"CAROL DAVILA" UNIVERSITY OF MEDICINE

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GENERAL MEDICINE

COMPARATIVE STUDY OF AORTIC WALL MORPHOLOGY DEPENDING ON THE CAUSE OF DEATH

PhD THESIS

ABSTRACT

PhD Coordinator:

Professor Iancu Emil PLEȘEA

PhD Student:

Doru Adrian ȘEICARU

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KEY WORDS

Aorta, Cause of death, Aortic wall morphology, Remodeling, Morphometry.

CURRENT STAGE OF KNOWLEDGE

INTRODUCTION

The multitude of morphological elements, some complex, which together constitute the human organism is in a permanent interaction, throughout life, with the environment, some of whose factors are harmful, contributing to the wear and tear of tissue structures, which in turn attracts a decrease in their functional capacities. Paradoxically, the increase in life expectancy determined by the improvement of living conditions has caused, in the last decades, the number and diversity of morbid conditions to increase through the appearance of diseases found only in the elderly.

Prolonged interactions with harmful factors in the environment, but also diseases and injuries caused by the passage of time, determine tissue structures to undergo transformation processes at both a structural and functional level, the structures of the cardiovascular system in general and the vascular wall in particular not being exempted from this procession of changes [Gallagher PJ and van der Wal AC 2019; Jaminon A et al 2019; Jani B et al 2006]. Lifestyle, blood glucose level, blood lipid level or even genetic factors are only some of the risk factors that can be at the origin of cardiovascular diseases, and they, in turn, are influenced by aging [Lakatta et Levy 2003].

The aorta, the most important segment of the arterial tree in the human body, is among the vascular structures most exposed to interaction with harmful environmental influences and, in particular, to those that accumulate with aging [Stary HC et al 1992; Nakashima Y et al 2002; Hansson GK 2005].

After finishing my license studies, I decided to deepen my knowledge related to the remodeling process of the arterial wall under the influence of various pathological conditions that can be found in the human body by enrolling in the doctoral studies program, under the coordination of Professor Iancu Emil Pleșea, who encouraged me to tackle this phenomenon.

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 K et Kirby M, 1998]. The muscle cells of the medial tunic derive from the cells of the neural crests, forming the mesenchymal cells of the dorsal aorta [Kuo CT et al, 1997; Suri C et Yancopolous GD, 1999; Hungerford JE et Little CD, 1999]. The transformation period of endothelial canal networks can be divided temporarily and spatially into two phases. [Johnson D, 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 L et Neagu N, 1977; Skandalakis LJ, 2004; Johnson D, 2005; Etesami M et al, 2014; Rosen RD et Bordoni B, 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. [Pakurar AS et Bigbee JW, 2004; Gartner LP et Hiatt JL, 2007; Junqueira LC et Carneiro J, 2007; Lindberg MR et Lamps LW, 2018]. 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) [Halloran BG et al, 1995; Gartner LP et Hiatt JL 2007; Tsamis A et al 2013; Kelley JD et al, 2022; Shahoud JS et al, 2022; Rosen RD et Bordoni B, 2022].

Microscopic morphology. Arterial vascular structures are divided according to the main morphological component in [Gartner LP et Hiatt JL, 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 [Bank AJ et al, 1996; Gasser TC et al, 2006; Fortier A et al, 2014].

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. [Junqueira LC et Carneiro J, 2007; Kentish JC, 2008].

CAUSES OF DEATH ÎN MEDICAL PRACTICE

It is important to know why people die because then we will know how to improve their way of living. Highlighting the number of people who die each year is useful in assessing the effectiveness of health systems and directing resources where they are most needed [WHO, 2020].

Generally agreed definition. Cause of death is defined by the World Health Organization (WHO/WHO) in the International Classification of Diseases (ICD) as: "The condition or injury that triggered the sequence of morbid events that directly led to death", or as " The circumstances of the accident or act of violence that produced the fatal injury"**.**

The classifications may differ, either in the situation where the cause of death is multiple or difficult to assess, or when the procedures are recorded differently. The consequence is that statistical data from different countries are not entirely

comparable, although an international harmonization of definitions has been attempted as far as possible.

Types of causes of death. The general definition of the cause of death, however, has several nuances depending on the profile of the cause of death, namely: Antecedent Cause of Death (CDA), Avoidable Cause of Death (CDEv), Concurrent Cause of Death (CDC), Cause of Death Contributing (CDD) or External Cause of Death (CDEx).

Classification of causes of death. Pathological conditions causing death have become more numerous and diverse in recent decades [Omran AR,1971; Heidenreich PA et al, 2011; ; Kennedy BK et al, 2014; Townsend N et al, 2016; OECD, 2017; GBD 2017 Causes of Death Collaborators, 2018; Ritchie H et al, 2018; Bergeron-Boucher MP et al 2020, 2016]. As a consequence, over time, there have been numerous attempts to classify causes of death, some containing more and others fewer classification categories [GBD CN (Global Burden of Disease Collaborative Network) 2018; Teker AG et al, 2021].

