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The internal thoracic artery as a transitional type of artery: a morphological and morphometric study

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Summary. Coronary artery by-pass grafting (CABG) with arterial grafts is widely accepted as the procedure of choice in the treatment of coronary ischemic disease. It brings back focus on morphological studies of arteries used as conduits in this procedure. One of the most frequently used CABG grafts is the internal thoracic artery with an excellent graft prognosis and patency rate.

The aim of the study was a detailed morphological and morphometric description of the internal thoracic artery with an emphasis on its basic histological structure and its changes in aging and atherosclerosis. Therefore, 42 full-length arteries were obtained during forensic autopsies from 27 persons, aged between 20 and 81 years, who had died from non-vascular causes. The arteries were classified into three different age groups.

Analysis of the serial arterial segments has shown that the internal thoracic artery is an artery of the transitional type whose media is organized into two layers: the internal, muscular layer and the external layer with spirally oriented elastic lamellae and smooth muscle cells in between. The number of elastic lamellae progressively decreases throughout the length of the examined arteries.

As opposed to previous assumptions, we have proven that the grade of atherosclerosis is independent of the number of elastic lamellae in the external media. Perfectly formed elastic lamellae are not a persistent feature of the internal thoracic artery, as previously claimed. We have confirmed that the thickness of elastic lamellae decreases, while the number and the size of their fenestrations steadily increase with aging.

Key words: The internal thoracic artery, CABG

Introduction

Coronary artery by-pass grafting (CABG), as one of the mostly used approaches in the treatment of coronary ischemic disease, brings back focus on the issue of detailed morphological description of the arteries used as conduits in this procedure. Morphological studies are motivated by the important task of exploring known arterial grafts and predicting their patency rate and by the need for new, alternative grafts.

The most frequently used arterial graft in contemporary cardiac surgery is the internal thoracic artery, with an excellent prognosis of graft survival and its patency rate (Loop et al., 1986).

Pioneer studies on the histology of the internal thoracic artery indicated certain morphological characteristics of the internal thoracic artery as a possible explanation for its relative lack of atherosclerosis and its excellent patency rate (Sims, 1987; Van Son et al., 1990, 1993; Sims et al., 1993). Here we have briefly summarized the conventional attitudes on the histology of the internal thoracic artery.

The internal thoracic artery was earlier classified as an artery of the elastic type, unique for its well-formed internal elastic lamina, with a small number of

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Abbreviations. CABG: coronary artery by-pass grafting, IEL: internal elastic lamina (membrane), ITA: internal thoracic artery

fenestrations (Sims, 1987). The compact structure of the internal elastic lamina was one of the earliest recognized possible explanations for the low grade of atherosclerosis of this artery (Sims et al., 1993). Van Son et al. described the specific organizational pattern of the tunica media of the internal thoracic artery (Van Son et al., 1990, 1993) claiming that the histological appearance and the number of elastic lamellae of the internal thoracic artery media vary throughout the length of the artery. The presence of elastic lamellae was also one of the supposed causes for the lack of atherosclerosis in the internal thoracic artery (Sims, 1987).

Van Son described four basic patterns of the media altering throughout the length of the artery: 1). elastic pattern, with 8 to 18 well formed elastic lamellae (including the internal and the external elastic lamina); 2). elasto-muscular pattern with 5 - 7 elastic lamellae; 3). muscular pattern with rare elastic lamellae (mean: 3 lamellae) and a predomination of smooth muscle cells; 4). muscular pattern, with the tunica media formed by smooth muscle cells with no elastic lamellae except the internal and the external elastic lamina (Van Son et al., 1993).

We performed a detail morphological and morphometric analysis of the internal thoracic artery in persons aged between 20 and 81 years. The whole length of the arteries was included in the analysis. As indicated by our research, the internal thoracic artery is an artery of the transitional (mixed) type. In addition, we investigated several other morphological aspects originating from the specific morphology of the internal thoracic artery, namely: the transition of the number of elastic lamellae throughout the length of the artery, the thickness of the elastic lamellae and the number and the size of elastic lamellae fenestrations. Also, changes of these parameters during aging and the development of atherosclerosis were analyzed in the morphometric study. In a separate segment of the investigation we tested the hypothesis that the level of atherosclerosis was dependent on the number of elastic lamellae.

To the best of our knowledge, this is one of the largest studies of this kind thus far. The conclusions of the study are not of purely academic significance, but give a new insight into the histology of the internal thoracic artery, which could be directly connected to the behavior of this artery as a superior CABG graft in coronary circulation.

Materials and methods

Arterial samples

Arteries were collected during legal medical autopsies between December 1999 and January 2003 and between January 2004 and May 2004.

All examinees died suddenly from non-vascular causes. However, fourteen patients had had a previous history of cardiac or vascular disease. Ten patients had had a diagnosis of ischemic heart disease, including one patient with verified previous myocardial infarction (which was not the actual cause of death), two patients had had arterial hypertension, one had been diagnosed with mitral valve prolapse and in the case of one patient, ventricular arrhythmia had been confirmed and treated. In one patient chronic anemia and rheumatoid arthritis had been established. Only two patients, one male and one female, had been obese.

Harvesting the arteries

The arteries were harvested in keeping with the technical principles for surgical graft harvesting. The internal thoracic artery was harvested from the level of the second rib up to its terminal branching point.

The dissected arteries were oriented and marked with respect to the topographical principle, in order to distinguish proximal and distal parts. Afterwards, they were measured, cut into 1 cm long serial segments and immediately fixed, without any prior pharmacological treatment or distension. This sampling method enabled the analysis of sequential structural changes of the artery throughout the entire arterial length.

The arterial samples were obtained 12 to 48 hours after death. Wherever it was possible, both the left and the right internal thoracic artery were sampled. In some cases this was not possible due to multiple fractures of the ribs and penetrating gun-shot wounds of the thorax.

Study groups

The study of the internal thoracic artery included 42 arteries from 27 persons. The mean age of all the patients was 46.57±16.27 years. The mean length of all the analyzed arteries was 13.5±3.07 cm.

All arterial samples were divided into three groups according to the age of the examinees.

Table 1. Characteristics of the patients and arteries in the study groups.

Age - related groups (years)	Number of patients	Male patients	Female patients	Number of arteries	Average age of patients (years)	Average length of arteries (cm)
Group (1) (20-40)	9	5	4	15	27.25±2.96	13.18±3.32
Group (2) (41-60)	14	9	5	20	50.79±5.62	12.94±3.12
Group (3) (≥ 61)	4	3	1	7	70.50±8.35	15.5±1.5
Total	27	17	10	42	46.57±16.27	13.5±3.07

The characteristics of the study groups are presented in Table 1.

Preparation of arterial samples for analysis

Preparation of tissue for light microscopy and histomorphometry

Morphological analysis included the whole length of the harvested arteries. Serial, one cm long segments were routinely processed for morphological analysis.

Blocks of tissue were fixed by immersion in 4% neutral buffered formaldehyde. Previous studies have shown that this type of fixation procedure produces results as valid and repeatable as does perfusion fixation under the pressure of 100 mm Hg (Van Son et al., 1993).

The tissue was then dehydrated and embedded in Paraplast. Each block was serially sectioned and five, 5 μm thick sections were sampled at different levels. After that, the sections were routinely stained using selective techniques for elastic fibers. Three techniques were employed: the Weigert van Gieson technique with resorcin fuchsin, the Voerhoff van Gieson method and staining with the acid orcein method. Randomly sampled sections from each group were stained with hematoxylin and eosin or by applying the Masson trichrome technique.

Preparation of the Tissue for Immunohistochemistry

The same blocks of tissue, previously prepared for classical histology, were used for immunohistochemical analysis. Two sections of 4 μm thickness were sampled from each analyzed specimen. A broad spectrum of antibodies was used in the study. The antigens, demasking procedures and staining kits used for the detection of vascular smooth muscle cells are summarized in Table 2.

