Remodeling of the aortic wall layers with ageing

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Abstract

**Aim:** The authors aimed to evaluate the correlations between the variation of two of the main morphological parameters of the aortic wall (intima and media thicknesses) and ageing. **Materials and Methods:** Aortic cross sections (base region, cross region, thoracic region, and abdominal region) were collected from 90 cases of all ages died and autopsied in the hospital. Tissue samples were processed using the classical histopathological technique (formalin fixation and paraffin embedding) and stained with Orcein and Goldner's trichrome. The obtained histological slides were transformed into virtual slides. Intima and media thicknesses were determined on virtual slides using a custom-made software, developed in MATLAB (MathWorks, USA). **Results and Discussions:** The intima layer underwent an obvious and continuous process of thickening both from the aortic base region to its terminal (abdominal) region and from young ages to old age. The processes were similar in men and women but almost always more pronounced in men than in women. The media layer underwent a thickness reduction process from the aortic base to the terminal (abdominal) region whereas with age, the thickness of the layer increased. This divergent profile of evolution was similar in both men and women but with some variations depending on either topography or ageing. **Conclusions:** Each of the main layers of the aortic wall revealed dynamic individual evolutionary profiles related to age, gender and topography along the aortic path. Studies must be continued in a more detailed, standardized and integrated way. **Keywords:** aorta, aortic wall layers, ageing, morphology, morphometry.

Introduction

The various physiological processes of the human body suffer a decline with and related to aging, because of decades of “wear and tear”. Aging, together with the diseases and their subsequent lesions, can lead to a process of arterial remodeling that consists of structural and functional changes of the vascular wall [1–3]. The aorta, which is the largest artery of the human body, of elastic type and, usually, divided into four major regions (ascending aorta, aortic arch, thoracic aorta, and abdominal aorta) is among the most susceptible to injury regions of the vascular tree as we grow older [4–7]. Any of the segments of the aortic artery can undergo different types and degrees of remodeling during the aging process so that the structural response is distinct depending on the different stress situations along the entire aorta.

The algorithm for the occurrence of structural changes induced by aging is “bottom-up”, the distal segments being affected at a younger age than the proximal segments [8, 9]. However, an important part of this diffuse process of remodeling, consisting mainly of thickening of the aorta, was considered to be a normal developmental and aging process and not a part of a particular lesion, like, for instance, arteriosclerosis [10]. The main morphological change of the aortic wall is the increase with age of both average and maximal wall thickness [11–13]. The remodeling process is however non-uniform. At the level of intima layer, for instance, in the first to fourth decades, the thickness is higher in the aortic distal regions than in the proximal ones [10]. Tunica media, on the other hand, occupies greatest proportion of vessel wall in all ages but it reaches the maximum proportion
(72.4%) in the 4th decade, to decrease then after 40 years [14]. Finally, it has been shown that, in general, men had greater average and maximal wall thicknesses than women [11].

**Aim**

The present study is part of a larger research project that aims to identify morphological changes in the various compartments of the cardiovascular system (heart, blood vessels) with age. Its goal is to assess the quantitative remodeling of the aortic wall layers in relation with ageing process but also with topographic regions and cases’ gender.

**Materials and Methods**

The analyzed material consisted of specimens of aortic wall obtained from 90 patients deceased in the hospital who underwent necropsy in the Department of Pathology to establish the final diagnosis of death, coming from previous studies [15–17].

The study was performed in agreement with the Ethical Standards of the Helsinki Declaration. All patients’ relatives signed an informed consent agreement for the necropsy.

The only inclusion criterion used for selecting the cases was the age, in the sense that cases’ ages had to cover as much as possible the four main age periods (APs) of life.

The study material came from two main sources:

- Patient’s clinical documents (clinical medical records and autopsy protocols);
- Samples of aortic wall tissue collected during the necropsies.

The study was carried out as a single-center study and had two main components:

- A prospective component consisting of sampling tissue fragments;
- A retrospective component consisting of collecting data from deceased patient’s documents and from histologically processed tissue specimens.

Tissue samples, consisting of aortic wall rings, were obtained following an aortic artery modified protocol we designed [15] and used [16, 17] previously. The algorithm consisted of cross sections at four different levels, with no visible lesions of aortic wall, illustrated in Figure 1, namely:

- Section No. 1, at the ascending aorta level, 2–3 cm above the sinotubular junction, labeled “01 B” (base);
- Section No. 2, at the aortic arch level, in the left side close proximity of the left subclavian artery, labeled “02 C” (arch);
- Section No. 3, at the thoracic aorta level, in the inferior segment, above the diaphragm aortic hiatus, labeled “03 T” (thoracic region);
- Section No. 4, at the abdominal aorta level, between the celiac trunk and superior mesenteric artery, labeled “04 Ab” (abdominal region).

The set of parameters established to be analyzed were grouped into two main categories, clinical and morphological (Table 1).

The tissue samples were processed using the classical histopathological technique (formalin fixation and paraffin embedding) and then stained with a set of classical procedures to identify the two main layers of the aortic wall. The staining procedures are presented in Table 2.

For transformation in virtual slides of all histological slides included in the study, a Leica Aperio AT2 scanner with the ×20 objective was used.

For acquisition, processing, and morphometric determinations on virtual slides an Aperio ImageScope [v12.3.2.8013] specialized software was used (Figure 2a). For each cross-section of each case, four values were obtained for each parameter [intima thickness (INTh) and media thickness (MEDTh)]. The average of these four values was calculated and used further as representative for each cross-section of the aortic wall (Figure 2, a and b).

For age evaluation, patients were grouped in a scale with four groups following the main APs of life (Table 3). The statistical indices assessed for each of the numerical parameters were: (a) the lowest value – VMIN; the highest value – VMAX; the mean value – AV; the standard deviation – STDEV; the AV+STDEV; the AV-STDEV.