The International Classification of Diseases, 10th revision (ICD-10) defines 19 groups and 211 subgroups of diseases. Moreover, in the first classification level of the GBD (Global Burden Disease) study, the causes of death were divided into three groups, namely [Ritchie H et al, 2018; GBD, 2017, 2019; WHO, 2020; Teker et al, 2021]: Group I or Communicable Diseases (CDs) group, Group II which includes Non-Communicable Diseases (NCDs) and Group III which includes Injuries.

Incidence of causes of death. As time passes, the causes of death have become, more and more diverse, the main reason being the introduction of new systems for measuring their variability [Bergeron-Boucher MP et al 2020]. However, they tend to focus on a few overarching causes [Iszak J, 1986].

Causes of death vary significantly from country to country. Thus, if non-communicable diseases dominate in countries with high per capita income, in countries with low per capita income, infectious diseases are still common, diseases that constitute the most important contingent from the group of communicable diseases.

Sex/gender and causes of death. Gender differences in disease prevalence, disease manifestations, and response to treatment originate in genetic differences between men and women [Arnold AP, 2017]. Sex-specific gene expression due to genomic copying also extends to autosomes and these copied genes show sex- and tissue-specific expression in humans [Baran Y et al, 2015].

Gender is as important a variable as sex in human health and influences the behavior of communities, clinicians and patients [Pelletier R et al, 2015, 2016].

PERSONAL CONTRIBUTION

The working hypothesis from which this study started was the evaluation of the influence that the morbid condition that led to the patient's death can exert on the morphology of the wall of the aorta artery, the most important segment of the arterial vascular tree.

The main objectives. The study has two main objectives: (a) to evaluate the size of the main layers of the aortic wall and the average diameter of the aortic artery and (b) to evaluate the density of the main morphological elements of the aortic wall. The sets of parameters established were evaluated in relation to Cause of death, to Cases' Sex and to Aortic topographic regions.

MATERIAL 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 Cause of death and Aortic topographic region, as follows: The group of patients who died due to a vascular disease (V_P), The group of patients who died due to a non-vascular disease (NV_P) and the group of patients who died due to a condition causing suspicious or violent death (MV/VDth).

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 single-center 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 (Cause of death and Gender) and Morphological Parameters (Thickness of the Aortic Wall Intima Tunic; Thickness of the Aortic Wall Media Tunic, Aortic Diameter, Media Tunic Elastic Fiber Density (FE), Media Tunic Collagen Fiber Density (FCOL), Media Tunic Smooth Muscle Cell (FM) Density, 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) [Șeicaru DA et al, 2021]. 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.

DISCUSSIONS

CAUSE OF DEATH

The two scales for evaluating the different causes of death found in our cases were very different from each other. Using online accessible interactive international databases [Şeicaru DA et al, 2023, GBD CN 2018, Ritchie H et al, 2018], we were able to collect data related to the hierarchies of the causes of death in the world and in our country during the same period in which we established the group our study (year 2013).

The only perfect overlap between the two scales was between the "Aggressions" group from the international scale and the "violent/suspicious causes of death" group from our scale across all three data sets compared. Otherwise, the group of causes of death from non-communicable diseases included, in all three case series, all deaths

from vascular diseases and most of the deaths from non-vascular diseases in our scale.

We included the group of deaths from communicable diseases in our group of deaths from non-vascular diseases. We could see, on the one hand, that at the national level this group represented the lowest percentage of all causes of death compared to world statistics and even to our group. On the other hand, our Aggression/Violent Death group had a significant extension (just over 30% of cases). The explanation is somewhat technical, in the sense that, in order to obtain a wide range of ages, we included in our observation group also forensic autopsies, found mostly in younger deceased and with causes of death belonging to the group mentioned above.

VASCULAR WALL PROFILES DEPENDING ON SEX

In men, regardless of cause of death, ***aortic diameter*** decreased continuously and significantly, nearly twofold, from the proximal to the distal end of the aorta. The ***Tunica Intima***, on the other hand, thickened steadily and also significantly, twice, from the base to the level of the abdominal region. ***The tunica media*** suffered, like the aortic diameter, a phenomenon of continuous but more attenuated reduction of its thickness by 25% from the level of the base to the level of the abdominal region [Albu M, Șeicaru DA et al, 2022]**.**

Regarding the fibrillary component, ***FE density*** had a continuous trend, but with small fluctuations, of reduction from the proximal to the distal end of the aorta, decreasing by 3%. ***FCOL*** density showed large fluctuations between the different topographic regions of the aorta with an overall increase of only 2.3% between the proximal and distal ends, with the lowest values in the thoracic region and the highest values in the abdominal region. ***FM density*** also fluctuated along the aortic trajectory, registering an overall increase of only 1.2% between the base and the abdominal region, with the lowest values in the trunk region and the highest values in the thoracic region [Albu M, Șeicaru DA et al, 2021].

In women, ***aortic diameter*** also decreased continuously and significantly, also nearly twofold, from the proximal to the distal end of the aorta. ***The Tunica Intima*** also thickened, as in men, constantly and significantly from the base to the level of the abdominal region, but more than twofold. ***The tunica media*** underwent, as in men, a phenomenon of continuous reduction of its thickness, but approximately 30% from the level of the proximal end to the level of the distal end [Albu M, Șeicaru DA et al, 2022].

