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Assessment of the aortic wall histological changes with ageing

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Abstract

Aim: The authors aimed to quantitatively assess the variation with age of three of the main components of the aortic wall, namely elastic fibers (FE) and collagen fibers [FC(COL)], and smooth muscle cells (SMCs). **Materials and Methods:** Four aortic cross sections (base, cross, thoracic, abdominal) were collected during autopsy from 90 cases of all ages, processed using the classical histopathological (HP) technique (formalin fixation and paraffin embedding) and stained with Orcein and Goldner's trichrome. The obtained histological slides were transformed into virtual slides. Quantitative measurements of the three components were made on identical regions of interest (ROIs) selected from two-paired slides stained with the above-mentioned techniques using custom-made software, developed in MATLAB (MathWorks, USA). **Results and Discussions:** FE revealed an obvious decreasing general trend with age, present at all four levels of investigation. Smooth muscle fibers (FM) density showed almost no variation with age regardless of the level at which the measurement was made. FC(COL) density had an obvious increasing trend with age, expressed in all four aortic regions. FE densities and FM densities were higher in men than in women, while FC(COL) densities were higher in women than in men in three of the aortic regions excepting, in all cases, the cross region. **Conclusions:** There were differences between men and women concerning the composition of aortic wall cellular and extracellular compartments. FE and FC(COL) dominated the age-related remodeling process of the aortic wall. The process evolved in the same way in all regions of the aorta. Studies need to be continued to define more clearly this complex process of vascular wall remodeling with aging.

Keywords: aorta, ageing, morphology, morphometry.

Introduction

Aorta is the main representative of the group of arteries that transport large volumes of blood from the heart, named elastic arteries. This type of arteries is characterized by a so-called lamellar unit, consisting of concentric ring-like structures made up of smooth muscle cells (SMCs) and thin laminae of elastic fibers (FE) (measuring about 11 μ m in thickness each of them and being at least 50 in the adult life) with a sandwich-like disposal that are tied together by collagen fibers [FC(COL)] with radial orientation [1, 2].

From the main components of any arterial wall, namely

different types of cells [endothelial cells (ECs), vascular smooth muscle cells (VSMCs), fibroblasts and pericytes] and structural extracellular matrix (ECM) components (mucopolysaccharides elastin and collagen), there is a high density of FE relative to SMCs in the media of the aorta [1, 3–5]. These fibers give to aortic wall a high degree of elasticity on one hand, which supports the propulsion of blood downstream to the systemic vasculature, and, on the other hand, a microstructure that aids this function [6–8].

There is an age-related decline in various physiological body processes as result of decades of “wear and tear”.

Aging, together with injuries and diseases, can determine arterial remodeling consisting of structural and functional changes of the vessel wall [3, 9, 10].

Not always chronological age corresponds to biological age, but vascular ageing has consistently been found to be related closely to the chronological age, as Thomas Sydenham stated centuries ago that “A man is as old as his arteries” [3, 10, 11]. In other words, arterial aging is “the cornerstone” of systemic aging and is mainly driven by chronic exposure to low levels of sterile inflammation, known as “proinflammation”, contributing to cellular senescence [12–16].

There are three main key vascular changes occurring with aging [17–20]: (i) generalized endothelial dysfunction; (ii) loss of central arterial distensibility (including aorta) with consecutive stiffness; (iii) a decrease in the cellularity of the *tunica media*.

The decline of arterial elasticity is firstly the result of structural changes consisting of increase in collagen (fibrosis) with a corresponding decrease in elastin content due to its fragmentation and degradation, and the formation of advanced glycation end-products [21, 22].

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 three of the main components of the aortic wall during aging, *i.e.*, SMCs, FE, and FC(COL).

Materials and Methods

We analyzed in our study specimens of aortic tissue obtained from 90 patients deceased in the Hospital who underwent necropsy in the Department of Pathology to establish the final diagnosis of death.

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 was to cover as much as possible all age periods (APs) of life.