The test used for statistical assessment was t-test for two independent samples (two-tailed test).

Graphs that illustrated evolutionary trends of the different parameters, as well as the statistical comparisons...
between them, were made using the “Graph” tool from “Word” and “Excel” modules of the Microsoft Office 2019 Professional software suite and the XLSTAT 2014 add-on for the “Excel” module.

Figure 2 – Intima and media measurements. Windows of Aperio ImageScope program. Orcein staining: (a) Overview with the four random measurements, ×0.8; (b) Detail with the up-right measurement, ×6.4.

Table 3 – Stratification scale for age

| Age period (AP) | Description                      |
|-----------------|----------------------------------|
| AP1             | 0–24 years Infancy childhood and adolescence |
| AP2             | 25–44 years Young adult           |
| AP3             | 45–64 years Mature adult          |
| AP4             | >64 years Elderly                |

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Results

Tunica intima

In different topographic regions

During childhood and adolescence (AP1), the intima tunic thickness (INTh) average value (AV) did not exceed 60 μm and recorded only an upward trend from the proximal extremity to the distal extremity of the aorta with a minimal difference between the values recorded at the two extremities of only 5.56 μm. In addition, the general ascending trend had oscillations at the level of the aortic cross, where the highest value of the INTh AV was recorded (56.28 μm), and at the level of the thoracic region, where the value of the INTh AV decreased, being lower than that from the abdominal distal segment (56.28 μm vs 54.53 μm) (Figure 3, blue dotted line).

This evolutionary trend was statistically confirmed, on the one hand, by the p values of the t test which were constantly much higher than the 0.05 reference value and, on the other hand, by the observed values of the t test, which were sometimes negative and sometimes positive (Figure 3, AP1 curve and Figure 4 AP1 – left and blue line – right).

During the young adult period (AP2), INTh AV showed a difference between extremities of 57.19 μm with an obvious and constantly increasing evolution from one topographic region to another, from proximal to distal end (Figure 3, yellow dotted line).

This evolutionary trend was statistically confirmed, on the one hand, by the p values of the t test which were constantly much higher than the 0.05 reference value and, on the other hand, by the observed values of the t test which were constantly negative and decreasing towards the distal regions (Figure 3, AP2 curve and Figure 4 AP2 – left, and yellow line – right).

During the mature adult period (AP3), the INTh AV showed a difference between extremities of 74.25 μm. In this life period too, the evolutionary trend was obvious and constantly increasing from one topographic region to another, from proximal to distal end but with an attenuation at the level of the terminal topographic segments (Figure 3, brown dotted line). This evolutionary trend was statistically confirmed, on the one hand, by the p values of the t test which were much lower than the reference value 0.05 up to the level of the thoracic region only to become much higher between the terminal segments, and, on the other hand, by the observed values of the t test which were constantly negative and decreasing towards the distal regions (Figure 3, AP3 curve and Figure 4 AP3 – left, and brown line – right).

During the senescence period (AP4), we observed the same situation encountered during the mature adult period (AP3), except that the difference between the extremities was 102.65 μm (Figure 3, red dotted line). The statistical evaluation also confirmed here the constantly increasing evolution from the proximal extremity to the distal extremity of the aorta, on the one hand, by the p values of the t test which were much lower than the reference value 0.05 up to the thoracic region to become much larger between the terminal segments, and, on the other hand, by the observed values of the t test which were constantly negative and decreasing towards the distal regions (Figure 3, AP4 curve and Figure 4 AP4 – left, and red line – right).
As a concluding remark, the general trend of the INTn AV values was an increasing one from the base of the aorta to the abdominal region in each of the four main periods of life but with notable differences between the ways in which this increase was achieved in each period of life.

In different periods of life

At the base of the aorta (1_B), the INTn AV value did not exceed $72.4 \mu m$ and showed only an increasing trend from AP1 to AP4, with a minimum difference between the values recorded at the two extremities of only $23.27 \mu m$. The general ascending trend was a constant one, more pronounced between AP1 and AP2 ($64.57 \mu m$ vs $48.97 \mu m$) and then more attenuated towards senescence ($64.57 \mu m$ vs $70.24 \mu m$ vs $72.24 \mu m$) (Figure 5, blue dotted line).

In different periods of life

At the base of the aorta (1_B), the INTn AV value did not exceed $72.4 \mu m$ and showed only an increasing trend from AP1 to AP4, with a minimum difference between the values recorded at the two extremities of only $23.27 \mu m$. The general ascending trend was a constant one, more pronounced between AP1 and AP2 ($64.57 \mu m$ vs $48.97 \mu m$) and then more attenuated towards senescence ($64.57 \mu m$ vs $70.24 \mu m$ vs $72.24 \mu m$) (Figure 5, blue dotted line).

At the aortic cross level (2_C), the INTn AV value recorded a difference of $61.62 \mu m$ between AP1 and AP4, with an obvious and constantly increasing evolution from one period of life to another, from young ages to old, with an attenuation towards senescence (Figure 5, yellow dotted line).

At the base of the aorta (1_B), the INTn AV value did not exceed $72.4 \mu m$ and showed only an increasing trend from AP1 to AP4, with a minimum difference between the values recorded at the two extremities of only $23.27 \mu m$. The general ascending trend was a constant one, more pronounced between AP1 and AP2 ($64.57 \mu m$ vs $48.97 \mu m$) and then more attenuated towards senescence ($64.57 \mu m$ vs $70.24 \mu m$ vs $72.24 \mu m$) (Figure 5, blue dotted line).

In the thoracic region (3_T), the INTn AV value showed a difference of $110.4 \mu m$, between AP1 and AP4, with an obvious and constantly increasing evolution from one period of life to the other, from young ages to old age, more pronounced between the period of childhood and adolescence and the period of young adult (Figure 5, brown dotted line).