Regarding the densities of the fibrillary components, they recorded significant fluctuations and different trends of evolution along the entire vascular path. Thus, the ***FE density*** had an oscillating tendency to decrease from the proximal to the distal end of the aorta, decreasing overall by 0.9%, but registering a significant increase at the trunk region. ***FCOL density*** registered an overall increase of only 0.3% between the proximal and distal ends, but with large fluctuations between the different topographical regions of the aorta with the lowest values at the club region (3% lower than at the base) and a return to slightly higher values at the distal end compared to the proximal one. ***FM density*** also showed fluctuations along the aortic trajectory, but much more attenuated, registering, overall, a discrete increase of only 0.7% between the base and the abdominal region, with the highest values in the middle regions, with 2% higher than those at the base level [Albu M, Șeicaru DA et al, 2021].

THE PROFILES OF EACH PARAMETER IN THE THREE GROUOPS

The behavior of the average diameter of the aorta artery related to the type of cause of death of the person can be characterized as follows:

The mean value of the aortic diameter decreased consistently and significantly (around 40%) from the proximal end to the distal end of the aortic trajectory regardless of the type of cause of death and was lower in the control group compared to the groups with deaths due to disease states (vascular or non-vascular) by around 20% at all topographical levels of the aortic pathway.

In the case of groups with deaths due to disease states, the average values of the diameter recorded at the level of each group in the different topographical regions were almost identical.

The same pattern of evolution was preserved in the two sexes taken separately, with the mentions that the aortic diameter was always slightly larger in men than in women in general, also slightly larger in men who died as a result of non-vascular diseases than in those who died from vascular diseases and the difference between the mean values of the diameter in the case of the control group in women compared to the groups with deaths from pathological causes was significantly greater than the same difference in men along the entire course of the vessel even if it was reduced towards the distal end (33% $\rightarrow$ 25% vs 15% $\rightarrow$ 14%).

The behavior of the thickness of the Intima tunic related to the type of cause of death (pathological or not) can be characterized as follows:

Tunica Intima increased in thickness consistently and significantly (around 50%) from the proximal end to the distal end of the aorta regardless of the type of cause of death and was thinner in the control group compared to the group of deaths due to disease states (vascular or non-vascular) at all topographical levels of the aortic tract with up to 17% at the terminal end.

In the case of the groups with deaths due to disease states, the average values of the thickness of the tunica Intima recorded at the level of the group with deaths from vascular causes in the different topographical regions were, in general, slightly higher, except for the terminal end of the vessel.

The same pattern of evolution was preserved in the two sexes taken separately, with the mention that the thickness of the Intima tunic was, in women, always slightly greater in the group of patients who died as a result of vascular diseases than in those who died as a result of non-vascular diseases and , also in women, the difference between the mean values of the thickness of the tunica Intima in the case of the control group compared to the groups with deaths from pathological causes was significantly greater than the same difference in men along the entire path of the vessel, being accentuated towards the distal end of the vessel.

The behavior of the thickness of the tunica media related to the type of cause of death (pathological or not) can be characterized as follows:

Tunica Media, in contrast, decreased in thickness consistently and significantly (around 27%) from the proximal end to the distal end of the aorta, regardless of the type of cause of death, and was slightly thinner in the control group compared to groups with deaths due to disease states (vascular or non-vascular) at all topographical levels of the aortic tract by between 1.5% and 7.5%.

In the groups with deaths due to disease states, mean tunica media thickness values recorded at the level of the group with deaths from vascular causes were generally slightly higher in the different topographic regions, except for the proximal end of the vessel.

The same pattern of evolution was preserved in the two sexes taken separately, with the mention that the thickness of the tunica Media was, in men, slightly higher in the group of patients who died as a result of vascular diseases than in those who died as a result of non-vascular diseases only in the proximal segments of the vessel while, in women, the thickness of the tunica Media was slightly higher in the group of patients who died as a result of vascular diseases than in those who died as a result of non-

vascular diseases in the first three segments of the vessel and the difference between the mean values of the tunica Media thickness in the control group versus the groups with deaths from pathological causes was significantly greater than the same difference in males along the entire course of the vessel.

The behavior of elastic fiber density (FE) related to the type of cause of death (pathological or not) can be characterized as:

Elastic Fibers (FE) had a synchronous variation, with a continuous decreasing trend along the aortic length, whether the person presents a pathological condition or not. Their lowest levels are encountered in patients died from vascular pathology and they are always more reduced than FCOL in people died from any pathological condition, vascular or nonvascular and their highest densities were observed in control group [Șeicaru DA, Albu M et al, 2023].

The evolution pattern was somewhat preserved in the two sexes taken separately in the sense of the hierarchy of the average values of the three groups. However, the FE density in the control group was higher in women compared to men, the trend of decreasing density values was more pronounced in the groups of patients who died due to a pathological condition in men compared to women, the lowest density values were found in patients who died due to vascular pathological conditions and the difference between the control group and the groups with deaths due to pathological causes was greater in women.

