

## ORIGINAL PAPER



# Anatomical remodeling of the aortic wall in relation with the cause of death

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## Abstract

**Aim:** The authors set out to evaluate the correlations between three of the main morphological aortic parameters (aortic diameter, intima, and media thickness) and the cause of death. **Materials and Methods:** Study group included 28 people died of a cardiovascular (CV) disease and 62 people died of a noncardiovascular (NCV) disease. Four aortic cross-sections (base, cross, thoracic, abdominal) were collected during autopsy from the selected cases, fixed in 10% buffered formalin and photographed together with a calibrating ruler. Then, they were processed using the classical histopathological (HP) technique (formalin fixation and paraffin embedding), stained with Hematoxylin–Eosin (HE) and Orcein, and the obtained histological slides were transformed into virtual slides. Aortic diameters were determined on calibrated photos using a custom-made software, developed in MATLAB (MathWorks, USA). Intima and media thicknesses were determined on virtual slides using a dedicated image analysis software. **Results and Discussions:** The most frequent CV causes of death were the ischemic heart diseases and the most frequent NCV causes of death were the inflammatory diseases. Aortic diameter decreased from the aortic origin till the aortic end, with larger values in women than in men and in CV diseases than in NCV diseases. The difference in the remodeling of the aortic diameter between the two groups is smaller towards the abdominal region. Intima thickness increased from the aortic origin till the aortic end and was larger especially in women died of CV diseases, whereas in men there were some shifts at the extremities of the aorta. The difference in the remodeling of the intimal thickness between the two groups is extremely variable. Media was thicker in almost all of its segments in CV group than in NCV. It was a divergent evolution of the correlation degree trends in the two groups. **Conclusions:** The three morphological parameters of the aorta (diameter, intima, and media thicknesses) are more or less influenced by the pathological status that caused patient's death by the patient's sex and by the topographic region where the measurement was made.

**Keywords:** aorta, cause of death, remodeling, morphometry.

## Introduction

Vessel walls are three-dimensional structures fulfilling complex vascular mechanic functions [1, 2]. The walls of all blood vessels have trilaminar structure consisting of distinct layers or tunicae: the *tunica intima* (the innermost layer), *tunica media* (the middle layer) and *tunica adventitia* (the outer layer) [3–5].

The arterial walls especially are the site of a continuous process of adaptation to different biochemical and biomechanical stimuli, process defined also as arterial remodeling [6, 7].

This process evolves in two opposite ways: (i) outward, with hypertrophy (thickening); (ii) inward, with hypotrophy (thinning) of the vessel wall and is influenced by a wide range of biological processes, such as: inflammation, oxidative stress, lipid accumulation, and degradation of the extracellular matrix (ECM). Large conduit vessels show hypertrophic remodeling because they don't respond to stress by constriction [8–10].

The major structural and functional changes appearing in the arterial bed, including aorta, are: (i) elongation with subsequent tortuous appearance; (ii) progressive and linear increase in diameter with subsequent lumen

enlargement; (iii) fragmentation of elastic lamellae with elasticity loss and widening of interlamellar spaces; (iv) eccentric or diffuse wall thickening as a result of collagen accumulation trend in “gaps” arising between the lamellar units because of the lesser amount of elastin mentioned above and the lesser amount of smooth muscle cells with fewer cellular focal adhesions, mainly affecting the intima and the media even in populations with a low incidence of atherosclerosis; (v) calcification; (vi) focal amyloid deposits and (vii) thickening of walls of *vasa vasorum* [11–20].

All of these processes are intervening with age and are more evident in large arteries, but also occur in the peripheral vascular bed [21, 22]. The aortic and coronary arteries are two of the most lesion-susceptible regions in the human vasculature with age [23–25].

It seems that there are intrinsic differences in arterial properties between men and women and these differences vary also across the life course [26]. For instance, men have greater endothelial dysfunction and arterial stiffness than women across the age spectrum until the sixth decade, when age-related arterial dysfunction progresses at a faster rate in women [27].

International Classification of Diseases 10<sup>th</sup> revision (ICD-10) defines 19 groups and 211 subgroups of diseases. Further, in the first level classification of the Global Burden of Disease (GBD) study, the causes of death were divided into three groups, namely: (i) group I of communicable diseases (CDs), including infectious, maternal, perinatal, and nutritional conditions; (ii) group II, including non-communicable diseases (NCDs); and (iii) group III of injuries, including external causes among which both intentional and unintentional injuries [28, 29].