In brief, the demasking procedure was used as the first step in the immunohistochemical procedure. For the purpose of antigen demasking, a 10 mM citrate buffer (pH 6) was used for 21-25 minutes in a microwave oven at maximum power of 800W or, alternatively, proteolytic digestion with proteinase K (0.04 mg/ml) for 10 minutes followed by a 10 mM citrate buffer (pH 6) for 40 minutes in a water bath at 99°C. The sections were then washed with TBS [Tris (hydroxymethyl) aminomethane buffer saline] and incubated with the primary antibody diluted at an adequate ratio (Table 2). The sections were treated applying the standard streptavidin biotin technique using the commercial DAKO LSAB+/HRP kit or the EnVision kit (Table 2). Immunoreactions were subsequently developed by using DAB (diaminobenzidine) or AEC (3-amino-9-ethylcarbazole) as chromogens. The sections were counterstained with Mayer's haematoxylin.

The quality and the specificity of the developed immunoreactions were controlled by negative controls performed by omitting the primary antibody and applying TBS instead.

All the slides were analyzed using an Olympus CH microscope or an Olympus BX 41 microscope and were photo documented by a Carl Zeiss Jenaval microscope and a Pentacon camera.

Morphometric analysis

The following parameters were measured in this segment of the study: 1). the number of elastic lamellae; 2). the thickness of the internal elastic lamina (membrane) (IEL); 3). the number and the size of fenestrations of the IEL; 4). the thickness of the elastic lamellae; 5). the number and the size of fenestrations of the elastic lamellae.

In a separate segment of the analysis we measured the thickness of the intima and the media and subsequently calculated the intima-to-media ratio. The intima thickness and the intima-to-media ratio were used for the estimation of the level of atherosclerosis. The integrated results of the intima and the media thickness and the intima-to-media ratio were grouped in the statistical data base.

Also, the grade of atherosclerosis was established for different sections according to the classification of the American Heart Association Committee on Vascular Lesions of the Council of Atherosclerosis (American Heart Association Committee on Vascular Lesions of the Council of Atherosclerosis, 1995).

Table 2. List of Antigens Used in the Immunohistochemical Analysis.

Primary antibody	Immunogen	Manufacturer serial number	Antigen demasking procedure	Ratio	Visualization method/ chromogen
Desmin (monoclonal mouse anti-desmin)	Desmin extracted from a pig's stomach	DAKO M 0724	10 mM citrate buffer - pH 6.0, 25 minutes, microwave oven (800 W)	1:50	EnVision+/HRP AEC
Smooth muscle actin (monoclonal mouse anti-human)	N-terminal deca-peptide of the human α-smooth muscle actin	DAKO M 0851	10 mM citrate buffer - pH 6.0, 21 minutes, microwave oven (800 W)	1:25	EnVision+/HRP AEC LSAB+/HRP DAB
Myosin heavy chain (monoclonal mouse anti-human)	Crude extract of the uterus	DAKO M 3558	Proteolytic digestion with proteinase K (0.04 mg/ml) 10 min; then 10 mM citrate buffer - pH 6.0, 40 minutes, in the water bath at 99°C	1:50	EnVision+/HRP AEC

The values of the intima thickness and the intima-to-media ratio for different grades of atherosclerosis have proven to be statistically significant (data not shown).

Histomorphometry was performed with an ocular micrometer. Only the cross sections were analyzed. Terminal parts of each block and the oblique sections, as well as the branching points were excluded from the analysis. The systematic field sampling method was used.

In order to determine the thickness of the intima and the media and the number of the elastic lamellae, as well as the thickness of the internal elastic lamina and the thickness of the elastic lamellae, the following method was used. Each section was inspected in 10 microscopic fields uniformly distributed over the circumference of the arterial section. The axis of the ocular micrometer was oriented normally to the wall of the vessel in the position marked by the arrow in Fig. 1E. The thickness of the intima and the media and the number of the elastic lamellae were determined using the medium magnification (100x) of the Olympus CH microscope (Japan), while 400x magnification was used for determining the thickness of the internal elastic lamina and the thickness of the elastic lamellae.

The number and the size of fenestrations were analyzed at 1000x magnification for the whole circumference of the arterial vessel.

The methodology was developed using previous similar studies (Ferro et al., 1991; Sims et al., 1993) and based on the experience from the pilot study performed prior to the final study.

Using this method, we obtained results for all parameters for each centimeter of the arterial length separately. For the purpose of this study we analyzed the mean values of the parameters for the entire arterial length.

Statistical analysis

Statistical methods were used to evaluate morphometric results. Data were expressed as the mean value ± SD. In addition, quantitative descriptions of sets of morphometric values included the standard error (SE) and a 95% confidence interval (95% C.I.). The following statistical methods were used: the One Sample Smirnov-Kolmogorov Test, the One-way ANOVA with the Bonferroni as the Multiple Comparison Test and the Pearson Correlation Coefficient. The value of $p<0.05$ was considered statistically significant.

The tests were performed with the SPSS version 10.0 for Windows.

All the results are presented in tables and graphs. The statistical analysis was performed per graft and not per patient. The units for statistical description and analysis were mean values of parameters obtained for serial, one centimeter long segments of the arteries.

Prior to the analysis all the data were tested with the Kolmogorov-Smirnov Test for the normality of their distribution.

Results

The internal thoracic artery as a transitional type of artery

The morphology of the internal thoracic artery

The internal thoracic artery is an artery of the transitional (mixed) type. The wall of the internal thoracic artery consists of three well defined layers: the *tunica intima, the tunica media* and *the tunica adventitia* (Fig. 1A).

The *tunica media* of the internal thoracic artery is organized into two sublayers: the internal layer and the external layer (Fig. 1A,E). The internal medial (muscular) layer consists of circularly oriented sheets of smooth muscle cells with rare, distinct elastic fibers, but without formed elastic lamellae (Figs. 1A-E, 2A-D). The presence of this layer was proven in all the examined arteries by histochemical techniques (Figs. 1A-E, 2A,C,D) and by immunohistochemical staining for α actin, desmin and myosin heavy chains. The representative immunohistochemical microphotograph is presented in Fig. 2B.

The external layer is similar to those in arteries of the elastic type with respect to its organization, the arrangement of smooth muscle cells and the presence of well-defined elastic lamellae. Spirally oriented smooth muscle cells are positioned between the elastic lamellae (Fig. 2A,B). In the adjacent lamellar units, sheets of smooth muscle cells are positioned approximately normally to each other (Fig. 2A,B). The lamellae are interconnected by interlaminar elastic fibers (Figs. 2C,D).

The number of lamellae varies throughout the length of the artery and it is different in various segments of the artery. Four different patterns of the external medial layer are recognized as to the number of lamellae (this number does not include the internal and the external elastic laminas):

1. the elastic pattern (E), which contains between 7 and 15 well-formed circular, elastic lamellae and spirally oriented smooth muscle cells (Fig.1A-C);
2. the elasto-muscular pattern (EM), with four to six concentric elastic lamellae and a well-formed, thicker, internal, medial (muscular) layer (Fig. 1E);
3. the musculo-elastic pattern (ME), with one to three delicate, but clearly discernible elastic lamellae and with the domination of smooth muscle cells (Fig. 1D);
4. the muscular pattern (M), with circularly oriented smooth muscle cells and rare, solitary elastic fibers (Fig. 1F).

The transition of different patterns through the length of the artery

We performed a detailed analysis of the number of elastic lamellae in the external medial layer for all the analyzed arteries and the transition of different media patterns throughout the length of the arteries.