The behavior of collagen fiber density (FCOL) related to the type of cause of death (pathological or not) can be characterized as:

Collagen Fibers (FCOL) have a synchronous variation with an oscillating but stable trend along the aortic length only in people died from a non-vascular pathological condition and in people died from a violent/suspicious cause of death (considered control group). Their highest levels are encountered in patients died from vascular pathology and they are always more elevated than FE in people died from any pathological condition, vascular or non-vascular but more reduced than FE in people died from a violent/suspicious cause of death (considered control group) [Șeicaru DA, Albu M et al, 2023].

The oscillating evolution pattern was also preserved for the two sexes taken separately as well as the hierarchy of the average values of the three groups, specifying that the differences between the groups were more pronounced in women compared to men and the average values were generally higher in women versus men.

The behavior of smooth muscle fiber (FM) density related to the type of cause of death (pathological or not) can be characterized as:

Smooth Muscle Fibers (FM) had a synchronous variation with an oscillating but slightly ascending trend along the aortic length, in people died from a pathological condition (vascular or non-vascular), more pronounced in the group of patients dead due to a vascular disease. In people died from a violent/suspicious cause of death (considered control group), the trend, also oscillating, was a descending one.

Their highest levels are encountered in the first three aortic regions of people died from a violent/suspicious cause of death (considered control group). Their amount is always at least two times lower than that of the other types of fibers, regardless of whether the person died due to a pathological condition or not.

The amount of FM was always at least twice that of the other two types of fibers, regardless of whether the person died from a pathological condition or not [Șeicaru DA, Albu M et al, 2023].

In the two sexes taken separately, similar evolutionary patterns were observed, but the ranking of the average density values in the three groups was totally different in the sense that, in men, the highest values were found in the control group, while in women, they were found in the group of patients who died due to non-vascular diseases, at a significant distance from the other two groups.

COMPARISONS WITH FOREIGN STUDIES

We didn't find too many data in the literature to compare with our results. În general, most of the consulted studies were either reviews, with general considerations, either were analyzes of the aortic wall as a whole.. In most of these few studies, the quantitative assessment of the aortic wall resulted from ultrasound investigation, only one study excepting ours, was a pathological one [Schriefl AJ et al, 2012 Bulut A et al, 2019; Koc AS & Sumbul HE, 2018 Sumbul HE and Koc AS, 2019].

In the consulted literature, we found only one study that compares the amounts of FE and FCOL along different aortic regions [Dobrin PB, 1997].

CONCLUSIONS

The present study, including an individual and comparative analysis of the layers of the aortic wall, the diameter of the vessel and the main morphological elements of the aortic wall in relation to the different causes of death, led to some conclusions that may have importance and applicability in medical practice:

- The different pathological conditions that lead to death can influence both the geometry of the aortic wall and the fibrillar composition of the tunica media of the aorta. Thus, all the pathological conditions causing death determine an increase in the aortic diameter and the thicknesses of the intima and media tunics compared to the values found in deaths from violent or suspicious causes considered as a control group.
- These changes occur against a general background of decreased aortic diameter and tunica media thickness and increased tunica intima thickness along the course of the aortic artery, regardless of whether death occurred due to a pathological condition or not.
- All pathological conditions causing death (vascular or non-vascular) cause, at the level of the tunica media, an increase in the amount of collagen fibers and a decrease in the amount of elastic fibers compared to non-pathological conditions causing death.
- In all these cases, the level of the density of collagen fibers is higher than the level of the density of elastic fibers while, in cases that died due to non-pathological conditions, the ratio is reversed, the amount of elastic fibers being higher than that of collagen fibers.
- The sex of the deceased patients also left its mark on the phenomenon of remodeling of the aortic wall along the path of the vessel, highlighting particularities in the three groups of deceased patients such as:
    - • higher diameter values in non-vascular diseases in men than in women,
    - • both tunics with greater mean thicknesses in women than in men in vascular diseases,
    - • divergent evolution of the main fiber types in women compared to men in vascular diseases, consisting of a lower value of the density of elastic fibers and a higher value of the density of collagen fibers.
- • We believe that the studies must be continued and expanded to reveal other changes in the morphology of the aortic wall in particular and of the vascular wall in general that could be linked to the pathological conditions that lead to death but which, until this moment, in fact, affects the whole body.

PERSONAL CONTRIBUTIONS

The present study is part of a more extensive project to evaluate the phenomenon of aging of the component structures of the cardiovascular system - cardiac wall and arterial wall, a project that began more than 10 years ago within the Discipline of Pathological Anatomy and the Doctoral School of the University of Medicine and Pharmacy in Craiova under the coordination of Professor Iancu Emil Pleșea and continued at the Doctoral School of the "Carol Davila" University of Medicine and Pharmacy in Bucharest.

The evaluation of the complex morphological remodeling of the macroscopic and microscopic component elements of the wall of the most important arterial vascular segment in the human body at all its topographical levels in relation to the pathological conditions that cause death is, to our knowledge, the only one of its kind in the specialized literature in our country.

