to-media ratio and the morphological grade), age and gender with the morphometric parameters of the elastic skeleton. The results are presented in table 7. The number of fenestrations of the IEL increased significantly with aging, while the elastic lamellae thickness decreased significantly with aging and the development of atherosclerosis. For some parameters, such as the size of fenestrations of the internal elastic lamina, the male gender was predictive for larger fenestrations (Table 7).

At the same time, aging was accompanied by higher values of the thickness of the internal, medial (muscular) layer (Table 2, Fig. 5E). Also, there was a statistically significant difference (ANOVA F=7.883, p<0.001) between sections without atherosclerosis and those with different grades of atherosclerosis in the thickness of the internal medial layer (Table 3). The thickness of this layer correlated highly and positively with the grades of atherosclerosis (Pearson Correlation Coefficient=0.334; p<0.001). Therefore it can be concluded that once the elastic skeleton starts to progressively decay during aging and atherosclerosis, the elastic lamellae gradually perish and the thickness of the internal, medial layer steadily increases.

Discussion

This study included the integral data on a large sample of internal thoracic arteries. Using the benefits of the autopsy technique, all arteries were analyzed throughout the entire length and the mean values of morphometric parameters were presented. This approach enabled us to obtain an insight into the thorough and unified morphometric features of the ITA. The formation of examined groups according to the age or the grade of atherosclerosis enabled the analysis of the changes that occurred during aging and atherosclerosis.

Morphometric data - comparison with the previous studies. Previously established morphometric parameters of the ITA varied substantially, depending on the sampling method (entire arteries or terminal parts discarded after the CABG procedure) (Ferro et al., 1991; Ruengsakulrach et al., 1999), the analyzed areas (areas with the minimal thickness of the intima or with the maximal thickness, atherosclerosis free areas or lesion areas) (Ferro et al., 1991; Ruengsakulrach et al., 1999),

and on the chosen morphometric parameters or the age of the individuals included in the examined group.

According to the results of a previous study, the thickness of the intima of the ITA was 2.5 micrometers and the thickness of the medial layer was estimated to be 180 micrometers (Landymore and Chapman, 1987). We have briefly summarized the morphometric data from previous similar studies in table 8.

At this point, we have to accentuate that results of our previous analysis indicated that the smallest value of the intimal thickness was noted in segments with the musculo-elastic pattern and the largest values were determined in the parts with the elasto-muscular pattern (Labudović Borović et al., 2010). However, these differences were not statistically significant. We also correlated the thickness of the intima and the intima-to-media ratio with the following parameters: the morphological pattern of the medial layer, the mean number of lamellae in the external medial layer, the minimal and the maximal number of elastic lamellae, and the minimal and the maximal number of elastic lamellae, together with the internal and external elastic laminas, and proved that the mean difference was statistically insignificant (Labudović Borović et al., 2010), as opposite to previous studies (Van Son et al., 1993).

For the estimation of the level of atherosclerosis, two morphometric parameters were chosen in this study: the intima thickness and the intima-to-media ratio. These two morphometric parameters are highly correlated and their combined analysis proved that during aging the level of atherosclerosis gradually increases, which could influence the postoperative expectations in older patients. However, the most frequent in all age groups is intimal hyperplasia. The incidence of intimal hyperplasia appears to increase with aging, as proven by our study.

These data are especially important considering the current research on influence of aging to prognosis of bilateral internal thoracic artery grafting (Kieser et al., 2011) and are in agreement with the study of Cizek et al. (2007). Our study and the study of Cizek et al. have proven that aging is a significant factor for the development of both preatherosclerotic intimal thickening and atherosclerotic lesions in the ITA. At the same time, these data are opposite to the results of Reddy et al., who did not find any significant increase in the intima thickness during aging (Reddy et al., 2011).

According to morphometric data from our study (Tables 2, 3), the wall thickness of the ITA was safely beneath the critical 350 micrometers. Namely, it has generally been established that all arteries within the distance of 350 micrometers from the lumen are adequately perfused and nourished from the lumen. In distances larger than 350 micrometers, or 29 lamellar units, the arteries need the supportive nutrition from the vasa vasorum (Geiringer, 1951; Scotland et al., 1999). Based on this data, the conclusion can be drawn that the wall of the ITA could be nourished entirely from the lumen, which provides the morphological basis for the fact that the ITA could be used as a skeletonized graft without dangerous ischemic damage to the wall and with an excellent clinical outcome and with the preserved basic structure of the wall (Gaudino et al., 1999; Deja et al., 1999). This pattern of wall nourishing makes the free ITA an equally good option for revascularization. Creative grafts (T, Y anastomoses) with a part of the skeletonized ITA are also acceptable. The use of bilateral skeletonized ITA is less frequently complicated by deep sternal wound infections (Toumpoulis et al., 2007). Eventually, skeletonization of the ITA improves the quality of grafts and prognosis in a high-risk population (Hu and Zhao, 2011).

