”CAROL DAVILA” UNIVERSITY OF MEDICINE

AND PHARMACY BUCHAREST

DOCTORAL SCHOOL

GENERAL MEDICINE

THE STUDY OF MORPHOLOGICAL CHANGES OF THE AORTIC WALL IN THE AGING PROCESS

Phd THESIS

ABSTRACT

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KEY WORDS

Aorta, Aging, Morphology, aortic wall, Remodeling, Morphometry.

CURRENT STAGE OF KNOWLEDGE

INTRODUCTION

The increase in life expectancy in recent decades due to improved living and working conditions has led to a paradox, namely the increase and diversification of morbidity by adding a whole family of pathological conditions that appear with age and are specific to the IIIrd age. The explanation could be that the morphological structures of the human body are subject to not infrequently harmful interactions with the environment for a longer period of time, which leads to an increase in the degree of wear and, consequently, their functional availability.

The various physiological processes of the human body suffer a decline with and related to aging, as a result of decades of wear and tear. Aging, along with the diseases and their subsequent lesions can lead to a process of arterial remodeling that consists of structural and functional changes in the vascular wall. [Gallagher and van der Wal 2019; Jaminon et al 2019; Jani et al 2006].

The aortic artery is among the most susceptible to injury regions of the vascular tree as we age [Stary et al 1992; Nakashima et al 2002; Hansson 2005].

Starting from the assertion of Professor Mingyi Wang and his collaborators that I quote: "Arterial aging is the cornerstone of systemic aging" [Wang M et al 2018], I decided to deepen my knowledge about this pathology.

MORPHOLOGY OF AORTA

The embryonic development of the vessels and especially of the arterial system begins at 20-22 days of age. It has a segmental and symmetrical character and is produced by two mechanisms, under the control of vascular endothelial growth factor (VEGF) and other growth factors [Bareliuc et Neagu 1977; Sadler 2006; Etesami et al 2014]: Vasculogenesis and Angiogenesis. The lumen of vascular primordia appears as they fuse to form wider channels [Waldo et Kirby 1998]. The muscle cells of the medial tunic derive from the cells of the neural crests, forming the mesenchymal cells of the dorsal aorta [Hungerford et Little 1999; Suri et Yancopolous 1999; Kuo et al 1997]. The transformation period of endothelial canal networks can be divided temporarily and spatially into two phases. [Johnson 2005]: Pharyngeal Phase and Post-Pharyngeal Phase. In relation to the embryonic sketch, the Aortic Artery develops, starting from the aortic sac, as follows: (a) The base of the aorta - through the compartmentalization of the cardiac bulb and the arterial trunk by the spiral septum, around the 5th week; (b) The ascending proximal portion (right) of the Aortic Arch - from the right horn of the Ventral Aorta; The left part of the aortic arch - through the persistence of the 4th embryonic aortic arch; (d) Descending aorta with the Thoracic and Abdominal segments - from the fusion of the two embryonic dorsal aorta, below the level of the 7th intersegmental artery. [Bareliuc et Neagu 1977; Skandalakis 2004; Johnson 2005; Etesami et al 2014; Rosen et Bordoni 2022].

Macroscopic morphology. Arteries are the efferent blood vessels that carry blood from the heart to the capillary bed. Arteries are classified according to their relative dimensions and their morphological characteristics into three broad categories: Transport Arteries; Distribution Arteries and Arterioles. [Lindberg et Lamps 2018; Junqueira et Carneiro 2007; Gartner et Hiatt 2007; Pakurar et Bigbee 2004]. The aortic artery is the main representative of the group of arteries that carry large volumes of oxygenated blood from the heart to the systemic circulation, called elastic arteries that have a high level of elasticity. It can be divided, from a topographic point of view, in relation to the diaphragm, into two large segments, each being then subdivided into several segments: Thoracic Aorta (Ascending Aorta; Aortic Arch / Aortic Cross; Descending Thoracic Aorta) and Abdominal Aorta (Supra Celiac Segment, Supra Renal Segment, Infrarenal Segment) [Rosen and Bordoni 2022; Shahoud et al 2022; Kelley et al 2022; Fortier et al 2014; Tsamis et al 2013; Holzapfel et al 2007; Laurent et al 2005; Westerhof et al 2005; Skandalakis 2004; Gartner et Hiatt 2007; Halloran et al 1995].