Apart from this, the original but also innovative character at the same time is supported by the evaluation algorithm of different morphological parameters, which was based on the concept of their quantitative, objective analysis, which is possible thanks to the use of Artificial Intelligence represented by dedicated computational analysis tools for macroscopic and microscopic images, some created especially for the project, an element of originality but also of novelty at the same time both in the methodology of morphological investigation in our country and abroad.

Another element of originality of the investigation algorithm is the individual and comparative approach of each morphological, macroscopic or microscopic parameter in relation to three major criteria: the topography at the level of the aorta artery, the type of cause of death and the sex of the cases.

The validation of the results obtained during the study was consistent, namely: four articles published in ISI rated journals as first author (2) or main author (2) and six communications at prestigious scientific events both in our country and abroad.

SELECTIVE BIBLIOGRAPHY

1. Albu M, **Şeicaru DA**, Pleşea RM, Mirea OC, Gherghiceanu F, Grigorean VT, Şerbănescu MS, Pleşea IE, Liţescu M. Remodeling of the aortic wall layers with ageing. Rom J Morphol Embryol. 2022, 63(1):71-82. https://doi.org/10.47162/RJME.63.1.07. PMID: 36074670; PMCID: PMC9593119
2. Albu M, **Şeicaru DA**, Pleşea RM, Mirea OC, Gherghiceanu F, Grigorean VT, Cordoş I, Liţescu M, Pleşea IE, Şerbănescu MS. Assessment of the aortic wall histological changes with ageing. Rom J Morphol Embryol. 2021, 62(1):85-100. doi: 10.47162/RJME.62.1.08. PMID: 34609411; PMCID: PMC8597368

3. Arnold AP. A general theory of sexual differentiation. J Neurosci Res. 2017, 95(1-2):291-300. https://doi.org/10.1002/jnr.23884. PMID: 27870435; PMCID: PMC5369239
4. Bank AJ, Wang H, Holte JE, Mullen K, Shammas R, Kubo SH. Contribution of collagen, elastin, and smooth muscle to in vivo human brachial artery wall stress and elastic modulus. Circulation, 1996, 94(12):3263–3270. https://doi.org/10.1161/01.cir.94.12.3263 PMID: 8989139
5. Baran Y, Subramaniam M, Biton A, Tukiainen T, Tsang EK, Rivas MA, Pirinen M, Gutierrez-Arcelus M, Smith KS, Kukurba KR, Zhang R, Eng C, Torgerson DG, Urbanek C; GTEx Consortium; Li JB, Rodriguez-Santana JR, Burchard EG, Seibold MA, MacArthur DG, Montgomery SB, Zaitlen NA, Lappalainen T. The landscape of genomic imprinting across diverse adult human tissues. Genome Res. 2015, 25(7):927-936. https://doi.org/10.1101/gr.192278.115. PMID: 25953952; PMCID: PMC4484390.
6. Bareliuc L, Neagu N. Dezvoltarea Aparatului Cardiovascular În: Bareliuc L, Neagu N. (Editors) Embriologie Umană, Editura Medicală, 1977, 146
7. Bergeron-Boucher MP, Aburto JM, van Raalte A. Diversification in causes of death in low-mortality countries: emerging patterns and implications. BMJ Glob Health, 2020, 5(7):e002414. https://doi.org/10.1136/bmjgh-2020-002414 PMID: 32694219 PMCID: PMC7375425
8. Bergeron-Boucher MP, Bourbeau R, Légaré J. Changes in cause-specific mortality among the elderly in Canada, 1979–2011. Can Stud Popul, 2016, 43(3–4):215–233. https://doi.org/10.25336/P69W3W. https://journals.library.ualberta.ca/csp/index.php/csp/article/view/25526
9. 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
10. Dobrin PB. Physiology and pathophysiology of blood vessels. In: Sidawy AN, Sumpio BE, DePalma RG (eds). The basic science of vascular disease. Futura Publishing Co., Armonk, New York, USA, 1997, 69–105. https://www.worldcat.org/title/35229112
11. Etesami M, Ashwath R, Kanne J, Gilkeson RC, Rajiah P. Computed tomography in the evaluation of vascular rings and slings. Insights Imaging. 2014, 5(4):507-521. https://doi.org/10.1007/s13244-014-0343-3. PMID: 25008430; PMCID: PMC4141344.
12. Fortier A, Gullapalli V, Mirshams, RA. Review of biomechanical studies of arteries and their effect on stent performance, IJC Heart & Vessels, 2014, 4: 12-18, https://doi.org/10.1016/j.ijchv.2014.04.007.
13. Gallagher PJ and van der Wal AC. Blood vessels. In: Mills SE (Editor) Histology for Pathologists, 5th edition, Lippincott Williams & Wilkins, 2019 pp:190-216 https://www.wolterskluwer.com/en/solutions/ovid/histology-for-pathologists-3488
14. Gartner LP, Hiatt JL, Color Teextbook of Histology, Third Edition, Elsevier Inc, 2007, 253-259
15. 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
16. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet, 2018, 392(10159):1736–1788. https://doi.org/10.1016/S0140-6736(18)32203-7. Erratum in: Lancet, 2019, 393(10190):e44. Erratum in: Lancet, 2018, 392(10160):2170. PMID: 30496103 PMCID: PMC6227606
17. GBD CN (Global Burden of Disease Collaborative Network. Global burden of disease study 2017 (GBD 2017) covariates 1980–2017. Institute for Health Metrics and Evaluation (IHME), Seattle, WA, USA, 2018. http://ghdx.healthdata.org/record/ihme-data/gbd-2017-covariates-1980-2017
18. Halloran BG, Davis VA, McManus BM, Lynch TG, Baxter BT. Localization of aortic disease is associated with intrinsic differences in aortic structure. J Surg Res, 1995, 59(1):17-22. https://doi.org/10.1006/jsre.1995.1126. PMID: 7630123
19. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med, 2005, 352(16):1685–1695. https://doi.org/10.1056/NEJMra043430 PMID: 15843671
20. Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khera A, Lloyd-Jones DM, Nelson SA, Nichol G, Orenstein D, Wilson PW, Woo YJ; American Heart Association Advocacy Coordinating Committee; Stroke Council; Council on Cardiovascular Radiology and Intervention; Council on Clinical Cardiology; Council on Epidemiology and Prevention; Council on Arteriosclerosis; Thrombosis and Vascular Biology; Council on Cardiopulmonary; Critical Care; Perioperative and Resuscitation; Council on Cardiovascular Nursing; Council on the Kidney in Cardiovascular Disease; Council on Cardiovascular Surgery and Anesthesia, and Interdisciplinary Council on Quality of Care and Outcomes Research. Forecasting the future of cardiovascular disease in the United States: a