Patterns in the cause of death structure across countries are considered a key indicator of population well-being [30]. Cause-of-death variation has increased in recent decades for all countries and both sexes, most countries experiencing a diversification in causes of deaths mainly due to ageing populations with subsequent decline in lifespan variation and increasing life expectancy [31, 32].

The rise in life expectancy together with the decline in lifespan variation resulted from reductions in infant and maternal mortality, along with infectious diseases, and more recently by reduced cancer mortality, decline in mortality at older ages and from cardiovascular (CV) diseases [33–38]. However, CV diseases are the leading cause of morbidity and death globally in modern societies, 33.1% of all deaths in 2017, for instance, being caused by them [39–41].

### Aim

The present study is part of a larger research project that aims to assess morphological changes in different compartments of the CV system (heart, blood vessels) in relation with age. Its goal is to assess the quantitative remodeling of the dimensions of the aortic wall and its layers in relation with the cause of death.

### Materials and Methods

This study analyzed human aortic samples obtained from 90 deceased subjects who underwent necropsy for diagnosis in the Department of Pathology, Emergency County Hospital, Craiova, Romania. It was a prospective

study that followed a comparison design and was carried out as a single-center study.

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 inclusion criterion was the cause of death established by corroborating necropsy diagnostic with clinical data and, depending on which the dead patients were divided in two groups:

(I) The **cardiovascular (CV) group**, including **28 cases** whose cause of death was a CV disease;

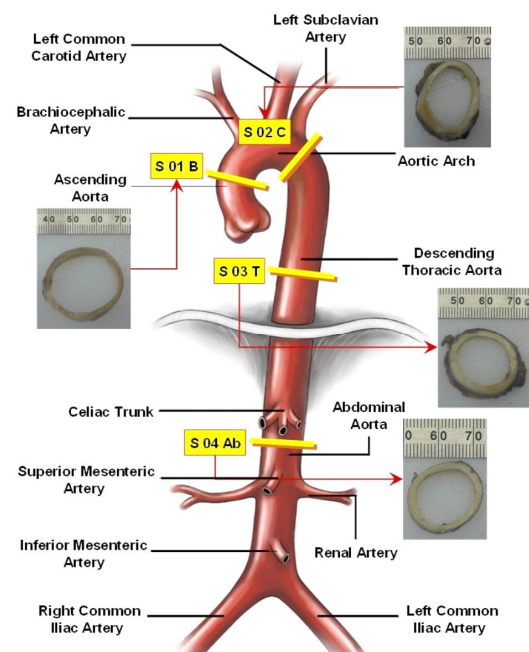
(II) The **noncardiovascular (NCV) group**, including **62 cases** whose cause of death was a NCV disease.

The studied material came from two sources:

- Patient's clinical documents (medical charts and autopsy protocols);
- Samples of aortic wall tissue collected during 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 [42] consisting of cross-sections at four different levels, without visible lesions of aortic wall. The algorithm is illustrated in Figure 1, namely:



**Figure 1 – Sites of sampling modified after [42, 43].**

• **Cross-section No. 1**, at the ascending aorta level – 2–3 cm above the sinotubular junction, labeled “01 B” (base);

• **Cross-section No. 2**, at the aortic arch level – in the left side close proximity of the left subclavian artery, labeled “02 C”;

• **Cross-section No. 3**, at the thoracic aorta level – in the inferior segment, above the diaphragm aortic hiatus, labeled “03 T”;

• **Cross-section No. 4**, at the abdominal aorta level – between the celiac trunk and superior mesenteric artery, labeled “04 Ab”.

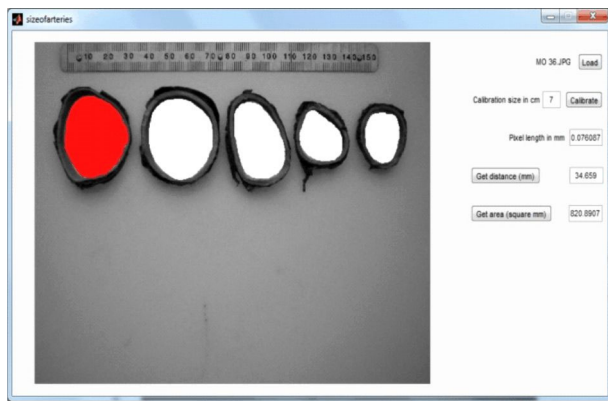
The assessed parameters were grouped into two main categories and listed below in Table 1.