The studied material came from two sources:

- patient’s clinical documents (medical charts and autopsy protocols);
- samples of aortic wall tissue collected during the necropsies.

The study had a prospective part in terms of sampling tissue fragments and a retrospective part in terms of collecting data from deceased patient’s documents and from histologically processed tissue specimens and was carried out as a single-center study.

Tissue samples, consisting of aortic rings, were obtained following an aortic artery modified protocol we designed previously [23] consisting of cross sections at four different levels, with no visible lesions of aortic wall, illustrated in Figure 1, namely:

- Slice No. 1 at the ascending aorta level – 2–3 cm above the sinotubular junction, labeled “01 B” (base);
- Slice No. 2 at the aortic arch level – in the left side close proximity of the left subclavian artery, labeled “02 C”;

- Slice No. 3 at the thoracic aorta level – in the inferior segment, above the diaphragm aortic hiatus, labeled “03 T”;
- Slice No. 4 at the abdominal aorta level – between the celiac trunk and superior mesenteric artery, labeled “04 Ab”.

Figure 1 – Sites of sampling. Modified after [23, 24].

The set of assessed parameters were grouped into two main categories, presented in Table 1.

Table 1 – The set of studied parameters

Parameter type	Parameter
Clinical	Sex
	Age
Morphological	The amount of elastic fibers (FE)
	The amount of smooth muscle fibers (FM)
	The amount of collagen fibers [FC(COL)]
	FE/FC(COL) ratio
	FM/FC(COL) ratio
	FE/FM ratio

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

Table 2 – The protocol of staining procedures

Staining type	Description
Hematoxylin–Eosin (HE)	General orientation on the sample
Orcein	Identification of FE
Goldner's trichrome	Identification of FM
	FC

FC: Collagen fibers; FE: Elastic fibers; FM: Smooth muscle fibers.

All histological slides were transformed into virtual slides with a Leica Aperio AT2 scanner, using the $\times 20$ objective.

Acquisition, processing, and morphometric determinations were done using specialized software Aperio

ImageScope [v12.3.2.8013] (Figure 2), and a morphometry module designed in the MATLAB (MathWorks) program.

Figure 2 – Windows of Aperio ImageScope program: (a) Selection of areas for measurement of different fibrillary components of aortic wall: HE (up), Orcein (middle) and Goldner's trichrome (bottom) staining, $\times 0.6$; (b) Detail – areas selected for elastic fibers measurement (left) stained with Orcein and smooth muscle fibers and collagen fibers stained with Goldner's trichrome (right), $\times 20$. HE: Hematoxylin–Eosin.

The algorithm for the quantitative assessment of the histological arterial wall components was the following:

- Four identical regions of interest (ROIs) were selected from each of two paired slides stained with Orcein and Goldner's trichrome techniques using the Aperio ImageScope software (Figure 2, a and b);
- Then, the eight ROIs were assessed using custom-made software, developed in MATLAB (MathWorks, USA);
- For each stain, a color transfer algorithm that we have proposed in a previous paper [25] was applied to have homogeneous stain within the images – since we had no hardware marker, we set the marker to “zero”;
- One image of each dataset was selected, based on an expert's opinion, as the target for all the images to be assessed.

For FE (stained with **Orcein**) percentage estimation, we have used the same background-foreground threshold approach. Additionally, all pixels that had the green and blue channels values higher than the red one were also set to be part of the background.

- In the next step, the images were converted from red, green, blue (RGB) to the hue saturation value (HSV) equivalent color space – all values below a fixed threshold set empirically to .315 were considered background, and the remaining pixels were labeled as Orcein stained;
- The algorithm returned the segmented image and the percentage of each interest class: red percentage, and background percentage (Figure 3).

Figure 3 – Image processing of Orcein staining regions of interest (ROIs): (a) Original image; (b) Pre-processed image; (c) Background selection; (d) Saturation; (e) Resulted percentage of Orcein-stained material (dark blue) – 0.6 (60%).