This evolution trend was statistically confirmed, on the one hand by the $p$ values of the $t$ test which were much lower than the reference value of 0.05 between this period and the AP4 and, on the other hand, by the observed values of the $t$ test which were constantly negative and oscillating, with an increase in the senescence period (Figure 5, IN 2_C curve and Figure 6, 02 C – left, and yellow line – right).

In the thoracic region (3_T), the INTn AV value showed a difference of $110.4 \mu m$, between AP1 and AP4, with an obvious and constantly increasing evolution from one period of life to the other, from young ages to old age, more pronounced between the period of childhood and adolescence and the period of young adult (Figure 5, brown dotted line).

This evolution trend was statistically confirmed, on the one hand by the $p$ values of the $t$ test which were much lower than the reference value of 0.05 between this period and the AP4 and, on the other hand, by the observed values of the $t$ test which were constantly negative and oscillating, with an increase in the senescence period (Figure 5, IN 2_C curve and Figure 6, 02 C – left, and yellow line – right).
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throughout life periods and on the other hand, by the observed values of the \( t \) test which were constantly negative and slightly increasing towards senescence (Figure 5, IN 3_T curve and Figure 6, 03 T – left, and brown line – right).

The same situation encountered in the thoracic region was observed in the abdominal region (4_Ab), only that the difference between the extremities was greater, namely 120.37 \( \mu m \) (Figure 5, red dotted line). The statistical evaluation confirmed here also the constantly increasing evolution from the young ages to the advanced ages, on the one hand, by the \( p \) values of the test which were much lower than the reference value of 0.05 but attenuated to senescence and, on the other hand, by the observed values of the \( t \) test which were constantly negative and oscillating, with a significant decrease between the periods of the young and mature adult and a re-stabilization going into senescence (Figure 5, IN 4_Ab curve and Figure 6, 04 Ab – left, and brown line – right).

The general trend of the INTh AV values was an increasing one from young ages to advanced ages in each of the four topographic segments of the aorta but with notable differences between the ways in which this increase was achieved at each topographic segment.

Relation with person’s gender

In different topographic regions

Analysis of the INTh AV evolution along the topographic regions of the aorta in both genders showed that in both men and women the INTh AV values obviously increased from the proximal end to the distal end of the vessel. The evolutionary trend was parallel between the two genders (the Pearson’s correlation test’s \( p \) value was 0.005 – lower than the reference value of 0.05). It should also be noted that, at all topographic levels, the average thickness of the intima tunic was higher in men than in women (Figure 7a). Overall, however, this difference was not validated as significant by the \( t \) test (the \( p \) value was 0.7486 – much higher than the 0.05 reference value).

Tunica media

In different topographic regions

During childhood and adolescence (AP1), the thickness of the media tunic (MEDTh) AV did not exceed 1200 \( \mu m \) and showed a continuous decreasing trend from the proximal extremity to the distal extremity of the aorta with a significant difference between the values recorded at the two extremities of 452.52 \( \mu m \). The general decreasing trend was very pronounced between the base and the cross, where the biggest difference between the average thicknesses was measured (334.9 \( \mu m \)), representing almost three quarters of the total reduction between the extremities of the aorta. From the cross level, the tendency of the average thickness to decrease reduced more and more towards the abdominal region (724.84 \( \mu m \)) (Figure 8, blue dotted line).

In different periods of life

Analysis of the evolution of INTh AV over the four periods of life in both genders showed that in both men and women the INTh AV values increased visibly from the period of childhood and adolescence (AP1) to senescence (AP4). The evolution trend was parallel between the two genders (the Pearson’s correlation test’s \( p \) value was 0.029 – much lower than the reference value of 0.05). It should also be noted that, in general, the INTh AV was higher in men than in women, with one exception, namely the period of childhood and adolescence, where INTh AV value was slightly higher in women compared to men (Figure 7b). Overall, however, this difference was not validated as significant by the \( t \) test (the value of the \( p \) was 0.7083 – much higher than the 0.05 reference value).

In different topographic regions

During childhood and adolescence (AP1), the thickness of the media tunic (MEDTh) AV did not exceed 1200 \( \mu m \) and showed a continuous decreasing trend from the proximal extremity to the distal extremity of the aorta with a significant difference between the values recorded at the two extremities of 452.52 \( \mu m \). The general decreasing trend was very pronounced between the base and the cross, where the biggest difference between the average thicknesses was measured (334.9 \( \mu m \)), representing almost three quarters of the total reduction between the extremities of the aorta. From the cross level, the tendency of the average thickness to decrease reduced more and more towards the abdominal region (724.84 \( \mu m \)) (Figure 8, blue dotted line).

This evolutionary trend was statistically confirmed, on the one hand, by the \( p \) values of the \( t \) test which were constantly lower than the reference value of 0.05 up to
the level of the thoracic region but increased discreetly after which they become much higher than the reference value of 0.05 towards the abdominal terminal segment. On the other hand, the evolutionary pattern was also confirmed by the observed values of the $t$ test which were constantly positive but decreasing, very accentuated towards the abdominal region (Figure 8, AP1 curve and Figure 9 AP1 – left and blue line – right).

\[ \begin{array}{|l|c|c|c|c|} 
\hline 
\text{Life Periods} & \text{Regions} & 01B-02C & 02C-03T & 03C-04Ab \\
\hline 
\text{t(Observed value)} & 6.787 & 6.5796 & 0.3697 \\
\text{DF} & 5 & 5 & 5 \\
\text{p-value} & <0.0001 & 0.0025 & 0.7268 \\
\hline 
\text{t(Observed value)} & 10.5801 & 1.3087 & 1.8951 \\
\text{DF} & 13 & 13 & 13 \\
\text{p-value} & <0.0001 & 0.2133 & 0.0806 \\
\hline 
\text{t(Observed value)} & 5.5337 & 5.5465 & 4.7418 \\
\text{DF} & 40 & 40 & 40 \\
\text{p-value} & <0.0001 & 0.0091 & 0.0187 \\
\hline 
\text{t(Observed value)} & -0.968 & 0.8901 & 2.4741 \\
\text{DF} & 28 & 28 & 28 \\
\text{p-value} & 0.3413 & <0.0001 & 0.0197 \\
\hline 
\end{array} \]

Figure 9 – Results of $t$ test and evolution diagram of $t$ (observed value) between aortic regions for each age period (AP). 1_B: Aortic base; 2_C: Aortic arch; 3_T: Thoracic aorta; 4_Ab: Abdominal aorta.