Microscopic morphology. Arterial vascular structures are divided according to the main morphological component in [Gartner et Hiatt 2007]: Elastic vessels (are transport arteries) and Muscular vessels (are distribution vessels), which, depending on caliber are of two kinds: Arteries and Arterioles.

The walls of the entire cardiovascular system have, over a certain diameter, the same structural plane, comprising three layers or concentric tunics that are continuous from the heart to the vessels.

The thickness and structural constituents of these layers vary depending on the mechanical and metabolic functions of each segment of the cardiovascular system. These are, from the lumen to the outside: the Inner Tunic, called the Endocard at the Heart, and the Intima Tunic at the Vessels; The middle tunic, called the Myocardium at the heart and the Media tunic at the vessels; The outer tunic, called the Epicard (visceral pericardium) at the heart and the Adventicea tunic at the vessels [Junqueira et Carneiro 2007; Pakurar et Bigbee 2004].

There are two main categories of components of any arterial wall, namely: Different types of cells (Endothelial Cells (ECs); Smooth Vascular Muscle Cells (VSMCs); Fibroblasts; Pericites) and Structural Components of the Extracellular Matrix (ECMs) (Elastin Fibers; Collagen Fibers; Mucopolysaccharides). These constitutive morphological elements are present in different proportions in different regions of the vascular walls with the exception of capillaries that have a simple wall formed by endothelial cells, basement membrane and pericytes. [Kentish 2008; Junqueira et Carneiro 2007].

AGING OF VASCULAR STRUCTURES

Introduction. Aging has direct effects on the structure and thus the function of the vascular tree [Thijssen et al 2016]. Vascular aging is closely linked to chronological age, as Thomas Sydenham said centuries ago, “man is as old as his arteries” [Gallagher and van der Wal 2019; Jani et al 2006; Garrison 1928], in other words, arterial aging is the cornerstone of systemic aging [Wang M et al 2018]..

Mechanisms of aging. Among the mechanisms by which the structures of the human body age that would contribute to the aging of vascular structures are: Oxidative and nitric stress; Disruption of mitochondrial functions; Vascular inflammation; Poor adaptation to molecular stress; Loss of proteostasis; Genomic instability; Cellular senescence; Increased apoptosis and necroptosis and epigenetic alterations [Ungvari et al 2018].

Aortic changes induced by aging. Any of the segments of the aortic artery can undergo different types and degrees of remodeling during the aging process. The algorithm for the occurrence of structural changes induced by aging is "bottom-up" [Van de Geest et al 2004; Tsamis et al 2013]. *The length and thickness* of the aorta increase progressively with age, with variations and differences from one decade of age to another and between the two sexes, the processes continuing into old age [Gallagher PJ and van der Wal 2019]. *Thickness of the Intima* increases with age, with its subendothelial layer also varying with age [Gasser et al 2006]. *The thicknesses of the Intima and Adventicea* tunics increase along the Aortic path, while *the thickness of the Media tunic* decreases along the Aortic path [Tsamis et al 2013].

By old age, the *concentration of FE* in the aortic wall decreases due, in part, to the increase in the concentration of other components such as FCOL but the *FE content* remains unchanged. [Sans et Moragas 1993; Greenberg 1986; Schlatmann et Becker 1977; Faber et Oller-Hou 1952; Lansing et al 1950; Myers et Lang 1946; Hass 1942]. However, the FE content and concentration do not appear to vary significantly depending on the location on the circumference of the aorta [Cattell 1993]. *The content of collagen fibers* in the aortic wall increases with age, but the increase is not recorded in the Intimate [Sans et Moragas 1993; Greenberg 1986; Schlatmann et Becker 1977; Smith 1965; Clausen 1962; Kanabrocki et al 1960]. *FCOL as a whole* (both type I and type III fibers) have an uneven distribution from the proximal to the distal end of the aorta. [Maurel et al 1987].

PERSONAL CONTRIBUTION

Working hypothesis. The working hypothesis was the evaluation of the influence that aging can have on the morphology of the wall of the main structure of the arterial vascular tree, namely the aortic artery.

The main objectives. The study has two main objectives: to evaluate the size of the main layers of the aortic wall and the average diameter of the aortic artery and to evaluate the density of the main morphological elements of the aortic wall. The sets of parameters established were evaluated in relation to the Age of the cases and in relation to the Sex of the cases.