For the **Goldner's trichrome staining**, we used a threshold algorithm that separated the background (white) from the foreground, and a red-green switch to segment the remaining pixels in red or green based on the dominant value, followed by a color post-processing similar with what we have developed in previously [26].

- The threshold for the background was set as the maximum value of the differences between the average channel value to the minimum and maximum value for each channel.

- If the resulted index exceeded 10 points (empirically established), the pixel was classified as background. The algorithm returned the segmented image and the percentage of each interest class: red percentage, green percentage, and background percentage (Figure 4).

All the numerical obtained values from the computational algorithms were exported in “Excel” module of the Microsoft Office 2019 Professional software suite for the statistical assessment.

Figure 4 – Image processing for Goldner's trichrome staining regions of interest (ROIs): (a) Original image; (b) Pre-processed image; (c) Background selection; (d) Saturation; (e) Resulted percentage of smooth muscle fibers (red stained material) – 0.13 (13%).

For each cross section of each case four values were obtained for each parameter [FE, FM, and FC(COL)]. The average of these four values was calculated and used further as representative for each cross section of the aortic wall.

For each numerical parameter, the lowest value (VMIN), the highest value (VMAX), the mean value (AV), the standard deviation (STDEV) the AV+STDEV and AV-STDEV were calculated.

For age evaluation, the scale presented in Table 3 was used.

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

The set of statistical tools included: *t*-test for two independent samples (two-tailed test), analysis of variance (ANOVA) test for more than two independent samples, and the Pearson's correlation test for measurement of the intensity and direction of the association between the values of two of the studied parameters.

Graphs that illustrated evolutionary trends of the different parameters, the statistical comparisons between them, as well as comparisons with other studies 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.

Results

Clinical profile of the group

The study group of cases included 55 males and 35 females, in other words, almost two thirds of the cases were deceased men.

There were no significant differences between the group of men and the group of women concerning the distribution according to age (Figure 5). In both groups

the mean age was almost the same, lower in men. The age ranges had practically the same amplitude.

The interval including most cases (defined by the STDEV around the AV) had almost the same width as was pushed by AV, in both groups, in the upper part of the age range.

Figure 5 – Age distribution related to sex. AV: Mean value; MAX: Highest value; MIN: Lowest value; STDEV: Standard deviation.

However, there were some differences concerning the distribution by APs (Figure 6).

Figure 6 – Distribution on age periods (APs) related to sex. F: Female; M: Male; WG: Whole group.

Thus, women were more frequently elderly (almost one third of them) while half of the men were mature adults (aged between 44 and 64 years).

Evolution with age of aortic wall components in different aortic regions

Elastic fibers

FE revealed an obvious decreasing general trend with age, validated by the statistic instruments.

The extent of the reduction in FE density was around 10%, starting at around 45% and reaching around 28% of the aortic wall area.

This trend was present at all four levels of investigation of the aortic wall (base, cross, thoracic segment, and abdominal segment) and there was no significant difference between the way FE density decreased in the four different

parts of the aortic wall. These individual decreases with age were also validated from statistical point of view (Figure 7 – left side).

Smooth muscle cells

Density of SMCs showed almost no variation with age regardless of the level at which the measurement was made (Figure 7 – middle). However, if we take into consideration the values even if very small but negative of the correlation matrix and if we look closely to the trend lines, we could speculate that a very discrete decreasing trend of the SMC population with age is possible.

Elastic Fibres	Correlation matrix	p-value	Coefficients of determination (R2)
V - 01_B	-0.6353	< 0.0001	0.4037
V - 02_C	-0.6008	< 0.0001	0.3610
V - 03_T	-0.6232	< 0.0001	0.3884
V - 04_Ab	-0.5505	< 0.0001	0.3030

Muscle Fibres	Correlation matrix	p-value	Coefficients of determination (R2)
V - 01_B	-0.0399	0.7087	0.0016
V - 02_C	-0.1205	0.2579	0.0145
V - 03_T	-0.0881	0.4091	0.0078
V - 04_Ab	-0.0289	0.7868	0.0008