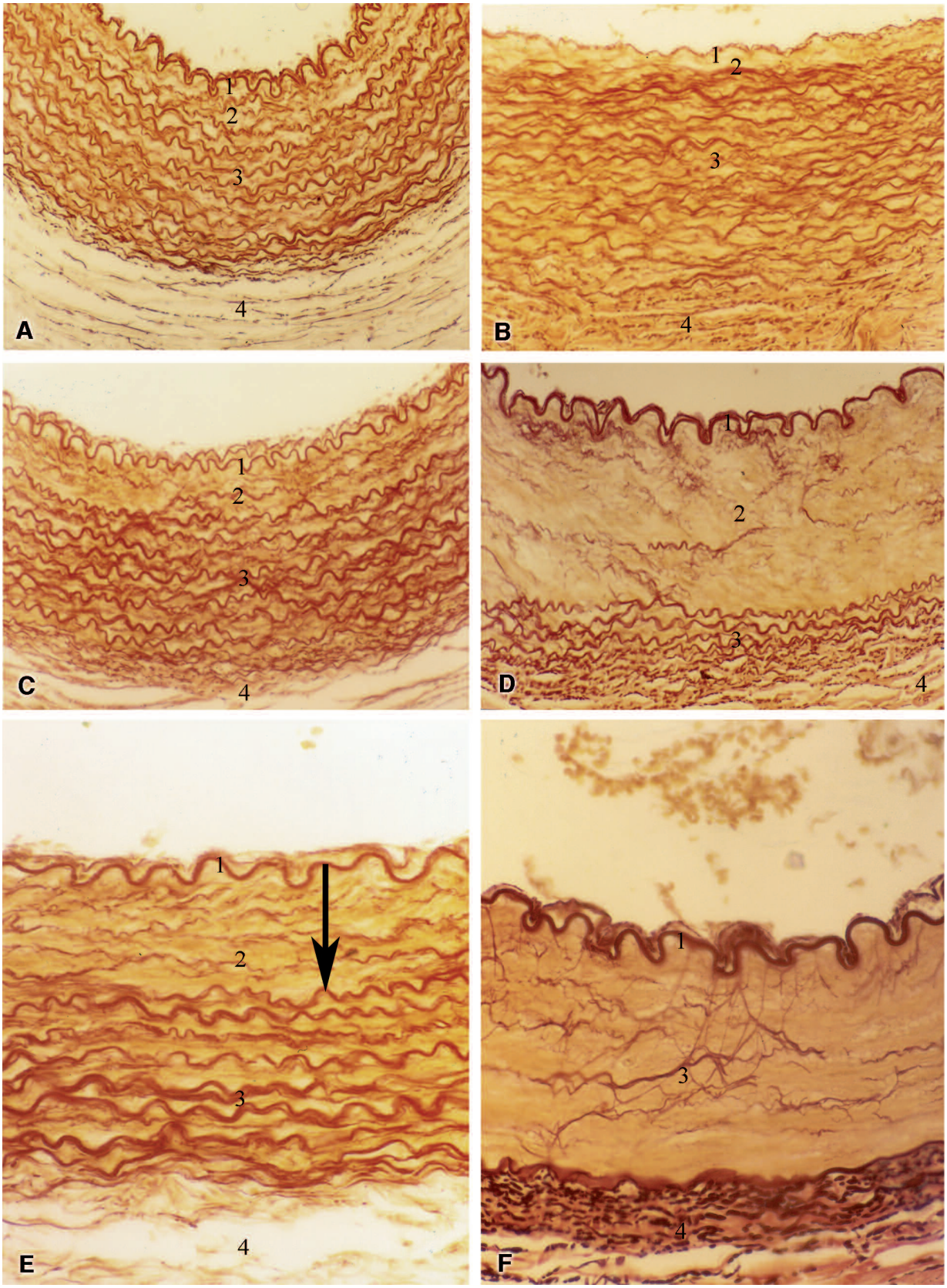


Fig. 1. The internal thoracic artery. **A-C.** Elastic pattern. **D.** Musculo-elastic pattern. **E.** Elasto-muscular pattern. **F.** Muscular pattern. 1: the IEL, 2: internal, muscular layer of the media, 3: external layer of the media (**A – E**) or tunica media (**F**), 4: tunica adventitia; arrow - internal, muscular layer of the media; arrow also suggests the position of the ocular micrometer axis during histomorphometry. Weigert van Gieson technique. A-C, x 25,6; D, x 32; E-F, x 64

A specific problem with this segment of the analysis was that the length of the arteries varied considerably. Firstly, we excluded the arteries with extremely small or large values of their length. In such a way, we were able to unify the length of all arteries in all groups. We also excluded arteries with missing data for some segments. Then, we calculated the relative distance according to the method already described by Van Son et al. (1993).

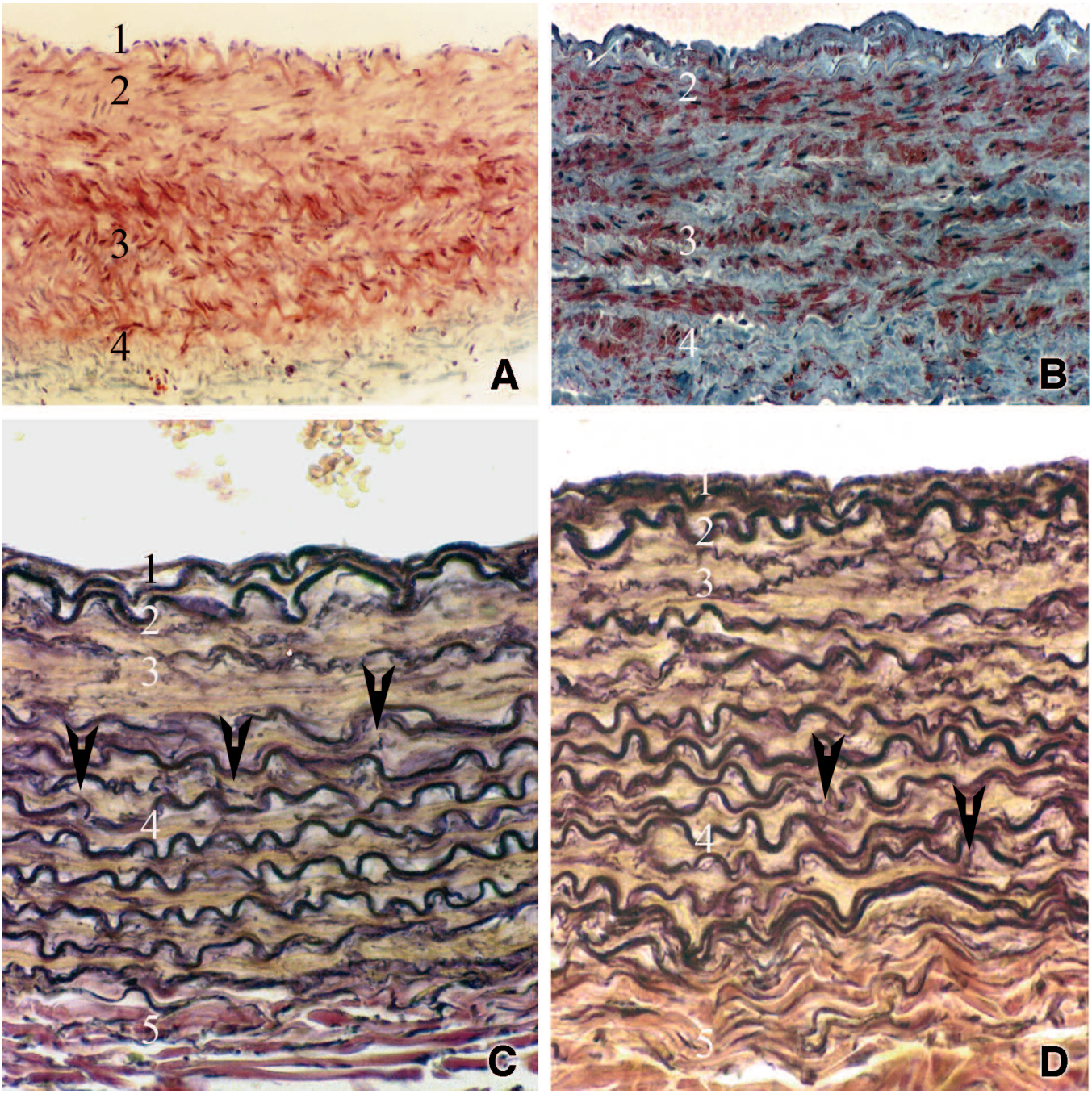


Fig. 2. The internal thoracic artery. **A** (Masson trichrome technique) **and B.** (α–smooth muscle actin; EnVision+/HRP, AEC). The circular arrangement of vascular smooth muscle cells in the internal layer of the media. Spiral arrangement of vascular smooth muscle cells in laminar units of the external medial layer. 1: tunica intima, 2: the internal, muscular layer of the media, 3: the external medial layer, 4: tunica adventitia. **C and D** (Weigert van Gieson technique). 1: tunica intima, 2: the IEL, 3: the internal, muscular layer of the media, 4: the external medial layer, 5: tunica adventitia; arrowheads: interlaminar elastic fibers. A, x 32; B, x 25.6; C, D, x 64