MATERIALS AND METHODS

The basis of the study for this paper was a group of 90 deceased patients who underwent necropsy to establish the final diagnosis. The study was conducted in accordance with the Standards of Ethics set by the Helsinki Declaration. All relatives of the deceased patients signed an informed consent for the autopsy.

The initial study group was subsequently divided into several sublots according to gender; Age and Topographic Region of the Aorta.

The study material was represented by two categories of data sources: Autopsy protocols and Human biological material harvested from the corpse represented by aortic wall fragments taken from different topographic levels (Section No. 1 - at the level of the ascending aortic region, labeled “01 B”, Section No. 2 - at the level of the aortic arch, labeled “02 C”, Section No. 3 - at the level of the thoracic region of the aorta, labeled “03 T” and Section No. 4 - at the level of the abdominal region, labeled “04 Ab”.

Type of study. The study was a unicenter study and had a prospective component in terms of sampling the study material and a retrospective component in terms of collecting data from the medical records of deceased patients.

The evaluated parameters were divided into two categories: Clinical Parameters (Gender and Age) and Morphological Parameters (Aortic Wall Thickness of the Intima Tunic; Aortic Wall Thickness of the Media Tunic, Aortic Diameter, Elastic Fiber Density (FE) at the Media Tunic of the aortic wall, Collagen Fiber Density (FCOL) at the Media Tunic of the aortic wall, Smooth Muscle Cell (FM) Density at the Aortic Wall Media Tunic, FE/FCOL Ratio, FE/FM Ratio, and FCOL/FM Ratio).

Investigation techniques used. The measurement of aortic diameters was performed on calibrated photographs of aortic wall rings fixed using specially built software using the MATLAB programming environment (MathWorks, USA). The determination of the other parameters was performed on virtual slides colored with Orcein and Tricromic Goldner using two dedicated programs: Aperio ImageScope [v12.3.2.8013] and another special morphometry module built using the MATLAB programming environment (MathWorks, USA).

Data processing and interpretation. Preliminary data processing from the cases entered in the database was performed using the Microsoft Excel module from the Microsoft Office 2019 professional software package and the XLSTAT 2014 "add in" program for the "Excel" module, trial version. The calculated statistical indicators were: Lowest value (VMIN); Highest value (VMAX), Average value (VMEDIE) and Standard deviation (STDEV). The statistical evaluation was performed using the following tests: The "t" test (Two-Sample Assuming Equal Variances) and the "Pearson" correlation test, the "chi square- $\chi 2$" correlation test.

DISCUSSIONS

THE EVOLUTION OF THE AORTIC WALL LAYERS WITH AGE

Intima tunic. The general trend of the values of the average thickness of the Intima tunic 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 and an increasing one from the young ages to the 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 the level of each topographic segment.

Media tunic. The general trend for the average thickness of the Media tunci values 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 and an inverse one, compared to the thickness of the Intima tunic, i.e. it was increasing from young ages to advanced ages in each of the four topographic segments of the aorta but with some notable differences between the ways in which this increase was achieved at the level of each topographic segment

THE EVOLUTION OF THE AORTIC DIAMETER WITH AGE

The general trend of mean aortic diameter (D_Ao) values was decreasing from the base of the aorta to the abdominal region in each of the four main life periods, with an almost identical pattern of evolution in each of the four life periods and was increasing from young ages to advanceds age in each of the four topographic segments of the aorta with minimal differences between the ways in which this growth was achieved at the level of each topographic segment.

THE EVOLUTION WITH AGE OF THE COMPONENT ELEMENTS OF THE AORTIC WALL

Elastic fibers. The general trend of evolution of the mean density values of FE showed different ways of variation along the aorta in each of the life periods. Thus, during Childhood and Adolescence (PA 1), the mean value of mean FE density did not exceed 43% and was not less than 41%, showing a discrete and oscillating growth trend from the proximal extremity to the distal extremity of the aorta, with an insignificant difference between the highest and lowest average values of only 1%. In the other three periods of life, on the other hand, the general trend of the evolution for the average values of the FE density was reversed, becoming more or less obvious a descending one but with some oscillations at the level of each of the periods of life. Throughout life, however, the trend was the same at the level of each of the four main topographic segments of the vessel, namely an obvious and constantly decreasing.