Collagen Fibres	Correlation matrix	p-value	Coefficients of determination (R2)
V - 01_B	0.4008	< 0.0001	0.1607
V - 02_C	0.4107	< 0.0001	0.1687
V - 03_T	0.4360	< 0.0001	0.1901
V - 04_Ab	0.2961	0.0046	0.0877

Figure 7 – Correlation between different types of fibers and age at the four aortic topographical levels. 01_B: Cross section at the aortic base level; 02_C: Cross section at the aortic cross level; 03_T: Cross section at the thoracic aorta level; 04_Ab: Cross section at the abdominal aorta level; COL: Collagen fibers; FE: Elastic fibers; FM: Smooth muscle fibers; V: Age.

Collagen fibers

In turn, as expected, the FC(COL) density had an obvious increasing trend with age, expressed in all four aortic regions and validated from statistical point of view.

The extent of this expansion of FE density was around 30%, starting at around 20% and reaching around 50% of the aortic wall area (Figure 7 – right).

Correlation with sex of aortic wall components in different aortic regions

Comparative analysis between men and women of the average values of the densities of the three types of components of the aortic wall calculated for each of the four aortic segments revealed some interesting observations (Figure 8).

FE densities and FM densities were higher in men than in women in three of the four aortic regions. The exception was the cross segment where FE and FM densities were slightly higher in women than in men. FC densities in turn, revealed a total opposite situation meaning that FC densities were higher in women than in men in three of the aortic regions excepting the same cross region where FC(COL) were denser in men than in women.

Figure 8 – Correlation between different types of fibers and sex at the four aortic topographical levels. 1_B: Cross section at the aortic base level; 2_C: Cross section at the aortic cross level; 3_T: Cross section at the thoracic aorta level; 4_Ab: Cross section at the abdominal aorta level; F: Female; FC: Collagen fibers; FE: Elastic fibers; FM: Smooth muscle fibers; M: Male.

FC densities had smoother general ascending trends in both men and women but with fluctuations in the supradiaphragmatic region where they recorded a significant decrease in women, followed by a continuous increase towards abdominal region, coupled with an obvious increase in men in the cross region, over the value registered in women, and followed by a decrease in the thoracic region, under the start value from the base region.

Evolution of aortic wall components by age periods in different aortic regions

The next step in our analysis was the assessment of the evolution over time of the density of each component of the aortic wall in each of the four aortic regions.

Elastic fibers

At the aortic base level, FE registered highest values in the young adult period (46%), values that slightly decreased till 40% in the elderly. The lowest values started from 38% in adolescence and significantly decreased till 28% in the elderly. Ranges of variation of density values became twice as large as deceased people got older. Intervals gathering most values (defined by the STDEV around AVs) were narrower but also had the tendency to enlarge from younger to older ages. Finally, mean values of FE density presented a descending trend with age, smoother at the beginning but evident after, with a density decrease of 8% from adolescence to old age. This evolution was validated also by the statistical instruments (Figure 9 – upper left graphs and table).

At the cross level, the situation was almost similar to that at the base level. Highest values varied between 46% and 40%. Lowest values varied between 37% and

Another observation concerned the pattern of density distribution along the aortic regions.

Thus, the density of FE had a decreasing trend from the aortic base to the abdominal region smoothed in the supradiaphragmatic regions in men whereas, in women, although the trend was the same, it registered a peak in the cross region, peak that had higher value than that registered in men in the same region.

FM density had, on the contrary, a general ascending trend in men but with peaks higher at the base and thoracic region than at cross region and abdominal region, respectively. In women, the trend was also ascending but smoother and with a flat look in the supradiaphragmatic regions and a discrete descent in the subdiaphragmatic region.

29%. However, ranges of variation of density values had a smoother enlargement with age and intervals gathering most values (defined by the STDEV around AVs) were more similar from one period to another. Finally, mean values varied in the same range with the same value of 8% and in the same way, with a gentler slope at the beginning and steeper after toward the old age. Statistical instruments (Pearson's correlation test) also confirmed this evolution (Figure 9 – upper right graphs and table).