Group (1). In the first group, 11 arteries were included in the study. The results are summarized in Graph 1A.

The elastic pattern predominated in the first two centimeters of the arterial length (the first 15.38% to 20% of the total arterial length). The elasto-muscular pattern prevailed from the third to the sixth centimeter (the following 30.77% to 40% of the total arterial length), while the musculo-elastic pattern became predominant from the seventh to the tenth centimeter (another 30.77% to 40% of the arterial length). The muscular pattern predominated from the eleventh centimeter onwards.

Group (2). Sixteen arteries were included in the second group. The results are summarized in Graph 1B.

Two out of 16 arteries were entirely elastic with 7-9 elastic lamellae in the external media. Of the remaining 14 arteries, half were elastic and half were elasto-muscular in the first centimeter and then again in the fourth centimeter (7.69% to 10% of the total arterial length). However, the elasto-muscular pattern was predominant from the third to the eighth centimeter (the following 46.15% to 60% of the total arterial length).

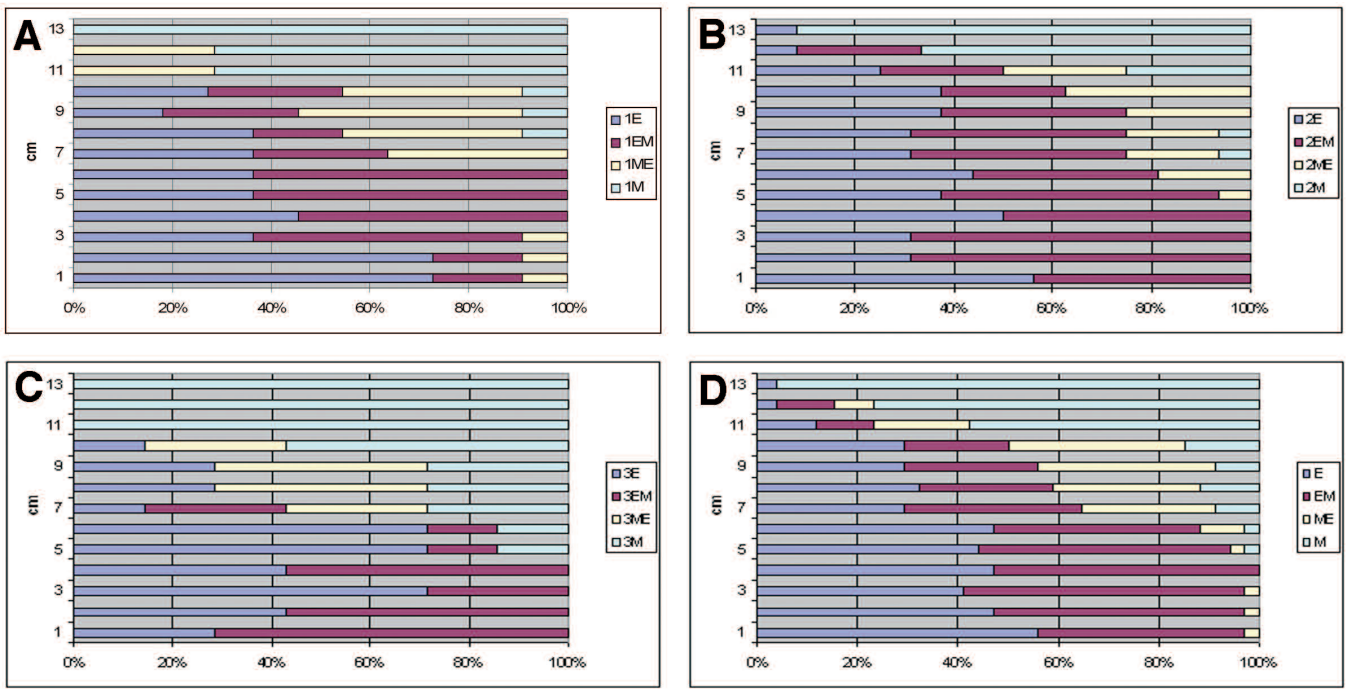
From the ninth to the tenth centimeter (another 15.38% to 20% of the total arterial length), the elastic, the elasto-muscular and the musculo-elastic patterns were almost equally represented. The muscular pattern emerged from the eleventh centimeter as equal to other patterns and became absolutely dominant in the twelfth and the thirteenth centimeter (15.38% to 20% of the total length).

Group (3). In the third group, all seven arteries were included in the study. The results are summarized in Graph 1C. One out of the seven arteries was entirely elastic.

The elasto-muscular pattern absolutely predominated in the first two centimeters of the arterial length (15.38% of the total arterial length) in the remaining six arteries. The elastic pattern dominated between the third and the sixth centimeter (the following 38.46% of the total arterial length). In the seventh centimeter, the musculo-elastic pattern emerged and became predominant in the eight and the ninth centimeter (another 15.38% of the total arterial length). In the final 30.77% of the total arterial length (from the tenth to the thirteenth centimeter and onwards) the muscular pattern was absolutely predominant.

Summary analysis. The summary report on the structure of all the analyzed arteries is presented in Graph 1D.

In brief, in the first centimeter (7.69% to 10% of the total length) of most arteries the elastic pattern was predominant. From the second to the fifth centimeter (30.76% to 40% of the total length) there was a slight predominance of the elasto-muscular pattern. On the sixth centimeter, the elastic pattern prevailed again, while from the seventh to the ninth centimeter (the following 30.77% to 40% of the total length) the elastic, the elasto-muscular and the musculo-elastic patterns were almost equally represented. In the tenth centimeter



Graph 1. Transition of the tunica media patterns. A: Group (1); B: Group (2); C: Group (3); D: All Groups.

(the following 7.69% to 10% of the total length) there was a domination of the musculo-elastic pattern, while in the final centimeters that followed, the muscular pattern was absolutely predominant.

In short, the number of elastic lamellae of the internal thoracic artery gradually decreased from the proximal part to the terminal branches. This finding is in accordance with its topographic localization and the histological structure of the typical transitional artery, interposed between large elastic arteries and classical muscular branches. In addition, variations of the number of elastic lamellae throughout the arterial length were highly variable from case to case. The rare examples of arteries with a purely elastic pattern of the external media were not an anomaly of the theory, but rather an exception that proves the rule.

In consistence with the gradual decrease of the number of elastic lamellae, the thickness of the internal muscular layer gradually increased (Fig. 1A-E). There was no statistically significant difference in the mean number of elastic lamellae between the groups as confirmed by the ANOVA with the Bonferroni as the Multiple Comparision Test (F= 2.194, p= 0.113).