Collagen fibers. The general trend of evolution of the average density values of FCOL was a discreetly increasing one but with small oscillations along the different topographic segments of the aorta in each of the life periods but, throughout life, it was the same at the level of each of the four main topographic segments of the vessel, namely an obviously and constantly increasing one but with oscillations.

Muscle fibers. The general trend of evolution of the mean density values of FM showed different ways of variation along the aorta in each of the life periods. Thus, in the periods of Childhood and Adolescence (PA 1) and that of the Mature Adult (PA 3), the average value of the average density of FM did not exceed 20% and was not less than 18%, showing an oscillating growth trend from the proximal extremity to the distal extremity of the aorta. During the Young Adult and Senescence periods, the general trend of evolution of the mean FM density values along the different topographic regions of the aorta was different from that observed in the other two life periods, namely it was a discrete decreasing one, with oscillations, from the proximal to the distal end of the aorta. The general trend of evolution for the values of the average density of FM over the lifetime was the same at the level of each of the four main topographic segments of the vessel, namely one obviously but oscillating decreasing.

THE EVOLUTION OF AORTIC WALL LAYERS BY SEX

The average thickness of the Intima tunic showed a constant increase from the origin of the aorta to the level of the abdominal region in both men and women, the values being obviously higher in men than in women throughout the anatomical trajectory of the aortic artery. It has grown steadily throughout life, both in men and women, being, with the exception of Childhood and Adolescence, higher in men than in women.

The average thickness of the Media tunic, on the other hand, showed a constant decrease from the origin of the aorta to the level of the abdominal region, the decreasing trend being observed in both men and women, and, with one exception, the values being higher at women than men. Throughout life, however, the average thickness of the tunic Media has increased steadily in both men and women, being higher in men in the first periods of life and higher in women in the last periods of life.

EVOLUTION OF D_AO BY SEX

The average diameter of the aorta also recorded a constant decrease from one topographic region to another, from proximal to distal in both sexes, the values being obviously higher in men than in women in all topographic regions of the aorta. Throughout life, however, the average diameter of the aorta increases steadily in both sexes, with the average values recorded in men being higher than those recorded in women in each of the four periods of life.

EVOLUTION OF THE AORTIC WALL COMPONENTS BY SEX

The average density of FE generally decreased along the topographic regions of the aorta, from the base to the abdominal region in both men and women, being higher in men than in women, except for the cross, where the ratio is reversed. This decreasing trend was also observed during the periods of life in both men and women, being higher in men in the beginning and end periods of life and higher in women in the active life periods.

The average density of FCOL generally increased along the topographic regions of the aorta, from the base to the abdominal region in both men and women. In general, the average density values of FCOL were higher in women than in men except for the cross where the ratio was reversed. In contrast, the average density of FCOL in both sexes increased steadily from childhood to senescence, with higher values in women in the first half of life and higher values in men in the second half of life.

The average density of FM also followed an upward general trend along the topographic regions of the aorta, from the base to the abdominal region in both men and women, with generally higher values in men than in women, except for the cross area. where the ratio was reversed. With age, however, the average density of FM showed a general downward trend in both sexes, with higher values in men in the first half of life and higher in women in the second half of life.

CONCLUSIONS

Our study, comprising both an individual and a comparative analysis of the aortic wall layers and aortic diameter as well as the main morphologic components of the aortic wall, led to several conclusions that may have importance and applicability in medical practice:

The average thicknesses of the main component layers of the aortic wall presented dynamic evolutionary profiles both in relation to the topographic regions of the aorta and with advancing age and in relation to sex. Thus:

- The Intima Tunic underwent a process of thickening both from the base of the aorta to the terminal (abdominal) region and from youth to senescence. Evolutionary patterns were similar in both sexes, and the thickening process was almost always more pronounced in men than in women.
- The Media Tunic was the site of a different remodelling in relation to the topographic regions of the aorta than in relation to aging. Thus, if, from the base of the aorta to the terminal (abdominal) region, the middle tunic underwent a process of reducing the thickness, with age, the thickness of the tunic increased.

  This divergent profile of evolution was similar in both sexes, stating that, compared to the topographic regions of the aorta, the values of the average thickness of the tunic were generally more pronounced in women, whereas, compared to aging, the average thickness of the tunic was higher in men compared to women in the first periods of life, while, after 45 years, the thickness of the tunic became greater in women.
- The evolution of the average thickness of the two tunics of the aortic wall was a parallel one for both men and women.