**Table 1 – The set of studied parameters**

Parameter type	Description
Clinical	Age
	Sex
	Cause of death
	Cardiovascular (CV) Noncardiovascular (NCV)
Morphological	Aortic diameter (D)
	Media thickness (MED)
	Intima thickness (IN)

Tissue samples were firstly fixed in 10% buffered formalin. Then, they were photographed together with a graduated ruler for image calibration.

For the measurement of aortic diameters, a software designed in MATLAB programming environment (Math Works, USA) was developed and used (Figure 2).



**Figure 2 – MathWorks software window.**

The program allowed tracing manually the internal perimeter of the photographed aortic samples (lumen

was marked with a single color). After color filling, a mathematical algorithm was used to determine the area of the arterial lumen in each image after prior calibration using the ruler within the picture.

Estimated diameter of each aortic segment was calculated using the formula:

$$\text{Diameter} = \text{SQRT} (4 \times \text{determined Area} / \pi)$$

where SQRT represents square root.

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

**Table 2 – The protocol of staining procedures**

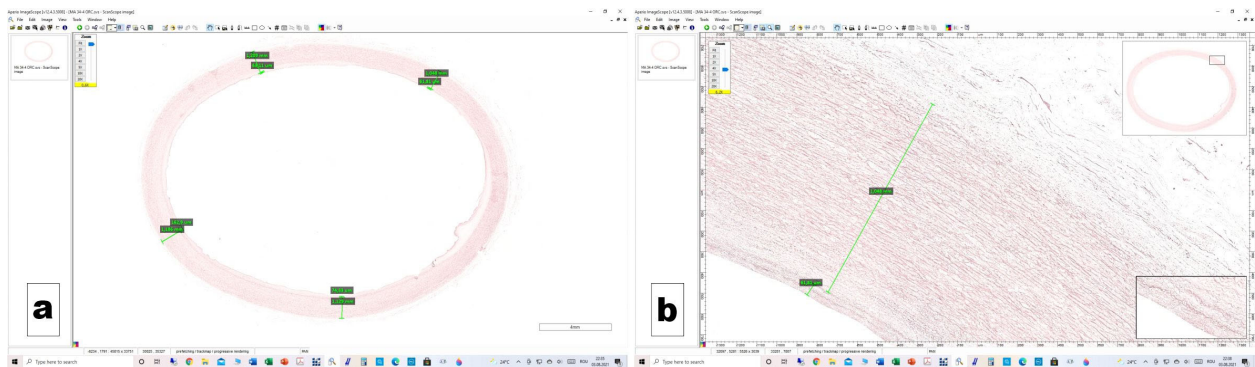
Staining type	Description
Hematoxylin–Eosin (HE)	General orientation on the sample
Orcein	Identification of
	Internal elastic membrane (IEM) External elastic membrane (EEM)

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 3, a and b).

For each cross-section of each case four values were obtained for each parameter [intima thickness (IN) and media thickness (MED)]. The average of these four values was calculated and used further as representative for each cross-section of the aortic wall (Figure 3, a and b).

For age evaluation, patients were grouped in four groups following the age periods (APs) of life (Table 3).



**Figure 3 – Intima and media measurements. Windows of Aperio ImageScope program. Orcein staining: (a) Overview with the four random measurements,  $\times 0.6$ ; (b) Detail with the up-right measurement,  $\times 6.2$ .**

**Table 3 – Stratification scale for age**

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

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.

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

Statistical tools used were 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.



## Results

### Cause of death

We analyzed the causes of death using two scales: the first one was the first level classification of the GBD study [28] and the second one was our scale (Table 4).

**Table 4 – Distribution of cases according to the cause of death**

IHME	No. of cases	OS
I	28	62
CDs	1	
NCDs	61	28
	33	
		CV

CDs: Communicable diseases; CV: Cardiovascular; I: Injury; IHME: Institute for Health Metrics and Evaluation; NCDs: Noncommunicable diseases; NCV: Noncardiovascular; OS: Our study.