policy statement from the American Heart Association. Circulation, 2011, 123(8):933–944. https://doi.org/10.1161/CIR.0b013e31820a55f5 PMID: 21262990
21. Holzapfel GA, Sommer G, Auer M, Regitnig P, Ogden RW. Layer-specific 3D residual deformations of human aortas with non-atherosclerotic intimal thickening. Ann Biomed Eng, 2007, 35(4):530-545. https://doi.org/10.1007/s10439-006-9252-z. PMID: 17285364.
22. Hungerford JE, Little CD. Developmental biology of the vascular smooth muscle cell: building a multilayered vessel wall. J Vasc Res. 1999, 36(1):2-27. https://doi.org/10.1159/000025622. PMID: 10050070.
23. Iszak J. Measuring the secular changes of the concentration of death causes. Genus. 1986, 42(3-4):197-208. PMID: 12280634.
24. 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
25. 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
26. Johnson D. Major blood vessels. Thorax – 6th Section. In: Standring S. Gray's Anatomy – The Anatomical Basis of Clinical Practice, 39th Edition, Elsevier Churchill Livingstone, 2005, 1021-1024
27. Junqueira LC, Carneiro J. Basic Histology – Text and Atlas. 11th Edition, The McGraw-Hill Companies, 2007, Chapter 11:266-288
28. Kelley JD, Kerndt CC, Ashurst JV. Anatomy, Thorax, Aortic Arch. [Updated 2021 Aug 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499911/. PMID: 29763086
29. Kennedy BK, Berger SL, Brunet A, Campisi J, Cuervo AM, Epel ES, Franceschi C, Lithgow GJ, Morimoto RI, Pessin JE, Rando TA, Richardson A, Schadt EE, Wyss-Coray T, Sierra F. Geroscience: linking aging to chronic disease. Cell, 2014, 159(4):709–713. https://doi.org/10.1016/j.cell.2014.10.039 PMID: 25417146 PMCID: PMC4852871
30. Kentish JC. Smooth muscle and the cardiovascular and lymphatic systems. In: Stranding S, ed. Gray's Anatomy: The Anatomical Basis of Clinical Practice. 40th ed. Philadelphia, PA: Churchill Livingstone Elsevier; 2008:127–144.
31. 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
32. Kuo CT, Veselits ML, Barton KP, Lu MM, Clendenin C, Leiden JM. The LKLF transcription factor is required for normal tunica media formation and blood vessel stabilization during murine embryogenesis. Genes Dev. 1997, 11(22):2996-3006. https://doi.org/10.1101/gad.11.22.2996. PMID: 9367982; PMCID: PMC316695.
33. Lakatta EG, Levy D. Arterial and Cardiac Aging: Major Shareholders in Cardiovascular Disease Enterprises. Part I: Aging Arteries: A "Set Up" for Vascular Disease. Circulation. 2003, 107:139-146. DOI: 10.1161/01.CIR.0000048892.83521.58
34. Laurent S, Boutouyrie P, Lacolley P. Structural and genetic bases of arterial stiffness. Hypertension. 2005, 45(6):1050-1055. doi: 10.1161/01.HYP.0000164580.39991.3d. PMID: 15851625.
35. Lindberg MR, Lamps LW. Diagnostic Pathology: Normal Histologiy, Second Edition, Elsevier, 2018, Chapter 21, 94-96
36. 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):279–288. https://doi.org/10.1007/s00428-002-0605-1 PMID: 12242525
37. Omran AR. The epidemiologic transition. A theory of the epidemiology of population change. Milbank Mem Fund Q, 1971, 49(4):509–538. PMID: 5155251
38. Organisation for Economic Co-operation and Development (OECD). Health at a glance 2017: OECD indicators. OECD Publishing, Paris, France, 2017. https://doi.org/10.1787/health_glance-2017-en https://www.oecd-ilibrary.org/social-issues-migration-health/health-at-a-glance-2017_health_glance-2017-en
39. Pakurar AS, Bigbee JW. Cardiovascular System In: Digital Histology An Interactive CD Atlas with Review Text. John Wiley & Sons, Inc., 2004, 85-92
40. Pelletier R, Choi J, Winters N, Eisenberg MJ, Bacon SL, Cox J, Daskalopoulou SS, Lavoie KL, Karp I, Shimony A, So D, Thanassoulis G, Pilote L; GENESIS-PRAXY Investigators. Sex Differences in Clinical Outcomes After Premature Acute Coronary Syndrome. Can J Cardiol. 2016, 32(12):1447-1453. https://doi.org/10.1016/j.cjca.2016.05.018. PMID: 27683172.