At the thoracic level, the highest values varied between 47%, registered in young adults and again 40% in elderly. Lowest values varied between 39% and 26%, the lowest value registered in all cases. Ranges of variation of density values became in this region again twice as large as deceased people got older while intervals gathering most values (defined by the STDEV around AVs) were narrower but quite stable, with only a discrete tendency to enlarge from younger to older ages. In turn, mean values of FE density presented a continuous descending trend with age, with a density decrease of 9% from adolescence to old age. This evolution was validated also by the statistical instruments (Figure 9 – lower left graphs and table).

At the abdominal level, the highest values registered the biggest value of all cases in adolescent group (49%) and decreased till 40% in the elderly.

The lowest values ranged in a narrower interval, from 36% in adolescence to 28% in elderly.

Intervals gathering most values (defined by the STDEV around AVs) were larger in younger ages but narrower and constant after. Mean values of FE density presented a continuous descending trend with age, with a density decrease of 8% from adolescence to old age.

This evolution was also validated by the statistical instruments (Figure 9 – lower right graphs and table).

Figure 9 – Distribution with age of whole values and mean values of elastic fibers (FE) percentages at the four aortic topographical levels. 01_B: Cross section at the aortic base level; 02_C: Cross section at the aortic cross level; 03_T: Cross section at the thoracic aorta level; 04_Ab: Cross section at the abdominal aorta level; AP: Age period.

Smooth muscle cells

At the aortic base level, FM registered highest values of 21% in adolescence and rose to 35% in mature adults. Lowest values went down till 9% in adulthood and rose till 14% in adolescence. These so dispersed limits defined ranges of variation of density values from very narrow in adolescence till very wide in mature adults and elderly. However, intervals gathering most values (defined by the STDEV around AVs) were much more reduced than ranges of variation, excepting the adolescence period and were placed toward their lower limit. Finally, mean values of FM density presented a general descending trend with age, but with an obvious reduction from adolescence to adulthood, followed by a stationary evolution till old ages. The overall density decrease was of only 3%. This evolution was confirmed by the statistical instruments (Figure 10 – upper left graphs and table).

At the cross level, FM expressed almost the same picture, with highest values ranging between 21% in young adults and 36% in mature adults, with lowest values varying between 9% in mature adults and 15% in adolescence, with narrow ranges of variation in the first two APs and wide ranges in the rest, with the same pattern of extension and distribution of intervals gathering the majority of values (defined by the STDEV around AVs) and the same pattern of evolution of the mean values like at the aortic base level. Therefore, this evolution too was confirmed by the statistical instruments (Figure 10 – upper right graphs and table).

At the thoracic level, FM density highest values ranged between 20% in young adults and 32% in mature adults and elderly, lowest values varied between 8% in elderly and 12% in adolescence. Ranges of variation were narrow in the first two APs and considerably wider in the rest. very wide in mature adults and elderly. Intervals gathering most values (defined by the STDEV around AVs) were much more reduced than ranges of variation, excepting the adolescence period and were placed toward their lower limit. Finally, mean values of FM density presented a general descending trend with age, with an obvious reduction from adolescence to adulthood, like previously but with a “down-up-down” fluctuation in the next three APs. Statistical instruments (Pearson’s correlation test) confirmed this evolution (Figure 10 – lower left graphs and table).

At the abdominal level, the situation was almost similar to that at the thoracic level. Highest values varied between 24% and 34%. Lowest values varied between 9% and 14%. Ranges of variation were narrow in the first two APs and considerably wider in the next two. Intervals gathering most values (defined by the STDEV around AVs) followed the same pattern of extension and distribution within ranges of variation and, finally, mean values of FM density revealed the same pattern of decreasing, with fluctuations between the second and the fourth APs. Statistical instruments (Pearson’s correlation test) confirmed this evolution too (Figure 10 – lower right graphs and table).