According to the international classification, two thirds of our cases died from a noncommunicable cause of death

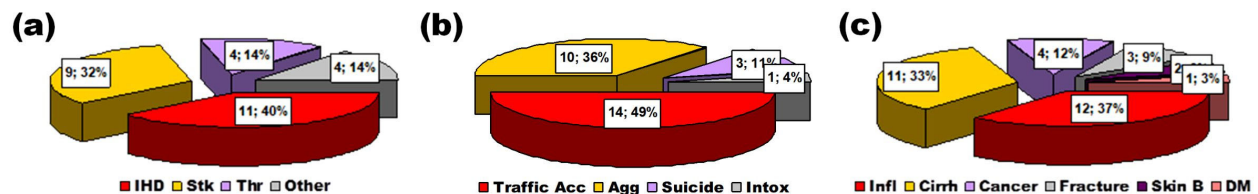
and less than one third died because of an injury. Only one case died by a communicable cause of death, namely by septicemia.

According to our classification, less than one third of the cases died from a CV cause of death and the rest died from a NCV cause of death.

In CV group, the most frequent causes of death were the ischemic heart diseases, especially myocardial infarctions, followed by strokes, especially cerebral infarctions (Figure 4a).

In the NCV group of “injury” type, the most frequent causes of death were the traffic accidents, followed by physical aggression, in both these situations the lesions were traumas and especially polytraumas (Figure 4b).

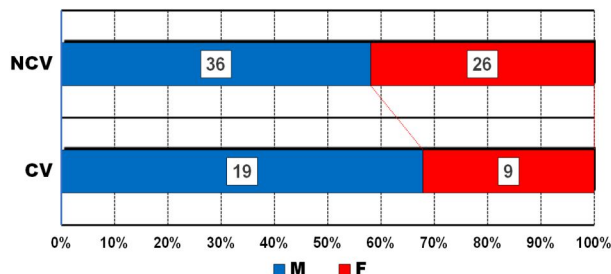
In the rest of NCV of NCD type, the most frequent causes of death were inflammatory diseases, especially those of the lung, followed by the terminal type lesions of the liver, and, at a distance by cancers, especially localized in the digestive tract, and skin burns (Figure 4c).



**Figure 4 – (a–c) Distribution of CV causes of death. Acc: Accident; Agg: Aggression; B: Burn; Cirrh: Cirrhosis; CV: Cardiovascular; DM: Diabetes mellitus; IHD: Ischemic heart disease; Infl: Inflammatory disease; Intox: Intoxication; Stk: Stroke; Thr: Thrombotic disease.**

### Sex distribution

In both groups, there were more men than women, more obviously in the CV group (Figure 5).



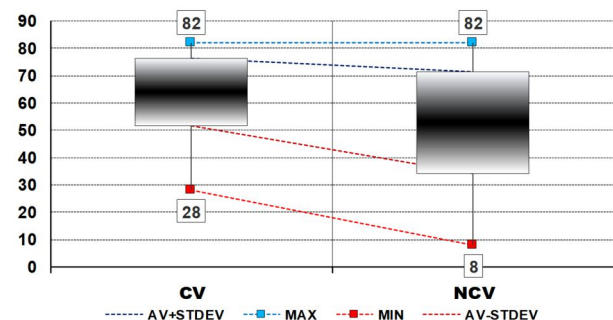
**Figure 5 – Sex distribution in the two groups. CV: Cardiovascular; F: Female; M: Male; NCV: Noncardiovascular.**

### Age distribution

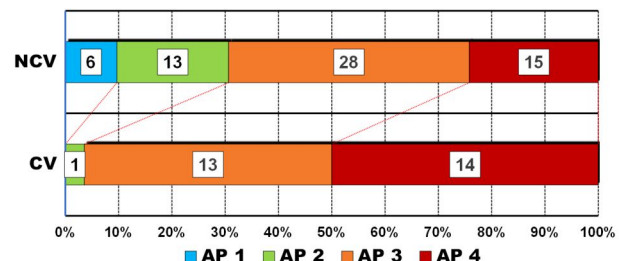
The analysis of cases' age revealed a wider age range of values and also a wider interval gathering most values (defined by the STDEV around AV) in the NCV group as compared with the CV group (Figure 6).