The composition of atherosclerotic lesions. Our morphometric results imply that with aging not only does the number of segments with hyperplasia increase, but the hyperplasia itself also advances. After an extensive morphological analysis, we may suggest that within the internal thoracic artery, there is a «natural» progression of intimal hyperplasia to atherosclerosis, which is not the universal rule for all arteries (Schoen, 2005). Namely, Schoen claims that the lack of harmful consequences of intimal hyperplasia suggests «that it is not an early lesion of atherosclerosis, nor is it necessarily a harbinger of other disease» (Schoen, 2005). The studies by Cizek et al. (2007) and, in certain aspects, our present study, indicate that the internal thoracic artery reacts by intimal hyperplasia not only in aging, but also in the case of other risk factors such as smoking or arterial hypertension. Intimal hyperplasia is subsequently augmented by atherosclerosis of a lower grade. Regarding this viewpoint, we preferred and actually used the term "grades" for different levels of atherosclerosis in this study, instead of the term "types" of atherosclerotic lesions (Figs. 2-4).

Inflammation in atherosclerotic lesions of the ITA is obscure, with macrophages as the main inflammatory elements, while lymphocytes are rare. A particular characteristic of the ITA is a preserved endothelium and a high proportion of CD34 immunoreactive endothelial cells, even in older patients and in advanced atherosclerotic lesions, which is not the case with coronary arteries and other arterial grafts, as shown by our previous results (Labudović Borović, 2003).

Parameters of the elastic skeleton and their relationship to age and atherosclerosis. Our previous and current results confirmed by Correlation Analysis and Multivariate and Multivariable Linear Regression Analysis indicate that gradual changes of the elastic skeleton develop during aging. This could be a part of the explanation why aging makes the artery prone to the development of atherosclerosis. The same is true for the male gender. In other words, age and the male sex, factors already confirmed as risk factors for atherosclerosis, operate even in the atherosclerosis-resistant internal thoracic artery.

This is also consistent with previous observations that in patients with hypercholesterolemia, hypertension and/or diabetes mellitus, atherosclerosis is extensive

even in the ITA (Loop et al., 1973; Frazier et al., 1974). We have to point out that the number of patients with risk factors in Group 3 was slightly larger than in Group 2 (Table 1) and we should bear in mind that aging is connected to an increase in risk factors for atherosclerosis, when discussing the issue of aging and its influence on morphometric parameters of the ITA.

At the same time, the thickness of the internal, medial (muscular) layer, previously described, in conjuncture with the structure of the ITA as the transitional type artery, gradually increased with age and the development of atherosclerosis (Table 3). This finding also corroborates well with our previous observations that aging and age-related developments of atherosclerosis are followed by progressive deterioration of the elastic skeleton (Labudović Borović et al., 2010) and with the results of a similar study that has taken into account only the number of elastic lamellae of the ITA during aging (Reddy et al., 2011).

The internal thoracic artery - the issue of positive remodeling. The combined results of the ANOVA, The Bonnfferoni Multiple Comparison Test, the Pearson Correlation Test and the Multivariate Regression Analysis have proven that with aging and the initial development of atherosclerosis, the thickness of the medial layer and of the wall increase. These results could correspond well with the enlargement of the cross sectional area and the external elastic membrane area, which are necessary for defining positive remodeling, and which indicate that the ITA is capable of positive remodeling during the lifetime of the patient and the progression of atherosclerosis. Also, these findings corroborate well with the previous observations of Singh and Sosa (Singh and Sosa, 1984). In a spirit of fair discussion, we have to point out that this conclusion is completely opposite from the conclusion of Reddy et al., who found that during aging, thickness of the media decreased, probably because of the reduced number of elastic lamellae (Reddy et al., 2011).