Morphometric analysis

The first goal of the morphometric analysis was to establish the morphometric parameters of the elastic lamellae and then to analyze their changes in aging and atherosclerosis. Secondly, we reexamined the hypothesis that elastic and elasto-muscular segments of the internal thoracic artery have a lower grade of atherosclerosis.

Morphometric characteristics of the internal elastic lamina

The following parameters of the internal elastic lamina (membrane) were examined: the thickness, the number of fenestrations per 100 μm and the size of the fenestrations.

According to our results the thickness of the internal elastic lamina differed significantly among the age

Table 3. Age dependent changes of the morphometric parameters of the IEL and the elastic lamellae.

Parameters								
Dependent variable	Age - related groups	Mean	SD	SE	95% C.I.		Minimum	Maximum
					Lower bound	Upper bound		
Thickness of the IEL (μm)	Group (1)	2.01	0.67	0.0514	1.90	2.11	0.50	4.00
	Group (2)	2.22	0.83	0.0724	2.08	2.37	1.00	7.00
	Group (3)	1.60	0.54	0.0967	1.41	1.80	1.00	3.00
	Total	2.05	0.75	0.0410	1.97	2.13	0.50	7.00
ANOVA F=9.960, p<0.001								
Number of IEL fenestrations per 100 μm	Group (1)	2.37	1.88	0.1214	2.13	2.61	0.00	9.00
	Group (2)	3.46	2.48	0.2123	3.04	3.88	0.00	11.00
	Group (3)	3.03	2.22	0.4054	2.20	3.86	0.00	9.00
	Total	2.79	2.18	0.1080	2.57	3.00	0.00	11.00
ANOVA F=11.659, p<0.001								
Size of IEL fenestrations (μm)	Group (1)	2.05	1.46	0.1232	1.81	2.30	1.00	8.00
	Group (2)	2.41	1.55	0.1381	2.14	2.68	0.50	7.00
	Group (3)	2.40	1.38	0.2513	1.89	2.92	1.00	5.00
	Total	2.24	1.50	0.0869	2.07	2.41	0.50	8.00
ANOVA F=2.075, p=0.127								
Thickness of elastic lamellae (μm)	Group (1)	1.61	0.46	0.0388	1.53	1.69	0.67	3.25
	Group (2)	1.51	0.42	0.0420	1.43	1.59	0.75	2.50
	Group (3)	1.17	0.29	0.0660	1.03	1.30	0.83	2.00
	Total	1.54	0.45	0.0278	1.48	1.59	0.67	3.25
ANOVA F=9.441, p<0.001								
Number of elastic lamellae fenestrations per 100 μm	Group (1)	2.31	0.97	0.0882	2.13	2.49	0.67	5.50
	Group (2)	2.07	1.56	0.1652	1.74	2.40	1.00	10.00
	Group (3)	2.00	1.23	0.3881	1.12	2.88	0.33	4.00
	Total	2.20	1.25	0.0846	2.03	2.37	0.33	10.00
ANOVA F=1.064, p=0.347								
Size of elastic lamellae fenestrations (μm)	Group (1)	2.88	1.42	0.1498	2.58	3.17	1.00	10.00
	Group (2)	2.94	2.07	0.2440	2.45	3.42	1.00	10.00
	Group (3)	4.59	1.44	0.4541	3.56	5.62	2.67	7.00
	Total	3.00	1.76	0.1342	2.74	3.27	1.00	10.00
ANOVA F=4.535, p=0.012								

groups (Tables 3, 4). However, in the group (2) of patients aged between 40 and 60 years the IEL was significantly thicker, while the gradual decrease in the internal elastic membrane thickness was observed after 60 years of age (Tables 3, 4; Graph 2A).

At the same time the number of fenestrations and their size increased significantly between 41 and 60 years of age (Tables 3, 4; Graph 2B), while the size of the fenestrations increased, but not significantly (Tables 3, 4; Graph 2C).

When the same data were crosschecked according to the grades of atherosclerosis, no significant differences were observed among different grades of atherosclerosis (Tables 5, 6). The thickness of the internal elastic membrane was slightly elevated in grade 1, subsequently decreasing in the following grades of atherosclerosis, but the difference was not statistically significant (Tables 5, 6). The number of fenestrations was somewhat higher in grade 1, but then in every subsequent grade the values of this parameter were relatively stable and without statistically significant differences. The size of the fenestrations was constant in different grades of atherosclerosis (Table 5). The Bonferroni as the Multiple Comparison Test confirmed the observed results (Table 6).

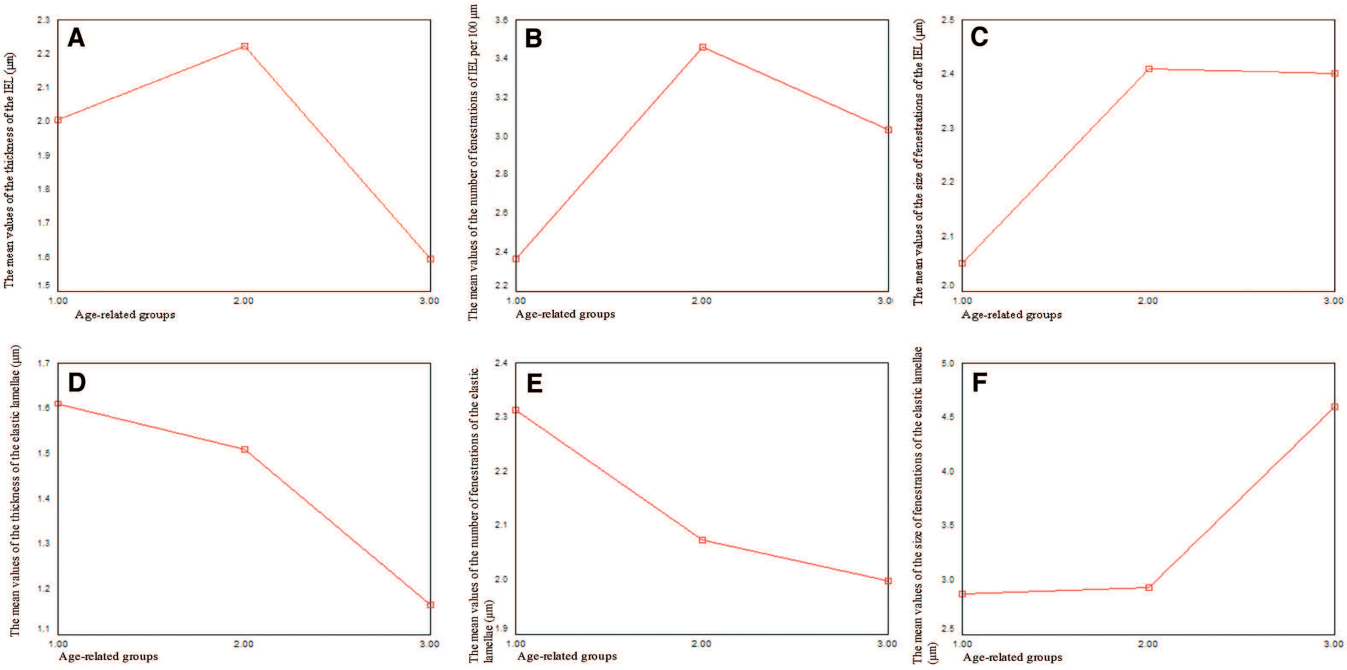
Morphometric characteristics of the elastic lamellae

The following parameters of the elastic lamellae (laminas) were examined: the thickness, the number of fenestrations per 100 μm and the size of the

Table 4. Multiple comparisons of the mean values of morphometric parameters of the IEL and the elastic lamellae among age-related groups.