In the NCV group, there were cases from all APs, with almost half of them grouped in the mature adulthood (45–64 years). In the CV group, in turn, most cases had more than 45 years of life and the most numerous group was that of elderly (Figure 7).

The above-mentioned differences were enhanced by the mean age values of the two groups: 53 years in NCV group and 64 years in CV group and were validated by the statistical apparatus (Table 5).



**Figure 6 – Age distribution in the two groups. AV: Mean value; CV: Cardiovascular; MAX: Highest value; MIN: Lowest value; NCV: Noncardiovascular; STDEV: Standard deviation.**



**Figure 7 – Age period (AP) distribution in the two groups. CV: Cardiovascular; NCV: Noncardiovascular.**

**Table 5 – Statistical assessment of age**

Age	ANOVA: single factor		
	F	F crit	p-value
CV group – NCV group	8.175	3.949	0.005

ANOVA: Analysis of variance; CV: Cardiovascular; NCV: Noncardiovascular.



## Aortic diameters

The first step in our analysis was the assessment of the aortic diameters' values evolutions in the two defined groups according to the cause of death in each of the four aortic regions.

### Individual values' variations

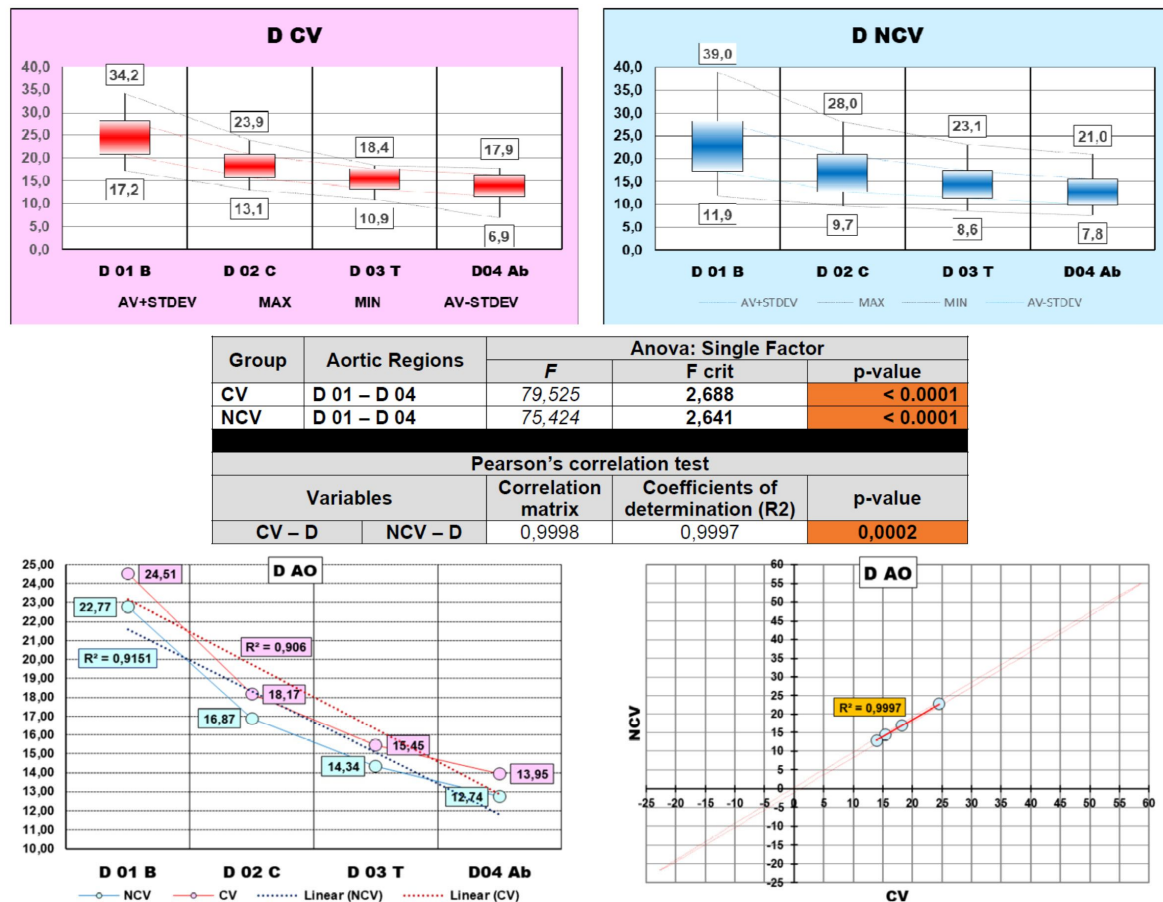
#### CV group

In CV group, the highest value was observed at the aortic base level and the lowest value was observed at the abdominal level. Ranges of values variation reduced in general their amplitude from aortic base level toward abdominal region. Both range limits decreased from one region to the other. The same trend was present also in intervals gathering most values (defined by the STDEV around AVs) which, in all regions, were obviously narrower