It is essential to emphasize, again, that 1cm long arterial segments were units for statistical analysis. This mode of analysis was necessary in order to reduce potential influence of specific arterial phenotype as well as significant differences in the thickness of medial layer amongst the most proximal and the most distal parts of the internal thoracic arteries and thus our conclusions apply to all segments and arterial medial phenotypes, as demonstrated in Fig. 4E,F.

This finding indicates that positive remodeling is characteristic for intimal hyperplasia and early atherosclerotic lesions in the internal thoracic artery, but it also could be the consequence of a larger number of sections with intimal hyperplasia and a low grade atherosclerosis. Figure 3K indicates that high grade atherosclerosis is linked to a decrease in the medial thickness at the level of the highest intimal thickness.

In brief, with aging, atherosclerosis develops and the elastic skeleton decays, but the thickness of the medial layer increases and the ITA is positively remodeled. In other words, although affected by aging and prone to atherosclerosis by elastic skeleton disruption, the ITA has a preserved interior *milieu* that antagonizes the devastating effect of ongoing aging and atherosclerosis. The question is, if the thickness of the medial layer steadily grows during aging, what is the source for medial renovation?

These findings and emerging questions, implicate potentially very complex biological interactions of all the elements of the arterial wall, including the endothelium, smooth muscle cells, the elastic skeleton and probably the adventitia. The molecular basis of such interactions should be investigated in further studies. Especially important, in that respect, could be the CD34+ vascular wall resident progenitor cells of the adventitia (Fig. 2E), which are very abundant in the internal thoracic artery, as suggested by previous studies (Labudović Borović, 2003; Zengin et al., 2006).

Gender specific differences. Of special importance in the description of the arterial grafts is the question of the difference in basic morphometric parameters between males and females. Should we expect a different outcome of CABG or total arterial revascularization in patients of different genders?

Studies with a detailed analysis of these parameters are rare. The results related to the radial artery, indicate that the blood vessels of females have a smaller perimeter than do the blood vessels of males, with a significantly larger ratio between the IEL surface and the luminal surface, but there are no statistically significant differences in the thickness of the intima and the medial layer (Mong et al., 2002). Our data indicate that all parameters are slightly larger in males than in females, but these differences are not of statistical significance.

The left-to-right specific differences. The next important question during the morphometric analysis was, are there any differences in the morphometric parameters between the left-sided and the right-sided arteries? This aspect is very important since the pedicled left ITA is insufficient to bypass the distal right coronary artery and the marginal branches of the circumflex coronary artery and there is a real need for another graft, the right ITA or the saphenous vein. Many clinical and angiographic studies have shown that the utilization of bilateral ITA grafts has a superior outcome in comparison to the combination of the ITA and the saphenous vein (Ascione et al., 2001; Tatoulis et al., 2011), with an extremely low rate of in-hospital mortality (0.5%) and a patency rate of 99% after 12 months of clinical follow-up (Lytle et al., 1999; Oster et al., 2005).

Our results indicate that there are no statistically significant differences in the morphometric parameters between the left-sided and the right-sided arteries, which is fully coherent with the contemporary clinical research showing no difference in patency rate amongst left and right arteries (Fukui et al., 2011).

Limitations of the study. The thickness of the intima and the intima-to-media ratio are illustrative and provide

an overall and unified global insight into the state of the intimal proliferation in the internal thoracic artery. However, the differences between various microscopic fields of the same cross section of the artery could be quite significant. It also seems that positive remodeling is characteristic of early atherosclerosis, in the ITA as well as in other arteries, while the remodeling process in advanced atherosclerosis should be studied further.

Another question is also, do all medial patterns/phenotypes have equal ability for remodeling, especially in advanced atherosclerosis?

That is why more measurements and morphometric models should be included in further analysis, including data on the cross sectional area, luminal area, the internal and the external elastic lamina areas and their changes throughout the length of the artery during aging and the development of atherosclerosis. The inclusion of these parameters would facilitate the formation of the final conclusions on the capability of the ITA for positive remodeling.

Conclusions. During aging, the following parameters of the ITA progressively increase: thickness of the intima, thickness of the medial layer and the wall, and the intima-to-media ratio. The increase in thickness of the medial layer could be an indicator of the ability for positive remodeling of the internal thoracic artery during aging and early atherosclerosis. There are no statistically significant differences between these morphometric parameters of left and right arteries or arteries belonging to subjects of a different gender. However, aging, atherosclerosis and the male gender increase the risk of disruption of the elastic skeleton.

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