Bonferroni Multiple Comparisons		
Dependent variable Age – related groups	Group (2)	Group (3)
Thickness of the IEL(μm)		
Group (1)	p=0.031(*)	p=0.014(*)
Group (2)		p<0.001(*)
Group (3)		
Number of IEL fenestrations per 100 μm		
Group (1)	p<0.001(*)	p=0.328
Group (2)		p=0.949
Group (3)		
Size of IEL fenestrations (μm)		
Group (1)	p=0.160	p=0.741
Group (2)		p=1.000
Group (3)		
Thickness of the elastic lamellae (μm)		
Group (1)	p=0.222	p<0.001(*)
Group (2)		p=0.004(*)
Group (3)		
Number of elastic lamellae fenestrations per 100 μm		
Group (1)	p=0.523	p=1.000
Group (2)		p=1.000
Group (3)		
Size of elastic lamellae fenestrations (μm)		
Group (1)	p=1.000	p=0.010(*)
Group (2)		p=0.015(*)
Group (3)		

* The mean difference is significant at the 0 .05 level.



Graph 2. Age dependent changes of the morphometric parameters of the IEL and the elastic lamellae.

fenestrations.

When the thickness of the elastic lamellae was analyzed during aging, a significant difference was observed and a gradual decrease in the thickness of the elastic lamellae was confirmed to exist (Tables 3, 4; Graph 2D).

As opposed to our expectations, the decrease of the number of fenestrations per 100 μm was not significant (Tables 3 and 4; Graph 2E). On the other hand, the size of the fenestrations increased dramatically (Tables 3, 4; Graph 2F).

When we analyzed the same data according to the grade of atherosclerosis, the thickness of the elastic lamellae seemed to decrease significantly, especially in the third grade of atherosclerosis (Tables 5, 6). The number of fenestrations and their size did not significantly change during the gradual development of atherosclerosis (Tables 5, 6).

Taken together, these data suggest that gradual devastation of the elastic skeleton develops primarily

Table 5. Changes of the morphometric parameters of the IEL and the elastic lamellae throughout the development of atherosclerosis.

Parameters								
Dependent variable	Grade of atherosclerosis	Mean	SD	SE	95% C.I.		Minimum	Maximum
					Lower bound	Upper bound		
Thickness of the IEL (μm)	0.00	2.05	0.83	0.0722	1.91	2.19	0.50	7.00
	1.00	2.13	0.75	0.0738	1.98	2.27	1.00	4.00
	2.00	1.75	0.35	0.2500	-1.43	4.93	1.50	2.00
	3.00	2.04	0.65	0.0711	1.89	2.18	1.00	3.50
	4.00	1.72	0.42	0.1133	1.47	1.96	1.00	2.50
	Total	2.05	0.75	0.0410	1.97	2.13	0.50	7.00
ANOVA F=1.033, p=0.390								
Number of IEL fenestrations per 100 μm	0.00	2.73	2.05	0.1710	2.40	3.07	0.00	9.00
	1.00	3.02	2.42	0.1824	2.66	3.38	0.00	11.00
	2.00	0.50	0.71	0.5000	-5.85	6.85	0.00	1.00
	3.00	2.41	1.84	0.2153	1.98	2.84	0.00	11.00
	4.00	2.69	1.70	0.4721	1.66	3.72	1.00	7.00
	Total	2.79	2.18	0.1080	2.57	3.00	0.00	11.00
ANOVA F=1.621, p=0.168								
Size of IEL fenestrations (μm)	0.00	2.35	1.47	0.1469	2.06	2.65	1.00	7.00
	1.00	2.15	1.46	0.1409	1.87	2.43	0.50	6.00
	2.00	1.00	0.00	0.0000	1.00	1.00	1.00	1.00
	3.00	2.23	1.63	0.1906	1.85	2.61	1.00	8.00
	4.00	2.31	1.32	0.3649	1.51	3.10	1.00	5.00
	Total	2.24	1.50	0.0869	2.07	2.41	0.50	8.00
ANOVA F=0.585, p=0.674								
Thickness of the elastic lamellae (μm)	0.00	1.70	0.53	0.0593	1.58	1.00	0.67	3.25
	1.00	1.54	0.37	0.0401	1.46	1.49	0.83	2.33
	2.00	1.00	0.00	0.0000	1.32	1.76	1.00	1.00
	3.00	1.40	0.39	0.0424	1.00	1.81	0.75	2.38
	4.00	1.46	0.49	0.1360	1.17	1.62	0.83	2.50
	Total	1.54	0.45	0.0278	1.48	1.59	0.67	3.25
ANOVA F=5.516, p<0.001								
Number of elastic lamellae fenestrations 100 μm	0.00	2.03	0.91	0.1290	1.78	2.29	1.00	5.50
	1.00	2.09	0.88	0.1021	1.89	2.29	0.33	4.50
	2.00	2.83	0.24	0.1667	0.72	4.95	2.67	3.00
	3.00	2.39	1.65	0.1844	2.03	2.76	0.33	10.00
	4.00	2.18	1.40	0.3883	1.33	3.03	1.00	5.00
	Total	2.20	1.25	0.0846	2.03	2.37	0.33	10.00
ANOVA F=0.968, p=0.426								
Size of elastic lamellae fenestrations (μm)	0.00	3.40	1.87	0.2959	2.80	4.00	1.00	10.00
	1.00	2.91	1.92	0.2609	2.38	3.43	1.00	8.00
	2.00	4.00	1.41	1.0000	-8.71	16.71	3.00	5.00
	3.00	2.72	1.47	0.1850	2.35	3.09	1.00	10.00
	4.00	3.37	1.98	0.5489	2.18	4.57	1.00	7.00
	Total	3.00	1.76	0.1342	2.74	3.27	1.00	10.00
ANOVA F=1.260, p=0.288								

with aging, but not with atherosclerosis.

To substantiate this thesis, we further tested the correlation between the thickness of the intima and the intima-to-media ratio (as morphometric markers of atherosclerosis) and all the parameters of the IEL and the elastic lamellae, with the Pearson Correlation Coefficient. These findings were consistent with the results obtained using the ANOVA and Bonferroni analyses. The only statistically significant positive correlation was confirmed between the number of fenestrations of the IEL and the intima thickness (Pearson Correlation Coefficient -0.108, $p<0.05$) and between the number of fenestrations and the intima-to-media ratio (Pearson Correlation Coefficient 0.140, $p<0.001$). The Pearson Correlation Coefficient also revealed two new correlations: 1. the negative and significant correlation between the number of fenestrations of the IEL and the thickness of the IEL (Pearson Correlation Coefficient -0.114, $p<0.05$); 2. the positive and highly significant correlation between the thickness of the IEL and the thickness of other elastic lamellae (Pearson Correlation Coefficient 0.186, $p<0.001$).

For all the other parameters the results of the correlation were insignificant.

The correlation between the number of elastic lamellae and the grade of atherosclerosis

To investigate the truth of the hypothesis that the number of elastic lamellae influences the level of atherosclerosis, we correlated the thickness of the intima and the intima-to-media ratio and the grade of atherosclerosis with the number of elastic lamellae in different arterial segments and morphological patterns. We made a summary analysis for all the groups.

The protective effect of a larger number of elastic lamellae could not be confirmed as a general rule. The results of the analysis that included all the arteries indicated that the smallest value of the intimal thickness

Table 6. Multiple comparisons of the mean values of morphometric parameters of the IEL and the elastic lamellae among different grades of atherosclerosis.