41. Pelletier R, Ditto B, Pilote L. A composite measure of gender and its association with risk factors in patients with premature acute coronary syndrome. Psychosom Med. 2015, 77(5):517-256. https://doi.org/10.1097/PSY.0000000000000186. PMID: 25984818.
42. Rachev A, Gleason RL Jr. Theoretical study on the effects of pressure-induced remodeling on geometry and mechanical non-homogeneity of conduit arteries. Biomech Model Mechanobiol. 2011, 10(1):79-93. https://doi.org/.1007/s10237-010-0219-5. PMID: 20473704; PMCID: PMC3098611
43. Ritchie H, Spooner F, Roser M. Causes of death. Our World in Data, Global Change Data Lab, 2018. https://ourworldindata.org/causes-of-death
44. Rosen RD, Bordoni B. Embryology, Aortic Arch. [Updated 2022 Feb 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553173. PMID: 31985966
45. Sadler TW. Langman's Medical Embriology. 10th Edigion, Lippincott Williams & Wilkins, 2006, ISBN-13: 978-0-7817-9485-5. 180-185
46. Schriefl AJ, Zeindlinger G, Pierce DM, Regitnig P, Holzapfel GA. Determination of the layerspecific distributed collagen fibre orientations in human thoracic and abdominal aortas and common iliac arteries. J. R. Soc. Interface 2012, 9:1275–1286. https://doi.org/10.1098/rsif.2011.0727 PMID: 22171063. PMCID: PMC3350738.
47. Shahoud JS, Miao JH, Bolla SR. Anatomy, Thorax, Heart Aorta. [Updated 2021 Jul 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK538140/. PMID: 30844167
48. Skandalakis LJ. Skandalakis Surgical Anatomy: The Embryologic and Anatomic Basis of Modern Surgery, Broken Hill, Paschalidis Medical Publications, 1st Edition, 2004
49. Stary HC, Blankenhorn DH, Chandler AB, Glagov S, Insull W Jr, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler W. 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
50. 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
51. Suri C, Yancopolous GD () Growth factors in vascular morphogenesis: insights from gene knockout studies in mice. In: Little CD, Mironov V, Sage H (eds) Vascular Morphogenesis: In vivo, in Vitro, In Menter. Birkhauser, Boston, 1999, 65–72
52. **Șeicaru Doru Adrian**, Lițescu M, Gherghiceanu F, Șerbănescu MS, Grigorean VT, Pleșea RM. Assessment of the aortic tunica media histological changes in relation with the cause of death. Rom J Morphol Embryol 2023, 642(3): 399-410. https://doi.org/10.47162/RJME.64.3.11. PMID: 37867357; PMCID: PMC10720938
53. **Șeicaru Doru Adrian**, Albu M, Pleşea RM, Gherghiceanu F, Cordoş I, Lițescu M, Alexandru DO, Pleşea IE, Grigorean 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: PMC: 8597380
54. Teker AG, Emecen AN, Ergör G. Cause-of-death distributions and mortality trends in Turkey between 2009 and 2017. Balkan Med J, 2021, 38(2):121–126. https://doi.org/10.4274/balkanmedj.galenos.2020.2020.4.200. PMID: 33053913
55. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. Eur Heart J, 2016, 37(42):3232–3245. https://doi.org/10.1093/eurheartj/ehw334. Erratum in: Eur Heart J, 2019, 40(2):189. PMID: 27523477
56. 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, 10:20121004. http://dx.doi.org/10.1098/rsif.2012.1004. PMID: 23536538. PMCID: PMC3645409
57. Waldo K, Kirby M. Development of the great arteries. In: de la Cruz MV, Markwald RR (eds) Living Morphogenesis of the Heart. Birkauser, Boston, MA, 1998, 187–217. DOI 10.1007/978-1-4612-1788-6
58. Westerhof N, Stergiopulos N, Noble MIM. Snapshots of hemodynamics: an aid for clinical research and graduate education. 2005, New York, NY: Springer Science + Business Media, Inc.
59. WHO. The top 10 causes of death. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death. 2020