Collagen fibers

FC(COL) behaved in the opposite way to FE with age.

At the aortic base level, FC registered highest values of 36% in the young adult period values that constantly increased till 51% in the elderly. The lowest values varied from 13% in adolescence and increased till 24% in the elderly. Ranges of variation of density values enlarged starting from the second AP. Intervals gathering most values (defined by the STDEV around AVs) were narrower but somehow constant in extension and placed toward the upper limits of the ranges of variation. Finally, mean values of FC density presented a clear ascending trend with age, more flattened in the adulthood, with a density increase of 14% from adolescence to old age. This evolution was validated also by the statistical instruments (Figure 11 – upper left graphs and table).

At the cross level, FC expressed almost the same picture, with highest values ranging between 35% in adolescence and 49% in mature adults, with lowest values varying between 16% in mature adults and 31% in young adults, with narrow ranges of variation in the first two APs and wide ranges in the remaining two APs and with the placement of intervals gathering the majority of values (defined by the STDEV around AVs) toward the upper limits of the ranges of variation in the last two APs. Mean values of FC density presented the same pattern of evolution as at the aortic base level, with a density increase of 15% from adolescence to old age. The evolution was validated by the statistical instruments (Figure 11 – upper right graphs and table).

At the thoracic level, FC density highest values ranged between 37% in adolescence, and 52% in elderly, lowest values varied between 16% also in adolescence and 30% in young adults. Ranges of variation were large and widened with age, excepting the range in young adults which was twice narrow than in elderly. Intervals gathering most values (defined by the STDEV around AVs) were more reduced than ranges of variation, excepting the adolescence period and were placed somehow toward their middle. Finally, mean values of FC density presented, like previously, a clear ascending trend with age, more flattened in the adulthood, with a density increase of 16% from adolescence to old age. This evolution was validated by the statistical instruments (Figure 11 – lower left graphs and table).

At the abdominal level, the situation was almost similar to that at the thoracic level. Highest values varied more restricted, between 45% and 52%. Lowest values varied slightly, between 20% and 28%. Ranges of variation were large and widened with age, excepting the range in young adults which was narrower than the others. Intervals gathering most values were more reduced than ranges of variation, excepting the young adult period and were placed somehow toward their upper limit. Finally, mean values of FC density presented, like previously, a clear ascending trend with age, but with a “gap” in the mature adult period. Density increased overall with 14% from adolescence to old age. This evolution was validated by the statistical instruments (Figure 11 – lower right graphs and table).

Figure 10 – Distribution with age of whole values and mean values of smooth muscle fibers (FM) percentages at the four aortic topographical levels. 01_B: Cross section at the aortic base level; 02_C: Cross section at the aortic cross level; 03_T: Cross section at the thoracic aorta level; 04_Ab: Cross section at the abdominal aorta level; AP: Age period.

Figure 11 – Distribution with age of whole values and mean values of collagen fibers (FC) percentages at the four aortic topographical levels. 01_B: Cross section at the aortic base level; 02_C: Cross section at the aortic cross level; 03_T: Cross section at the thoracic aorta level; 04_Ab: Cross section at the abdominal aorta level; AP: Age period.

Evolution with age of different relations between aortic wall components in different aortic regions

FE/COL ratio

One of the most important and therefore studied relationships connected with the aging phenomenon is the ratio between the amount of FE and FC(COL). Their ratio is the main expression of the morphological changes that are appearing in the aortic wall during aging.

The analysis of this ratio evolution with age in different

regions of the aorta revealed that everywhere along the aortic wall its value is greater than “1” at younger ages. However, after the age of 45 years more cases revealed a shift of ratio's value towards values smaller than “1” so that most of the old people had such kind of ratio values. This meant that the amount of FC(COL) increased with age, and, at the same time, the amount of FE decreased. This inverse correlation between the FE/COL value and the person's age was proved to be very highly significant by the statistical Pearson's correlation test in all regions of the aortic wall (Figure 12).