During the young adult period (AP2), the evolutionary pattern of the MEDTh AV was almost identical to that observed in children and adolescents. The MEDTh AV was higher than in childhood and adolescence at the base of the aorta (1486.7 μm vs 1177.36 μm) and showed, in this period of life, a continuous downward trend from the proximal extremity to the distal extremity of the aorta with an equally significant difference between the values recorded at the two extremities of 443.6 μm.

The average thickness initially “collapsed” between the base and the aortic cross, where the largest difference between the average thicknesses (356.4 μm) could be observed, representing 80% of the total reduction between the extremities of the aorta so that, from the cross, the tendency to decrease the average thickness should be reduced more and more to the level of the abdominal region (1043.1 μm) (Figure 8, yellow dotted line).

This evolutionary pattern was statistically confirmed, on the one hand by the $p$ values of the $t$ test which were initially much lower than the reference value 0.05, to then increase well above the reference value of 0.05 between the cross and the thoracic region and return around it to the distal segment of the aorta. On the other hand, this evolutionary pattern was also confirmed by the observed values of the $t$ test which were constantly positive and decreasing, very pronounced towards the terminal segments of the aorta (Figure 8, AP2 curve and Figure 9 AP2 – left and yellow line – right).

During the mature adult period (AP3), the evolutionary pattern of the MEDTh AV was similar to that observed in the other two life periods.

The average value for the thickness of the intima tunic showed a difference between extremities slightly less than only 391.94 μm and presented an obvious and constantly decreasing evolution from one topographic region to another, from proximal to distal, with a discreet attenuation between the cross and the thoracic region (Figure 8, brown dotted line).

The evolutionary pattern was statistically confirmed on the one hand by the $p$ values of the $t$ test which were much lower than the 0.05 reference value along the entire length of the aorta, and on the other hand by the observed values of the $t$ test which were constantly positive and decreasing towards the distal regions (Figure 8, AP3 curve and Figure 9 AP3 – left and brown line – right).

During the senescence period (AP4), however, the evolutionary pattern of the MEDTh AV was slightly different from that observed in the other three periods of life. The MEDTh AV also showed, for this period of life, a general decreasing tendency from the proximal extremity to the distal extremity of the aorta with a significant difference between the values observed at the two extremities of 312.88 μm.

For the third period of life, however, this trend was oscillating. Thus, between the base and the aortic cross, the media tunic had an inverse tendency, to thicken, as evidenced by the much higher $t$ test’s $p$ value than the reference value of 0.05 and the observed negative value of the $t$ test (Figure 8, AP4 curve and Figure 9 AP4 – left). Then, the trend was reversed, the MEDTh decreasing more sharply between the cross and the thoracic region and then more attenuated towards the abdominal region. This obvious and continuous reduction of the MEDTh towards the distal segments of the aorta was statistically confirmed by the $p$ values of the $t$ test which were much lower than the reference value of 0.05 and by the observed values of the $t$ test which were constantly positive but decreasing towards the distal regions (Figure 8, AP4 curve and Figure 9 AP4, brown line – right).

The general trend of the MEDTh AV was a decreasing one from the base of the aorta to the abdominal region in each of the four main periods of life with insignificant differences between the ways in which this reduction was achieved in each of the four periods of life.

In different periods of life

At the level of the aortic base (1_B), the MEDTh AV had a particular behavior in relation to ageing. It did not exceed 1500 μm and showed a spectacular increase from the period of childhood and adolescence to the period of the young adult, with a significant difference between the values recorded in these two periods of 309.34 μm.

After the young adult period, however, MEDTh AV showed a continuous downward trend until senescence. However, the general trend was an increasing but
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oscillating one, more pronounced between the periods of childhood and adolescence and the period of the young adult, as we showed before, and then more attenuated towards senescence (1407.21 μm vs 1177.36 μm), with a general increase to a value of only 229.85 μm (Figure 10, blue dotted line).

This evolutionary pattern was statistically confirmed on the one hand by the p values of the t test which were initially lower than the 0.05 reference value and then became constantly higher than this but with a decreasing trend and, on the other hand, the observed values of the t test which were initially negative but then became positive with an increasing trend (Figure 10, MED 1_B curve and Figure 11 01 B – left and blue line – right).

At the aortic cross segment (2_C), MEDTh AV did not exceed 1450 μm but registered a significant difference between the AP1 period and the AP4 of 594.51 μm, with an obviously and constantly increasing evolution from one period of life to another, from young ages to old age (Figure 10, yellow dotted line).

This evolutionary trend was statistically confirmed, on the one hand, by the p values of the t test which were much lower than the 0.05 reference value along the entire aortic path and, on the other hand, of the observed values of the t test which were constantly negative and relatively stable (Figure 10, MED 2_C curve and Figure 11 02 C – left and yellow line – right).

The same evolutionary pattern was observed in the thoracic region (3_T). The MEDTh AV showcased a smaller but still significant difference between the period of childhood and adolescence and the period of senescence of 454.03 μm, with an obvious and constantly increasing evolution from one period of life to the other, from younger ages to older ages.

The evolution was more pronounced between the period of childhood and adolescence and the young adult period, an evolution that then diminished towards senescence (Figure 10, brown dotted line).