Bonferroni Multiple Comparisons					
Dependent variable	Grade of atherosclerosis	1.00	2.00	3.00	4.00
Thickness of the IEL (μm)	0.00	p=1.000	p=1.000	p=1.000	p=1.000
	1.00		p=1.000	p=1.000	p=0.573
	2.00			p=1.000	p=1.000
	3.00				p=1.000
	4.00				
Number of IEL fenestrations (μm)	0.00	p=1.000	p=1.000	p=1.000	p=1.000
	1.00		p=1.000	p=0.458	p=1.000
	2.00			p=1.000	p=1.000
	3.00				p=1.000
	4.00				
Size of IEL fenestrations (μm)	0.00	p=1.000	p=1.000	p=1.000	p=1.000
	1.00		p=1.000	p=1.000	p=1.000
	2.00			p=1.000	p=1.000
	3.00				p=1.000
	4.00				
Thickness of the elastic lamellae (μm)	0.00	p=0.239	p=0.261	p<0.001(*)	p=0.719
	1.00		p=0.825	p=0.402	p=1.000
	2.00			p=1.000	p=1.000
	3.00				p=1.000
	4.00				
Number of elastic lamellae fenestrations per 100 μm	0.00	p=1.000	p=1.000	p=1.000	p=1.000
	1.00		p=1.000	p=1.000	p=1.000
	2.00			p=1.000	p=1.000
	3.00				p=1.000
	4.00				
Size of elastic lamellae fenestrations (μm)	0.00	p=1.000	p=1.000	p=0.578	p=1.000
	1.00		p=1.000	p=1.000	p=1.000
	2.00			p=1.000	p=1.000
	3.00				p=1.000
	4.00				

* The mean difference is significant at the 0 .05 level.

Table 7. Mean values of the intima thickness and the intima-to-media ratio for different arterial patterns.

Parameters								
Dependent variable	Pattern	Mean (μm)	SD	SE	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
Thickness of the intima	E	15.93	18.84	2.09	11.76	20.09	1.25	126.25
	EM	22.15	27.42	2.87	16.44	27.86	0.70	96.25
	ME	12.57	14.24	2.64	7.15	17.98	2.10	73.00
	M	13.37	10.67	1.47	10.43	16.31	0.00	44.50
	Total	17.24	20.99	1.32	14.65	19.83	0.00	126.25
ANOVA F=2.913; p=0.035								
Intima-to-media ratio	E	0.07	0.0755	0.0084	0.0533	0.0867	0.00	0.47
	EM	0.1063	0.1225	0.0128	0.0808	0.1318	0.01	0.51
	ME	0.0807	0.1340	0.0249	0.0297	0.1317	0.01	0.74
	M	0.1009	0.0887	0.0122	0.0765	0.1254	0.02	0.47
	Total	0.0907	0.1049	0.0658	0.0777	0.1036	0.00	0.74
ANOVA F=2.000; p=0.115								

Table 8. Multiple Comparisons of the Mean Values of the Intima Thickness and the Intima-to-media Ratio among Different Arterial Patterns.

Bonferroni Multiple Comparisons				
Dependent variable	Pattern	EM	ME	M
Thickness of the intima	E	p=0.304	p=1.000	p=1.000
	EM		p=0.188	p=0.090
	ME			p=1.000
	M			
Intima-to-media ratio	E	p=0.142	p=1.000	p=0.565
	EM		p=1.000	p=1.000
	ME			p=1.000
	M			

* The mean difference is significant at the 0.05 level. E: elastic pattern; EM: elasto-muscular pattern; M: muscular pattern; ME: musculo-elastic pattern

was present in segments with the musculo-elastic pattern, followed by purely muscular type segments and then by elastic pattern segments. The largest values were in the parts with the elasto-muscular pattern (Table 7).

However, the observed differences were not of statistical significance, although the hypothesis was tested in several ways. With the Bonferroni as the Multiple Comparison Test we examined whether the grade of atherosclerosis within the one cm long arterial segments depended on any of the following: the morphological pattern, the mean number of lamellae, the minimal and the maximal number of elastic lamellae, as well as the minimal and the maximal number of elastic lamellae together with the internal and external elastic laminas. The mean difference for all the tests performed was insignificant, with p values well above 0.05 ($p>0.05$).

In a separate analysis, we tested if there was a statistically significant difference in the thickness of the intima and the intima-to-media ratio between different arterial patterns (Tables 7 and 8). Initially, according to the ANOVA, the difference between the groups was established in the thickness of the intima ($F=2.913$, $p=0.035$) (Table 7). However, with the Bonferroni as the Multiple Comparison Test this difference could not be confirmed (Table 8). For the intima-to-media ratio, no statistically significant difference whatsoever could be found, regardless of the testing method used.

Discussion

The long-term benefits of the exclusive use of arterial grafts were established in several aspects: the survival benefit, a more favorable long-term clinical outcome, a decreased number of reinterventions and the improved patency of arterial grafts proved by angiograms (Korompai and Knight, 2005).

These surgical results make the research of the basic, but detailed histology of potential arterial grafts current again, since it represents the basis for the evaluation of the grafts and the estimation of the adequacy of new surgical approaches. Matching the arteries according to their morphometric parameters, together with advanced surgical techniques could be useful in an effort to minimize the discrepancies between arterial grafts being used and the coronary arteries as the source of turbulent blood flow and thrombosis at the anastomotic site.

Although morphometric analysis is an essential part of the detailed morphological description of potential and arterial grafts in use, there are few data available in this area and they vary very much depending on the mode of collecting specimens for the analysis. In addition, studies that take into account the whole length of the arteries are rare.

The first question important for the histological description of the potential or already well-known and used arterial graft is, to which type of artery it belongs. Although it seems trivial, there are some important

aspects of this finding that extend beyond purely academic dialogue.

Initially, the internal thoracic artery was described as the only peripheral artery in the human body that is elastic with a well-formed elastic lamina and the media formed by a network of circularly and longitudinally interlacing elastic lamellae. In between elastic lamellae, smooth muscle cells are dispersed in a spirally arranged fashion (Van Son et al., 1990, 1993).

Further, Van Son et al. defined four histological patterns in the media of the internal thoracic artery: 1) the elastic pattern, 2) the elasto-muscular pattern, 3) the muscular pattern, which primarily contains smooth muscle cells with rare elastic lamellae; and 4) the muscular pattern in which the media consists almost completely of smooth muscle cells (Van Son et al., 1993).

When the analysis of the pattern change was performed, Van Son et al. found that two out of the 11 analyzed arteries were elastic throughout their whole length. In the other 9 individuals an alternating histological pattern was observed: the first 20% to 30% were elasto-muscular, and then an abrupt transition to the elastic pattern followed for up to 70-80% of the total arterial length. At 70-80% of the total length, the second elasto-muscular segment started. In 5 out of 9 analyzed arteries this final, distal elasto-muscular segment extended to the epigastric bifurcation, while in 4 arteries it abruptly converted to the muscular pattern at 80-90% of the total length of the ITA (Van Son et al., 1993).

We have proven that the internal thoracic artery is a mixed or transitional type of artery with the media formed of the internal (muscular) layer and the external layer with well-formed, spirally distributed, elastic lamellae. The vascular smooth muscle cells in the inner media are circularly arranged and there are few solitary elastic fibers. On the other hand, the external media imitates elastic arteries with a spiral orientation of both the elastic lamellae and the smooth muscle cells. Our conclusions are substantiated by immunohistochemical studies and morphometric data.

In our study, we followed the initial description of four media patterns, although our mathematical and statistical model demonstrated a somewhat different number of elastic lamellae in the specific pattern and the distinct changing plan (Graphs 1A-D).

When we analyzed the whole sample we established two simple rules: 1). the number of elastic lamellae decreases gradually throughout the length of the artery; 2). variations in the number of elastic lamellae differ greatly from one individual case to another.

These features are highly predictable if the internal thoracic artery is observed in a way that we have proposed, namely as an artery of the transitional type interposed between the large elastic (subclavian) artery and the muscular arteries (the musculophrenic and the epigastric artery).

The final number of elastic lamellae is also influenced by their spiral arrangement and the progressive changes of their basic structure during aging (Tables 3, 4; Graphs 2A-F).

There are several important implications of this finding.

We suppose that, consistent with its mixed characteristics, the internal thoracic artery possesses special adaptability when it is used as a by-pass graft and exposed to higher values of blood pressure and blood flow. Namely, the combination of characteristics of the muscular and elastic arteries makes the mixed (transitional) arteries more compliant to the changes of blood flow and blood pressure as observed in previous studies (Fawcett, 1986; Singh et al., 1986; Sarabu et al., 1987; Von Segesser et al., 1989).