than the ranges of variation (Figure 8 – upper left graph).

#### NCV group

In NCV group, the situation was almost similar, but highest values of the ranges of variation in every aortic region were greater than those in the CV group and lowest values were smaller than their counterparts, resulting in larger ranges of variation of values than in CV group. Intervals gathering most values (defined by the STDEV around AVs) had the same decreasing trend in amplitude from the aortic origin toward abdominal region but they were larger in general than their counterparts in CV group (Figure 8 – upper right graph). These continuous decreases of aortic diameters from the aortic base towards the abdominal region in both groups of cases were statistically validated by the ANOVA single factor test (Figure 8 – center table, upper part).



**Figure 8 – Comparative study of aortic diameters in CV and NCV groups.** 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; ANOVA: Analysis of variance; AO: Aorta; AV: Mean value; CV: Cardiovascular; D: Diameter; MAX: Highest value; MIN: Lowest value; NCV: Noncardiovascular; STDEV: Standard deviation.

### Average values' variations

Average values of each aortic region diameters had a clear descending trend from the aortic base towards the abdominal region in both groups of cases. An interesting observation was that, in each region, the mean value of the aortic diameter was higher in the CV group than in the NCV group (Figure 8 – lower left graph; Figure 9). This direct correlation was validated by the Pearson's correlation test as very high significant (Figure 8 – lower right graph and center table, lower part).

### Comparisons between regions

#### Base region–cross region

The relationship between the diameter value evolution in the two neighbor aortic regions is a direct one, meaning that, when the diameter of the base region is increasing, the same thing is happening in the cross region. The phenomenon is similar in both CV and NCV groups. However, the correlation is more obvious in the NCV group, its slope being more elevated (Figure 10 – upper left graphs) and its correlation matrix being higher than

that of the CV group –  $0.919 > 0.634$  (Figure 10 – upper table, upper rows).

#### Base region–thoracic region

The relationship between the diameter value evolution in the base region as compared with thoracic region is also a direct one, meaning that, when the diameter of the base

region is increasing, the diameter of the thoracic region is increasing too. The trend is similar in both CV and NCV groups and is clearer in NCV group, its correlation matrix being higher than that of the CV group –  $0.907 > 0.670$  (Figure 10 – upper table, middle rows) although slopes are almost parallel (Figure 10 – upper center graphs).