This evolutionary pattern was statistically confirmed, on the one hand by the p values of the t test, which were much lower at first and then lower than the reference value of 0.05 between AP1 and AP3, only to become higher than the reference value of 0.05 at senescence and, on the other hand, by the observed values of the t test which were constantly negative and increasing slightly but oscillatingly towards senescence (Figure 10, MED 3_T3 curve and Figure 11 03 T – left and brown line – right).

The same evolutionary pattern was observed in the abdominal region (4_Ab). The MEDTh AV showing a smaller but still significant difference between the AP1 period and the AP4 period, of 369.49 μm, with an also obvious and constantly increasing evolution from one life period to the other, from young ages to old age.

The evolution was more pronounced between the periods of childhood and adolescence and the period of the young adult, and much diminished towards senescence (Figure 10, red dotted line).

The general trend of the values for the MEDTh AV was an inverse one as compared to that of the INTh, namely it was an increasing one from young ages to advanced ages in each of the four topographic segments of the aorta but with some notable differences in which this increase was achieved at the level of each topographic segment.

Relation with person’s gender

Within different topographic regions

Finally, an almost similar evolutionary pattern was observed in the abdominal region (4_Ab). The MEDTh AV showing a smaller but still significant difference between the AP1 period and the AP4 period, of 369.49 μm, with an also obvious and constantly increasing evolution from one life period to the other, from young ages to old age. The evolution was more pronounced between the periods of childhood and adolescence and the period of the young adult, and much diminished towards senescence (Figure 10, red dotted line).

This evolutionary pattern was statistically confirmed on the one hand by the p values of the t test which were much lower and then lower than the 0.05 reference value 0.05 from AP2 to AP4 and, on the other hand, by the observed values of the t test which were constantly negative and slightly but oscillatingly increasing towards senescence (Figure 10, MED 4_Ab curve and Figure 11 04 Ab – left and red line – right).

The general trend of the values for the MEDTh AV was an inverse one as compared to that of the INTh, namely it was an increasing one from young ages to advanced ages in each of the four topographic segments of the aorta but with some notable differences in which this increase was achieved at the level of each topographic segment.

Relation with person’s gender

Within different topographic regions

Analysis of the evolution of the MEDTh AV along the topographic regions of the aorta in both genders showed
that in both men and women the MEDTh AV was obviously reduced from the proximal end to the distal end of the vessel. The evolutionary trend was parallel in the two genders (the value of the \( p \) of the Pearson’s correlation test was 0.0131 – lower than the reference value 0.05). The evolution trend in the two genders was parallel (the value of the \( p \) of the Pearson’s correlation test was 0.0131 – lower than the reference value of 0.05). It should also be noted that, in general, the MEDTh AV was higher in women than in men with one exception, in the thoracic region, where the MEDTh AV was discreetly higher in men than in women (Figure 12a).

Overall, however, this difference was not validated as significant by the \( t \) test (the \( p \) value was 0.7375 – much higher than the 0.05 reference value).

**In different periods of life**

Analysis of the evolution of the MEDTh AV over the four periods of life in both genders showed that in both men and women the MEDTh AV increased clearly from childhood and adolescence AP1 to senescence AP4. This increase was very obvious between the periods of childhood and adolescence and the period of the young adult in both genders, after which the ascending trend greatly diminished until senescence and, in the case of men, reversed, the AV recorded in senescence being still higher than the one observed during young adulthood (Figure 12b).

The evolutionary trend was parallel in the two genders until the period of the mature adult, after which, at senescence, the evolutionary trends dissociated.

Overall, however, the trend was the same for both genders (the \( p \) value of the Pearson’s correlation test was 0.0315 – lower than the 0.05 reference value) (Figure 12b).

It is also worth highlighting the dissociation between the values of the average thickness of the media tunic in the two genders along with the ageing process. Thus, in the first part of life (AP1 and AP2) the MEDTh AV was higher in men than in women, while in the second part of life (AP3 and AP4) the MEDTh AV became higher in women as compared to men (Figure 12b).

Overall, however, this difference was not validated as significant by the \( t \) test (the value of the \( p \) was 0.9433 – much higher than the reference value of 0.05).

Figure 12 – Comparative diagram of the media (MED) tunic thickness evolution between the two genders (a) along the aorta length (b) during life. 1_B: Aortic base; 2_C: Aortic arch; 3_T: Thoracic aorta; 4_Ab: Abdominal aorta; AP: Age period; F: Female; M: Male.

** Discussions**

**Intima layer**

The length and thickness of the aorta proved to increase progressively with age, with variations and differences from one decade of age to another and between the two genders, processes which continue going into old age [1]. This increase of the INTh with age proved to be independent of the presence of atheromatous lesions, the subendothelial layer of the intima layer being the main component that varies with age [19, 20]. At the same time, the INTh proved to increase along the path of the aorta from the level of the ascending segment of the thoracic aorta where it measured 0.08±0.03 mm, up to the level of the infrarenal segment of the abdominal aorta where it measured 0.64±0.24 mm [9].

**Relation with age**

On the other hand, the intima layer also underwent a process of progressive thickening in each of the four topographic regions of the aorta that was more pronounced with age. The evolutionary patterns of the INTh AV values for each topographic region were stratified upwards from the proximal to the distal regions, with a single intersection, during childhood and adolescence, where the INTh AV was higher at the aortic cross than at the level of the thoracic region.

**Relation with topographic regions**

In our study, the intima layer underwent, on one hand, a process of progressive thickening along the aorta, which was pronounced from the proximal to the distal regions in all periods of life. The lines of evolution of the INTh AV values for each life period were stratified upwards, from the young ages to the advanced ages, without intersecting.

**Relation with person’s gender**

The INTh AV showcased a constant increase from the origin of the aorta to the abdominal region in both men and women (Table 4). The observed peculiarity was that the INTh AV values were obviously higher in men than in women along the entire anatomical path of the aortic artery, from the base to the terminal topographic region (abdominal segment) (Table 4A).