PUBLISHED PAPERS

PAPERS PUBLISHED IN ISI JOURNALS

1. **Șeicaru Doru Adrian**, Lițescu M, Gherghiceanu F, Șerbănescu MS, Grigorean VT, Pleșea RM. Assessment of the aortic tunica media histological changes in relation with the cause of death. Rom J Morphol Embryol 2023, 642(3): 399-410. doi: 10.47162/RJME.64.3.11. – **First Author**, quoted in text (Chapter IX – Discussions, pages 156, 169, 172, 174) and included in Bibliography

2. **Șeicaru Doru Adrian**, Albu M, Pleşea RM, Gherghiceanu F, Cordoș I, Lițescu M, Alexandru DO, Pleşea IE, Grigorean VT. Anatomical remodeling of the aortic wall in relation with the cause of death. Rom J Morphol Embryol 2021, 62(1):19–40. doi: 10.47162/RJME.62.1.03 – **First Author**, quoted in text (Chapter IV – General research methodology, pages 53-54, Chapter V – Clinical profile, page 64) and included in Bibliography.

3. Albu M, **Șeicaru Doru Adrian**, Pleșea RM, Mirea OC, Gherghiceanu F, Grigorean VT, Șerbănescu MS, Pleșea Iancu Emil, Lițescu M. Remodeling of the aortic wall layers with ageing. Rom J Morphol Embryol 2022, 63(1):71–82 – **Main Author**. quoted in text (Chapter V – Clinical profile, pages 66, 68, Chapter IX – Discussions, pages 157, 158, 159, 192, 193, 194) and included in Bibliography.

4. Albu M, **Șeicaru Doru Adrian**, Pleşea RM, Mirea O, Gherghiceanu F, Grigorean VT, Cordoș I, Lițescu M, Pleşea IE, Șerbănescu M-S. Assessment of the aortic wall histological changes with ageing. Rom J Morphol Embryol 2021, 62(1):85–100. doi: 10.47162/RJME.62.1.08. PMID: 34609411; PMCID: PMC8597368 – **Main Author** – Citation in: Liping D, Jia L, Guangyi L, Heng Y. Effect of aging on the ultrastructure of common iliac artery in rats. Chinese Journal of Tissue Engineering Research. 2022, 26(26):4123-4126. https://doi.org/10.12307/2022.813. quoted in text (Chapter V – Clinical profile, pages 69, 70, 71 and Chapter IX – Discussions, pages 157, 158, 159) and included in Bibliography.

STUDIES IN ABSTRACT BOOKS

1. Pleșea I.E., Șerbănescu M., **Șeicaru Doru Adrian**, Albu M., Gherghiceanu F., Avramescu I.D., Giuroiu F., Mirea O.C., Pleșea R.M. ***Morphometric analysis of arterial wall main components densities depending on the patient's cause of death***. 34th European Congress of Pathology, 3 – 7 September 2022, Basel, Switzerland, Virchows Archiv (2022) 481 (Suppl 1):S156

2. Plește I.E., Șerbănescu M., Albu M., **Șeicaru Doru Adrian**, Gherghiceanu F., Giuroiu F., Chiriac P.L., Mirea O.C., Plește R.M. ***Morphometric analysis of aortic wall main components depending on age***. 34th European Congress of Pathology, 3 – 7 September 2022, Basel, Switzerland, Virchows Archiv (2022) 481 (Suppl 1):S158

3. Albu M, **Șeicaru Doru Adrian**, Șerbănescu M-S, Gherghiceanu F., Avramescu I.D., Giuroiu F., Mirea O.C., Plește R.M, Plește I.E. ***The assessment of aortic wall Intimal layer with ageing***. XVIIIth National Symposium of Microscopic Morphology with international participation. Craiova, October 12-15, 2022

4. Șeicaru Doru Adrian, Albu M, Șerbănescu M-S, Gherghiceanu F., Giuroiu F., Chiriac P.L., Mirea O.C., Plește R.M, Plește I.E. ***The assessment of aortic wall Media layer with ageing.*** XVIIIth National Symposium of Microscopic Morphology with international participation. Craiova, October 12-15, 2022

5. Albu M, **Șeicaru Doru Adrian**, Șerbănescu M-S, Nicola (Roșu) G.C., Ofițeru (Istrate) A.M., Pleşea RM, Iordache L. Mirea OC, Gherghiceanu F, **Pleşea IE**. ***Evaluarea componentei fibrilare colagene din peretele aortic în procesul de îmbătrânire***. Al **XVII-lea Simpozion Național de Morfologie Microscopică cu participare internațională**, Craiova, 6-9 Octombrie, 2021, Abstract Book p 20

6. **Șeicaru Doru Adrian**, Albu M, Șerbănescu M-S, Nicola (Roșu) G.C., Ofițeru (Istrate) A.M., Pleşea RM, Iordache L. Mirea OC, Gherghiceanu F, **Pleşea IE**. ***Evaluarea remodelării tunicii media a peretelui aortic în funcție de cauza de deces***. Al **XVII-lea Simpozion Național de Morfologie Microscopică cu participare internațională**, Craiova, 6-9 Octombrie, 2021, Abstract Book p 21