The fact that the internal thoracic artery is an artery of the mixed type is also in agreement with the fact that when used as a CABG graft in low free flow conditions, the internal thoracic artery reacts with initial spasm, but soon after it is capable of increasing the blood flow in proportion to myocardial demands without any previous intraluminal maneuvers (Hata et al., 2000). Although initially, the potent system of endogenous nitrate was offered as the possible reason for this phenomenon (Hata et al., 2000; Buyukates et al., 2007), a potential explanation for this result could be the high adaptability of the internal thoracic artery, which originates from its characteristic histological structure. The basic structure of the artery and its ample endogenous nitrate potential form a powerful and effective union that enables easy adaptation of the internal thoracic artery.

Also, the described regional differences in the structure of the internal thoracic artery could explain the variations in reactivity manifested along the length of the artery. He et al. showed that the distal parts were more sensitive and more prone to spasm than the proximal parts, probably because of their primarily muscular structure (He et al., 1994).

The characteristic mixed or transitional nature of the internal thoracic artery explains in part its special adaptability in coronary circulation and its high patency rate, but not the lack of atherosclerosis. Contemporary studies indicate that the nonatherogenic properties of the internal thoracic artery may be in part due to the low level of the platelet-activating factor and the activity of the platelet-activating factor acetylhydrolase (Tsoukatos et al., 2008).

That is why the next important issue concerning the structure of the internal thoracic artery was examining the integrity and the composition of the elastic lamellae, including the IEL, which was proposed as one of the main reasons for the relative lack of atherosclerosis in the internal thoracic artery by some authors (Sims, 1987; Sims et al., 1993).

Morphometric investigations showed that the internal elastic lamina was well formed with a thickness of 2.5 μm (Landymore and Chapman, 1987). Van Son et al. estimated the number of fenestrations of the internal elastic lamina, which was 18±10 for the whole circumference in the elastic segments and 35±12 in the

elasto-muscular segments (Van Son et al., 1993). According to Wahba and Offerdal the number of fenestrations per mm of the arterial circumference was 4.62 (Wahba and Offerdal, 1994). Our results showed similar values of the thickness of the IEL (mean ± SD = 2.05±0.75 μm). The mean value of the number of fenestrations was detected by a different method and the results were somewhat different than suggested by previous authors (Table 3). Previous investigations showed that the thickness of the intima correlated well with the number of the internal elastic lamina fenestrations (Sims et al., 1993). In one part of our research, we have come to a conclusion that is completely in line with this data (Pearson Correlation Coefficient 0.108, $p<0.05$).

Also, previous morphometric analysis showed that the thickness of the elastic lamellae was 1.3 μm (Landymore and Chapman, 1987). The mean value of this parameter in our study was 1.54±0.45 μm (Table 3). We also established the mean for the number and the size of the elastic lamellae fenestrations (Table 3).

The exact thickness of the elastic lamellae, including the IEL, as well as the precise number and size of their fenestrations represented just the tool necessary to analyze other important facts.

Numerous studies proposed that during aging and the development of atherosclerosis the thickness of elastic lamellae decreased, while the number and the size of fenestrations increased (Nakashima et al., 1990; Ferro et al., 1991; Van Son et al., 1993; Ross, 1995).

Our investigations proved some new elements. When we analyzed the data regarding the gradual changes of elastic lamellae of the internal thoracic artery during the aging process and atherosclerosis separately, we proved that gradual devastation of the elastic skeleton developed primarily with aging, but not with atherosclerosis (Tables 3-6; Graph 2).

These results were surprising and interesting, but they were consistent with the observations of some authors that atherosclerosis and aging were two different processes. According to a group of vascular histologists, all the changes in the basic structure of arteries after the third decade of life are actually different grades of atherosclerosis and, morphologically, aging and atherosclerosis are the same process. However, there is another quite opposite point of view. Authors that support this different concept consider aging and atherosclerosis as two separate processes, where aging makes blood vessels more susceptible to the influence of different factors that promote atherosclerosis (Folkow and Svanborg, 1993).

Our results highly correlate with new studies in this area. As indicated in a study by Cizek et al., age (51-75 years of age and up), smoking and hypertension correlate highly with the intimal thickness of the internal thoracic artery (Cizek et al., 2007).

In other words, a perfectly formed internal elastic lamina is not a constant feature of the internal thoracic artery as previously claimed (Sims et al., 1993), but the IEL, as well as elastic lamellae in general change extremely during aging. These gradual alterations of the elastic skeleton during aging facilitate atherosclerosis.

This finding was a prelude into the investigation of the next frequently exploited issue. The analysis of changes of different media patterns throughout the length of the artery was initially very important because of the assumption that elastic or elasto-muscular patterns coincided with a lower grade of atherosclerosis and these segments were recommended as preferential for use in the CABG procedure (Van Son et al., 1990, 1993). This theory was rather contradictory from the beginning and there were some studies that showed exactly the opposite (Ferro et al., 1991; Van Son et al., 1993).

According to our results, there is no statistically significant difference among the different media patterns in the thickness of the intima and the intima-to-media ratio.

It was previously proposed that the presence and the integrity of elastic lamellae in the elastic and elasto-muscular segments had a protective effect against the development of intimal hyperplasia and atherosclerosis by stabilizing the media and slowing down the migration of smooth muscle cells of the internal thoracic artery (Van Son et al., 1993).

However, we have shown that the number of elastic lamellae does not correlate with the parameters of atherosclerosis. The number of elastic lamellae alone is not and cannot be of essential importance to the delay of atherosclerosis, it is rather the complex interaction of elastin and smooth muscle cells that has this effect, as already concluded by similar studies (Atkinson, 1998; Ortiz et al., 1998, 2000). The benefit of using the proximal 80% to 90% of the internal mammary artery length comes from the knowledge that the cross sectional luminal area of this segment corresponds better to the same parameter of the coronary arteries than the luminal area of the more distally positioned segments (Van Son et al., 1993). Also, this area is less prone to spasm than the distal segments of the artery, as shown by previous studies (He et al., 1994).

Finally, from the histological point of view these results bring back attention to the issue of mixed, transitional and specialized arteries. This is a diverse group of exceedingly adaptable and highly specialized arteries interposed topographically between the two extremes: the elastic and the muscular arteries. There are numerous arteries in the organism that could be included in this group. Some of them are already well-known: coronary arteries are specialized arteries with bundles of longitudinal smooth muscle fibers in the subendothelial tissue. The presence of these muscle cells enables adaptation to changes of length and diameter during systole and diastole (Fawcett, 1986).

Many other arteries are also included in this group: lienal, renal and hepatic arteries, as well as femoral, axillary and palmar arteries, arteries of the ovaries and

the uterus (Fawcett, 1986; Ortiz et al., 1998, 2000). The characteristic histological structure of these arteries is considered a form of adaptation of the arterial vessels subject to traction (Rhodin, 1974; Fawcett, 1986).

The popliteal and tibial arteries also have the structure of the mixed (transitional) type of arteries (Fawcett, 1986). It has been confirmed that the length of the popliteal artery changes up to 20% between flexion and extension of the knee joint (Browse et al., 1979). This easy adaptation is enabled precisely because of the characteristic mixed (transitional) and specialized structure of this artery.

Obviously, mixed arteries are very adaptable in different physiological conditions and if we search for a new arterial graft we should look for it among the transitional arteries. For example, such an artery is an already described graft - the inferior epigastric artery. (Labudović Borović, 2003).

The basic histological structure of an artery as a fundamental fact must be taken into account when discussing the process of the remodeling of arteries during aging and different pathological conditions, especially the presence of atherosclerosis. We should always bear in mind that the vascular system is a system of extreme regional heterogeneity, both in physiological and pathological conditions.

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