At the same time, throughout life, the INTh AV also presented a steady increase from childhood to senescence. This ascending trend was observed in both men and women. Except for the period of childhood and adolescence, where the average thickness of the intima tunic was only 1 \( \mu m \)
higher in women than in men, in the rest of the life periods it was obviously higher in men than in women (Table 4B).

### Table 4 – Relation with person’s gender of intima layer’s thickness

| Layer | Topographic regions | Trend |
|-------|---------------------|-------|
| Intima |                      |       |
| Life periods |                  |       |
| AP1   | M > F               |       |
| AP2   | M > F               |       |
| AP3   | M > F               |       |
| AP4   | M > F               |       |

1_B: Aortic base; 2_C: Aortic arch; 3_T: Thoracic aorta; 4_Ab: Abdominal aorta; F: Female; M: Male.

### Media layer

The tunica media has been shown to make up the bulk of the thickness of the arterial wall in all ages, with a maximum in the fourth decade of life and, as compared with intima layer, the MEDTh decreased along the aorta length from 1.41±0.09 mm at the ascending segment of the thoracic aorta level to 0.64±0.18 mm at the infrarenal segment of the abdominal aorta level [9, 14].

### Relation with age

The media tunic has undergone a process of progressive thickening throughout life in all topographic segments of the aorta. This process was more pronounced between the first two periods of life in all the topographic regions, after which it diminished until senescence, mostly at the base of the aorta and least at the level of the cross.

The lines of the evolution trends of the MEDTh AVs for each period of life were stratified upwards from childhood and adolescence to senescence, without intersecting. There was, however, one exception, namely, at the base of the aorta, where the MEDTh AV from the period of senescence (AP4) was smaller than that of the adult period (AP3).

### Relation with topographic regions

In our study, the media layer underwent a process of progressive narrowing that was more pronounced between the proximal regions of the aorta in all periods of life except senescence, where the MEDTh increased from the base to the aortic cross. Beyond the cross, the phenomenon diminished towards the distal end also for all periods of life.

The lines of the evolution trends of the MEDTh AVs for each topographic region were stratified descending from the proximal to the distal segments, with a single intersection, during senescence (AP4) where the MEDTh AV was higher at the cross than at the base.

### Table 6 – Clinical profile of series from the literature

| Sampling procedure | Our study | [21] | [22] | [23] | [23]* | [24] |
|--------------------|-----------|------|------|------|-------|------|
| Age [years]        | 56.4±17.8 | 63.0±15.3 | 50.2±10.1 | 47.1±12.3 | 46.3±12.8 | 56.9±9.8 |
| M/F ratio          | 1.57      | 0.8  | 2.8  | 0.9  | 0.8   | 0.5  |

F: Female; M: Male; US: Ultrasound examination; *Subgroup of patients with pathology; [21] Schrieff et al. (2012); [22]: Bulut et al. (2019); [23]: Koc & Sumbul (2018); [24]: Sumbul & Koc (2019).

We didn’t find, on the one hand, too many data in the literature to compare with our results and, on the other hand, most of the consulted studies were either reviews, with general considerations, either were analyzes of the aortic wall as a whole. Another observation related to the few studies we could use for comparison was that, in most of them, the quantitative assessment of the aortic wall resulted from ultrasound investigation, only one study [21] excepting ours was a pathological one (Table 6). Finally, if the mean age belonged to the third period of life (45–64 years) in all consulted studies, most of the studies included more women than men, excepting the Turkish study of Bulut et al. [22] and our study (Table 6).

### In different topographic regions

The MEDTh AV on the other hand, showed a constant decrease starting from the origin of the aorta and up to the level of the abdominal region, the decreasing trend being observed in both men and women.

Unlike the INTTh AV, the MEDTh AV was, with one exception, higher in women than in men. The observed peculiarity consisted in the fact that, as it advanced towards the terminal segments, the difference reduced gradually and even reversed at the level of the thoracic region where the AV Th of the tunic was only 4 μm higher in men than in women, but it came back at the last aortic segment where it was only 1 μm higher in women than in men (Table 5).

### Table 5 – Relation with person’s gender of media layer’s thickness

| Layer | Topographic regions | Trend |
|-------|---------------------|-------|
| Media |                      |       |
| Life periods |                  |       |
| AP1   | M > F               |       |
| AP2   | M > F               |       |
| AP3   | F > M               |       |
| AP4   | F > M               |       |

1_B: Aortic base; 2_C: Aortic arch; 3_T: Thoracic aorta; 4_Ab: Abdominal aorta; F: Female; M: Male.

### In different periods of life

The MEDTh AV recorded a steady increase throughout life for both men and women. The peculiarity of its evolution consisted in the fact that, in the first periods of life, the media tunic had a greater thickness in men as compared to women, this difference decreasing however with age. But, from the period of mature adult (after 45 years) MEDTh AV became higher in women as compared to men and more pronounced towards senescence (Table 5).

### Comparisons with the literature

We could compare the evolution with ageing with only one American study and only for the mean values of media layer thickness. The sampling procedure used imagistic techniques – magnetic resonance imaging (MRI), like most of the other consulted studies.

In both studies, the MEDTh AV values followed a general
increasing trend from young ages to old age. The trend was more pronounced in the American study, probably because the number of cases in each group of age was more consistent in their study (Table 7).

Table 7 – Comparative data with other studies of the MEDTh AV evolution on age groups

| Age Group | Our Study | [11] |
|-----------|-----------|------|
| 45–54 years | 1.40 | 2.03 |
| 55–64 years | 1.35 | 2.18 |
| 65–74 years | 1.37 | 2.26 |
| 75–84 years | 1.44 | 2.51 |

[11]: Li et al. (2004); MEDTh AV: Media thickness average value.

The same American study was our only reference point concerning the gender related behavior of only MEDTh AV. In our study, mean values of media layer thickness were slightly higher in women whereas in the American study media layer was thicker in men than in women (Table 8).