The average diameter of the aorta was the site of a divergent remodeling process in relation to the two reference landmarks: topography and age, similar to that observed in the Media tunic. Thus, the dimensions of the diameter decreased constantly from proximal to distal but increased constantly from youth to senescence.

This divergent profile was observed in both sexes, the diameter size values being always higher in men than in women, and throughout the aorta and throughout life.

The average densities of the main morphological components of the aortic wall also presented dynamic evolutionary profiles both in relation to the two reference landmarks - topography and age - but also in relation to sex. Thus:

- The average density of Elastic Fibers at the level of the Media tunic has been in a continuous process of reduction both from the base of the aorta to the abdominal region and with age.

  The evolutionary patterns presented small peculiarities in relation to sex. Thus, the mean density of FE was generally higher in men compared to women along the aortic path except for the area of the aortic cross where the values were reversed while, throughout life, the mean densities of FE did not differ.

significantly between the sexes, being slightly higher in men compared to women in Childhood and Adolescence and Senescence and slightly higher in women compared to men in the periods of active life of the Adult

- The average density of Collagen Fibers at the level of the Media tunic was, instead, in a continuous process of growth both with age and both from the base of the aorta to the abdominal region. The process was more pronounced from Childhood to Senescence but much more diminished and with oscillations from the proximal to the distal extremity of the Aorta.

  There were also peculiarities here regarding the evolution of the average density of Collagen Fibers in the two sexes. Thus, along the aortic trajectory, the values were significantly higher in women compared to men, except for the aortic cross, where the ratio was reversed and, throughout life, the values were slightly higher in women than in men in the first two periods of life so that, after 44 years, the ratio reverses, the values becoming slightly higher in men compared to women.

- The average density of Muscle Fibers showed, on the one hand, a general downward trend but oscillating with age, more pronounced from childhood to young adulthood and more attenuated to senescence. On the other hand, a very discrete upward trend also oscillating, from the base of the aorta the distal end of the artery in all periods of life was observed, except the Young Adult period, where the trend was slightly reversed. The peculiarities regarding sex consisted in the fact that, throughout life, the values of the average density of FM were higher in men than in women in the first two periods of life, the ratio reversing after the age of 45 years in time. which, along the aortic trajectory, the mean FM density values were generally higher in men than in women, except in the area of the club, where the ratio was reversed.

- Regarding the direct relationship between the evolutions of the values of aortic layers thicknesses and aortic diameter:

  - On the one hand, the thickness of the Intima was large where the thickness of the Media and aortic diameter were large, both in men and women
  - On the other hand, the thickness of the Media was large where the aortic diameter was large, both in men and women

- Regarding the direct relationship between the evolutions of the values for the three components of the aortic wall
  - the average density of FE was reduced where FCOL were denser and FMs were less both in men and women
  - FCOL were also denser where FM were less, both in men and women

The morphologic assessment of the aortic wall has to be continued by:

- the inclusion of new parameters in the study
- comparing their evolution with the evolution of the parameters already studied

in order to be able, finally, to define more clearly the complex process of vascular wall remodeling with advancing age.

PERSONAL CONTRIBUTIONS

The present study represents only one of the parts of a larger project larger project to analyze the remodeling process of the arterial wall in general and the aortic wall in particular with advancing age, project initiated by Professor’s Pleșea team in University of Medicine and Pharmacy of Craiova and continued in ”CarolDavila” University of Medicine and Pharmacy in Bucharest.

The complex assessment of the morphological changes of the aortic wall during the aging process at all topographical levels of the most important arterial vascular segment in the human body is among the very few if not the only one of its kind in the medical literature in our country.

The method of quantitative, objective assessment of different morphological parameters, using computer programs dedicated to the analysis of macroscopic and microscopic images, constitutes an element of originality and novelty at the same time both in the methodology of morphological investigation in our country and abroad.

Another element of originality is the individual and comparative approach of each morphological parameter, be it macroscopic or microscopic, in relation to three major criteria: the topography of the evaluation at the level of the aorta artery, the different periods of life and the sex of the cases.

The validation of the results obtained during the study was consistent, namely: three articles accepted for publication and published in ISI rated journals and three communications at prestigious scientific events in our country.

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