Figure 12 – Correlation with age of the FE/COL ratio at the four aortic topographical levels. 01_B: Cross section at the aortic base level; 02_C: Cross section at the aortic cross level; 03_T: Cross section at the thoracic aorta level; 04_Ab: Cross section at the abdominal aorta level; COL: Collagen fibers; FE: Elastic fibers; V: Age.

FM/COL ratio

The ratio between the amount of SMCs and the amount of FC(COL) was, in turn, smaller than “1” in any region of the aortic wall and tended to decrease with age more clearly expressed in the supradiaphragmatic regions and especially in the thoracic region. This trend was probably caused, on one hand, by the increase of the amount of FC(COL) described just before, and, on the other hand by the decreasing trend of SMCs also described previously (Figure 13).

FE/FM ratio

The ratio between the amount of FE and the amount of SMCs had a more discrete tendency of decreasing with age, illustrated by its gentler slope in all four regions of the aortic wall (Figure 14).

This was probably the result of the fact that both components of the aortic wall were proved previously to decrease with age, but FE demonstrated a more obvious trend, thus reducing the fraction numerator.

Discussions

The elastic properties and strength of the aorta are determined by the main fibrillary structures of its wall, elastin, and collagen whose quantity and/or architecture alterations result in mechanical, and functional impairments of the aortic wall [27–37].

These impairments are consisting basically of:

- The onset and development of large artery stiffness [38];
- Disturbance of endothelium-dependent dilation [39–41].

Figure 13 – Correlation with age of the FM/COL ratio at the four aortic topographical levels. 01_B: Cross section at the aortic base level; 02_C: Cross section at the aortic cross level; 03_T: Cross section at the thoracic aorta level; 04_Ab: Cross section at the abdominal aorta level; COL: Collagen fibers; FM: Smooth muscle fibers; V: Age.

Figure 14 – Correlation with age of the FE/FM ratio at the four aortic topographical levels. 01_B: Cross section at the aortic base level; 02_C: Cross section at the aortic cross level; 03_T: Cross section at the thoracic aorta level; 04_Ab: Cross section at the abdominal aorta level; FE: Elastic fibers; FM: Smooth muscle fibers; V: Age.

Arterial wall alterations are more pronounced in men until the sixth decade. After that, arterial stiffness and endothelial dysfunction are more pronounced and progresses at a faster rate in old women [42–46]. Elastic arteries, and particularly aorta, are the main candidates for age-related stiffening and especially in people aged over 60 years. This stiffening is the result of concomitant increasing in the amount of FC(COL) and decreasing in the amount of FE [17, 47–50].

Elastic fibers

Our study showed that the amount of FE in the aortic wall was clearly decreasing in general along the aortic regions from the aortic base to the abdominal region. This pattern of distribution was the same in men and in women. One should notice also that men revealed in general a larger amount of FE than women in all aortic regions excepting aortic cross region which proved to be richer in FE in women than in men.

Another observation was that FE amount was decreasing as individuals were getting older. This phenomenon was continuous and constant, with no fluctuation throughout life and affected equally any of the anatomical aortic segments. The magnitude of this decrease proved to be also almost the same in all aortic regions, of around 8% excepting the thoracic segment where it was slightly higher (9%).

Collagen fibers

The amount of FC(COL), in turn, was obviously increasing in general along the aortic length, general pattern observed in both sexes, less pronounced in women. Unlike FE, FC(COL) were, overall, more numerous in women than in men. There were, however, some striking differences between sexes. FC(COL) were more reduced in aortic cross region in men than in the neighboring regions and, moreover, than in thoracic region in women. In women, we observed also a clear fluctuation consisting of a remarkable diminution of FC(COL) amount in the thoracic region as compared with neighboring regions even lower than in the base region.

Another observation was that FC(COL) amount was increasing as individuals get older. The phenomenon was also continuous and constant but with a flattening during adulthood and a return of the upward trend towards old age, affected equally any of the anatomical aortic segments. All anatomical aortic regions were equally affected with almost the same magnitude of around 14% excepting the thoracic segment where it was slightly higher (16%).