Table 8 – Comparative data with other studies of the MEDTh AV evolution on gender

| Gender | Our Study | [11] |
|--------|-----------|------|
| M      | 1.32      | 2.32 |
| F      | 1.36      | 2.11 |

[11]: Li et al. (2004); M: Male; F: Female; MEDTh AV: Media thickness average value.

A possible explanation of this situation could be again the more consistent number of cases in the foreign study and the more homogenous distribution between genders (M/F ratio was 1 as compared with 1.57 in our study).

The assessment of the topographic behavior of the aortic wall layers thicknesses was somehow easier (we found the five studies with data described above, for comparison) but still difficult because in all these studies the aortic wall layers were measured together and only in one of the four regions we defined in our study, i.e., the abdominal region. Three of these studies, however, measured the whole thickness of the aortic wall in the thoracic region too.

The first observation was that the registered values of all studies were placed in a not so wide variation range, i.e., between 1.2 mm and 1.6 mm (Figure 13).

Our data were placed near the lower limit of the range. We could invoke the tissue contraction after fixation as compared with the in vivo measurements achieved by US examination, but this hypothesis could be contradicted by the results obtained by Sumbul & Koc using US [24].

Another interesting and intriguing observation was that, in the three studies were aortic wall thicknesses of both thoracic and abdominal regions were measured, the results were totally different. Thus, if in the American study of Tsamis et al. [9] aortic wall of the thoracic region was thicker than that of the abdominal region (like in our study), in the Turkish study of Bulut et al. [22] the situation is totally opposite, the aortic wall of the abdominal region being thicker. And, to complete the various picture of the comparison between the different topographic regions of the aorta, in the Austrian study of Schriefl et al. [21], there is no difference in aortic wall thickness between thoracic and abdominal regions.

There was only one American study, a review of Tsamis et al. [9], where measurements of both intima and media were mentioned and in two of the aortic regions: ascending aorta (at the proximal end of the artery) and abdominal aorta (at the terminal end of the artery). Our data were similar to those reported by the American researchers, in the sense that the evolutionary trends from the proximal to the terminal end of both layers’ thicknesses were the same (Figure 14, a and b).

The difference was that, in the American study, both the increasing trend of the intima layer thickness and the decreasing trend of the media layer thickness were more pronounced than those in our study (Figure 14, a and b).
Conclusions

The average thicknesses of the main component layers of the aortic wall presented dynamic evolutionary profiles in relation to the topographic regions of the aorta, with advancing age and in relation to person’s gender.

The intima layer underwent an obvious and continuous process of thickening on the one hand from the aortic base to its terminal (abdominal) region and, on the other hand, from youth to senescence. Evolutionary patterns were similar both in men and women, and the thickening process was almost always more pronounced in men than in women.

The media layer was the site of a different remodeling process in relation to the aortic topographic regions than in relation to aging. Thus, if, from the aortic base to the terminal (abdominal) region, the media layer underwent a thickness reduction process, with age, the thickness of the layer increased. This divergent profile of evolution was similar in both sexes. However, relative to the ageing process, the media layer was thicker in men as compared to women whereas relative to the ageing process, aortic regions, media layer was thicker in women as compared to men whereas relative to the ageing process, the layer increased. This divergent profile of evolution was similar in both sexes. However, relative to the ageing process, the media layer was thicker in men as compared to women in the first periods of life and became thicker in women as compared to men after the age of 45.

As a final general conclusion, we think we could at least reduce most of these conflicting results and consequently have more truthful data concerning the dynamics of the remodeling processes which occur in the arterial wall in general and the aortic wall in particular in relation with different factors like age, gender, topography, disease, by having more studies which use the same type of samples and the same protocols and techniques of measurement. In other words, the studies must continue but in a more detailed, standardized, and integrated way.

Conflict of interests

The authors declare that they have no conflict of interests.

Authors’ contribution

The first and the second authors had equal contribution to the achievement of this paper.

References

[1] Gallagher PJ, van der Wal AC. Blood vessels. In: Mills SE (ed). Histology for pathologists. 5th edition, Lippincott Williams & Wilkins, Philadelphia, PA, USA, 2019, 190–216. https://www.wolterskluwer.com/en/solutions/ovid/histology-for-pathologists-3488

[2] Jaminon A, Reesink K, Kroon A, Schurgers L. The role of vascular smooth muscle cells in arterial remodeling: focus on calcification-related processes. Int J Mol Sci, 2019, 20(22): 5694. https://doi.org/10.3390/ijms20225694 PMID: 31739395 PMCID: PMC6888164

[3] Jani B, Rajkumar C. Ageing and vascular ageing. Postgrad Med J, 2006, 82(968):357–362. https://doi.org/10.1136/pgmj.2005.036053 PMID: 16754702 PMCID: PMC2563742

[4] Komutrattananont P, Mahakkanukrauh P, Das S. Morphology of the human aorta and age-related changes: anatomical facts. Anat Cell Biol. 2019, 52(2):109–114. https://doi.org/10.1515/acb.2019.52.2.109 PMID: 31338225 PMCID: PMC6624342

[5] Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W Jr, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of the intima of human arteries and of its atherosclerosis-prone regions. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation, 1992, 85(1):391–405. https://doi.org/10.1161/01.cir.85.1.391 PMID: 1728483

[6] Nakashima Y, Chen YX, Kinukawa N, Sueishi K. Distributions of diffuse intimal thickening in human arteries: preferential expression in atherosclerosis-prone arteries from an early age. Virchows Arch, 2002, 441(3):279–286. https://doi.org/10.1007/s00428-002-0605-1 PMID: 12342525

[7] Hasson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med, 2005, 352(16):1685–1695. https://doi.org/10.1056/NEJMra043430 PMID: 15843671

[8] Vande Geest JP, Sacks MS, Vorp DA. Age dependency of the biaxial biomechanical behavior of human abdominal aorta. J Biomech Eng, 2004, 126(6):815–822. https://doi.org/10.1115/1.1824121 PMID: 15796340

[9] Tsamis A, Krawiec JT, Vorp DA. Elastin and collagen fibre microstructure of the human aorta in ageing and disease: a review. J R Soc Interface, 2013, 10(83):20121004. https://doi.org/10.1098/rsif.2012.1004 PMID: 23563583 PMCID: PMC3645409

[10] Movat HZ, More RH, Haust MD. The diffuse intimal thickening of the human aorta with aging. Am J Pathol, 1958, 34(6): 1023–1031. PMID: 13583094 PMCID: PMC1934788

[11] Li AE, Kamei I, Rando F, Anderson M, Kumbasar B, Lin JAC, Blumekme DA. Using MRI to assess aortic wall thickness in the multitiethnic study of atherosclerosis: distribution by race, sex, and age. AJR Am J Roentgenol, 2004, 182(3):593–597. https://doi.org/10.2214/ajr.182.3.1820593 PMID: 14979553

[12] Jaffer FA, O’Donnell CJ, Larson MG, Chan SK, Kissinger KV, Kupka MJ, Salton C, Botnar RM, Levy D, Manning WJ. Age and sex distribution of subclinical aortic atherosclerosis: a magnetic resonance imaging examination imaging of the Framingham Heart Study. Arterioscler Thromb Vasc Biol, 2002, 22(5):849–854. https://doi.org/10.1161/01.ATV.0000012662.29622.00 PMID: 12060401

[13] French Study of Aortic Plaques in Stroke Group; Amarencop, Cohen A, Hommel M, Moulin T, Leys D, Bousser MG. Atherosclerotic disease of the aortic arch as a risk factor for recurrent ischemic stroke. N Engl J Med, 1996, 334(19):1216–1221. https://doi.org/10.1056/NEJM199605093341902 PMID: 8606716

[14] Alex RB, Amma LKK. Microanatomical study of age changes in tunica media of ascending aorta. J Evol Med Dent Sci, 2016, 5(101):7409–7412. https://www.jemds.com/data_pdf/Reba%20Abu.pdf

[15] Mina OC, Anaoua AA, Šerbánescu MS, Postolache P, Uscatu CD, Marin C, Pleșea IE, Chițu LC. Analysis of aortic size in subjects died due to cardiovascular and non-cardiovascular events: a necropsy study. Rom J Morphol Embryol, 2014, 55(3 Suppl):1105–1109. PMID: 25607392

[16] Šeicaru DA, Albu M, Pleșea RM, Gherghiceanu F, Cordoș I, Lițescu M, Alexandru DO, Pleșea IE, Griorean VT. Anatomical remodeling of the aortic wall in relation with the cause of death. Rom J Morphol Embryol, 2021, 62(1):19–40. https://doi.org/10.47162/RJME.62.1.03 PMID: 34609406 PMCID: PMC8597380

[17] Albu M, Šeicaru DA, Pleșea RM, Mina OC, Gherghiceanu F, Griorean VT, Cordoș I, Lițescu M, Pleșea IE, Šerbănescu MS. Assessment of the aortic wall histological changes with ageing. Rom J Morphol Embryol, 2021, 62(1):85–100. https://doi.org/10.47162/RJME.62.1.08 PMID: 34609411 PMCID: PMC8597368

[18] Szalay D, Frolov M. Aortic aneurysms. In: Jaeschke R, Gajewski P, 12006401

[19] Szalay D, Frolov M. Aortic aneurysms. In: Jaeschke R, Gajewski P, O’Byrne P (eds), McMaster textbook of internal medicine. Medycyna Praktyczna, Kraków, Poland, 2022. https://empendium.com/mcmtextbook/chapter/B31.II.2.2.2

[20] Lindberg MR, Lamps LW. Chapter 20: Arteries. In: Lindberg MR, Lamps LW (eds). Diagnostic pathology: normal histology. 2nd edition, Elsevier, Philadelphia, PA, USA, 2018, 94–97. https://doi.org/10.2214/ajr.19879-032-54803-8.50026-7 https://www.sciencedirect.com/book/9780323548038/diagnostic-pathology-normal-histology

[21] Gasser TC, Ogden RW, Holzapfel GA. Hyperelastic modelling of arterial layers with distributed collagen fibre orientations. J R Soc Interface, 2006, 3(6):15–35. https://doi.org/10.1098/rsif.2005.0073 PMID: 16849214 PMCID: PMC1618483

[22] Schnief AJ, Zindlingner G, Pierce DM, Regitnig P, Holzapfel GA. Determination of the layer-specific distributed collagen fibre orientations in human thoracic and abdominal aortas and
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common iliac arteries. J R Soc Interface, 2012, 9(71):1275–1286. https://doi.org/10.1098/rsif.2011.0727 PMID: 22171063 PMCID: PMC3350738

[22] Bulut A, Acele A, Donmez Y, Pekoz BC, Erdogan M, Sumbul HE, Icen YK, Koc M. Aortic intima-media thickness can be used to determine target organ damage in adult patients with coronary artery disease risk factors. Arch Med Sci Atheroscler Dis, 2019, 4:e183–e190. https://doi.org/10.5114/amsad.2019.87002 PMID: 31538122 PMCID: PMC6747883

[23] Koc AS, Sumbul HE. Increased aortic intima-media thickness may be used to detect macrovascular complications in adult type II diabetes mellitus patients. Cardiovasc Ultrasound, 2018, 16(1):8. https://doi.org/10.1186/s12947-018-0127-x PMID: 29891012 PMCID: PMC5996542

[24] Sumbul HE, Koc AS. The abdominal aortic intima-media thickness increases in patients with primary hyperparathyroidism. Exp Clin Endocrinol Diabetes, 2019, 127(6):387–395. https://doi.org/10.1055/a-0664-7820 PMID: 30107624

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