Smooth muscle cells

Ageing is also accompanied by a decrease in the cellularity of the vascular *tunica media*, especially the VSMCs although the causes of this finding remain to be established. This process proved to be strongly correlated with both vascular stiffening and the arterial remodeling processes meaning that remaining VSMCs are embedded in a collagen-enriched ECM with fewer cellular focal adhesions [20, 51–54].

In our study, the amount of SMCs was also increasing in general but slowly and also less pronounced in women. Like FE, SMCs were, overall, more numerous in men than in women and had in both sexes a fluctuating evolution along the aortic length. There were, however, some clear differences between sexes. In aortic cross area of men FM revealed a “gap”, their amount being also lower than in the same area in women. Then, in men, FM reached a peak in the thoracic area and reduced again in the abdominal region but to a higher amount than at the aortic origin. In women, in turn, the amount of FM increased slowly, reached a flattened level in thoracic area, to decrease again slowly toward the abdominal region.

The amount of FM too decreased as individuals were getting older, like FE but in a but in a gentler way, with a flattening starting with adulthood in the first two segments and even with a “gap” in the young adulthood in the last two segments. The magnitude of this decrease proved to be almost the same in all aortic regions, of around 4% excepting the aortic cross segment where it was slightly higher (5%).

Ratios between the three types of aortic wall components

Calculation of ratios between the amounts of different aortic wall components proved to be useful for a better assessment of vascular wall remodeling. For instance, the inversion of the elastin/collagen ratio together with the nonenzymatic glycosylation of some proteins present in the arterial wall, such as collagen, capable of crosslinking adjacent proteins were closely related with the increase of the mechanical vessel rigidity and stiffness [17, 55–59] or elastin to VSMCs ratio proved to vary within arterial vessels [60].

Therefore, we calculated three ratios using the values of the three aortic wall components: FE/FC(COL), FM/FC(COL) and FE/FM. From all these three ratios, the one between FE and COL is the most representative for the remodeling process of the arterial wall because points out the evolution of both elements of the fraction in relation with age: FE – the numerator – is obviously decreasing with age and COL – the denominator – is obviously increasing with age, thus both contributing to the evident decrease of fraction value and the shifting from values >1 to values <1 .

The evolving trend with age of the second fraction – FM/COL – was not so clear and therefore most often not validate by the statistical instruments because the evident increasing trend of the FC(COL) amount was somehow balanced by the gentler decreasing trend of the SMCs amount. The ratio value was always <1 because of the small amount of SMCs and increasing amount of FC(COL).

Finally, the third fraction – FE/FM – was always >1 because of the amount of FE, even if on a downward trend. The gentle decreasing trend of the slope pointed out that even both elements of the fraction were decreasing with age, the FE amount decreasing was more pronounced and, thus, maintaining a fraction value smaller than <1 .

✉ Conclusions

There were differences between men and women concerning the composition of aortic wall cellular and extracellular compartments. While FE and SMCs were more pronounced in men than in women, FC(COL) were more pronounced in women. FE and FC(COL) dominated the age-related remodeling process of the aortic wall, the former by its continuous and constant decreasing and the latter by its also continuous and constant increasing with age. Their clear influence on the aging process was confirmed also by the evolution of their ratio. SMCs had a more discrete participation in this continuous transformation of the aortic wall related to aging. The remodeling process proved to evolve following the same pattern and with the same intensity in all regions of the aorta. However, the cross region exhibited some particularities. Both FE decreasing and FC increasing were more pronounced than in the other aortic regions. Then, there was a shift of the amounts of FE and FC between men and women from the general rule, meaning that FE were found more numerous in women while FC were found more numerous in men. Nevertheless, studies need to be continued by including new morphological parameters and compare their evolution with age with that of the parameters already assessed to define more clearly this complex process of vascular wall remodeling with aging.

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.

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