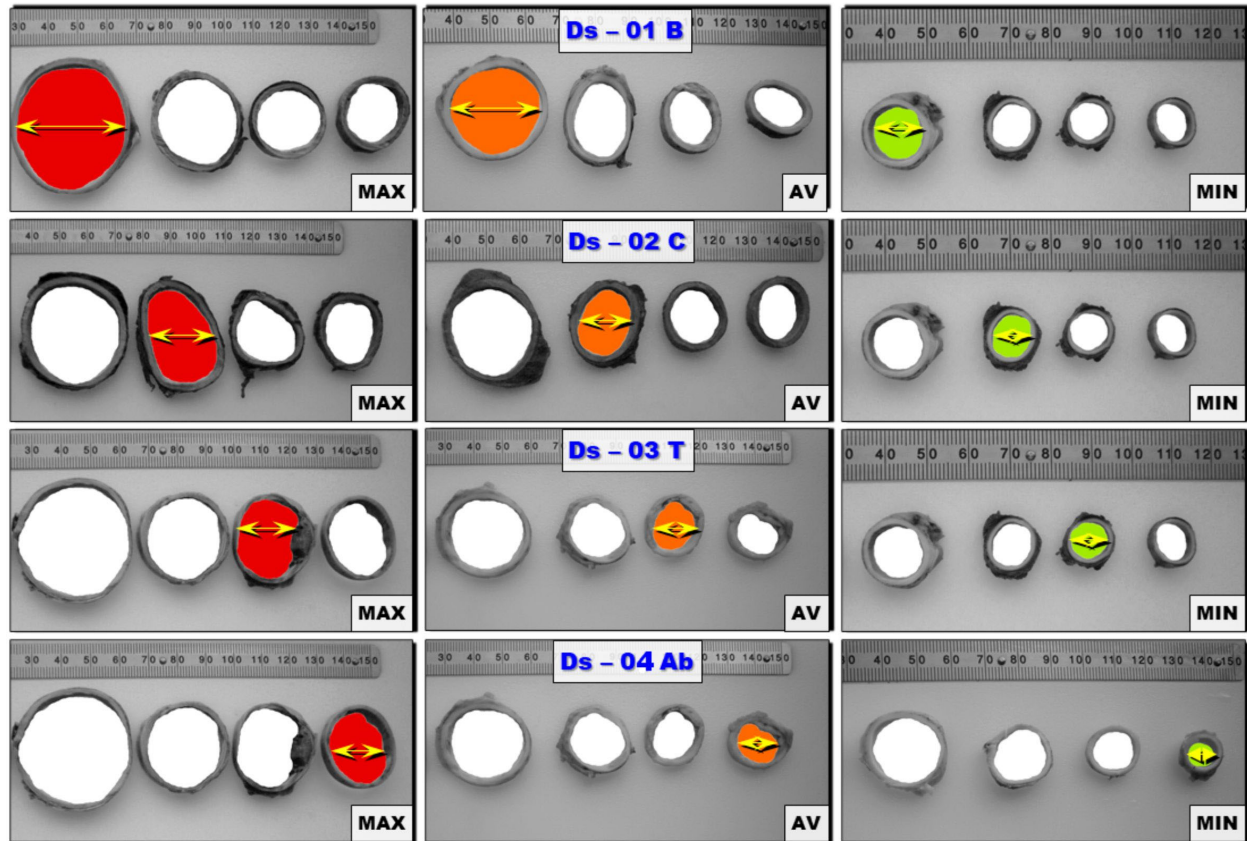


Figure 9 – Illustration of highest, mean, and lowest values of aortic diameters' determinations in the four aortic regions. MathWorks software windows. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; AV: Mean value; Ds: Diameters; MAX: Highest value; MIN: Lowest value.

#### Base region–abdominal region

The relationship between the diameter value evolution in the base region as compared with abdominal region is again a direct one meaning that, when the diameter of the base region is increasing the diameter of the abdominal region has the same behavior. Although the trend is similar in both groups, it is clearer in NCV group, where the correlation matrix is higher than that of the CV group –  $0.831 > 0.500$  (Figure 10 – upper table, lower rows) and where the slope is more elevated (Figure 10 – upper right graphs).

#### Cross region–thoracic region

The relationship between the diameter value evolution in the cross region as compared with thoracic region is a direct one, meaning that, when the diameter of the cross region is increasing, the same thing is happening in the thoracic region. The relationship has similar behavior in both CV and NCV groups, although the correlation is more obvious in the NCV group, its slope being slightly more elevated (Figure 10 – lower left graphs) and its correlation matrix being higher than that of the CV group –  $0.919 > 0.764$  (Figure 10 – lower table, upper rows).

#### Cross region–abdominal region

The relationship between the diameter value evolution in the cross region as compared with abdominal region is also a direct one, meaning that, when the diameter of the cross region is increasing, the same thing is happening in the abdominal region. The trend is similar in both CV and NCV groups but is slightly clearer in NCV group, its correlation matrix being just a little higher than that of the CV group –  $0.851 > 0.764$  (Figure 10 – lower table, middle rows) although slopes are almost parallel (Figure 10 – lower center graphs).

#### Thoracic region–abdominal region

The relationship between the diameter value evolution in the thoracic region as compared with abdominal region is, finally a direct one too. It means that, when the diameter of the thoracic region is increasing the diameter of the abdominal region has the same behavior. Although the trend is similar in both groups, it is a little bit clearer in NCV group, where the correlation matrix is higher than that of the CV group –  $0.892 > 0.796$  (Figure 10 – lower table, lower rows). However, the slopes seem to be parallel (Figure 10 – lower right graphs).

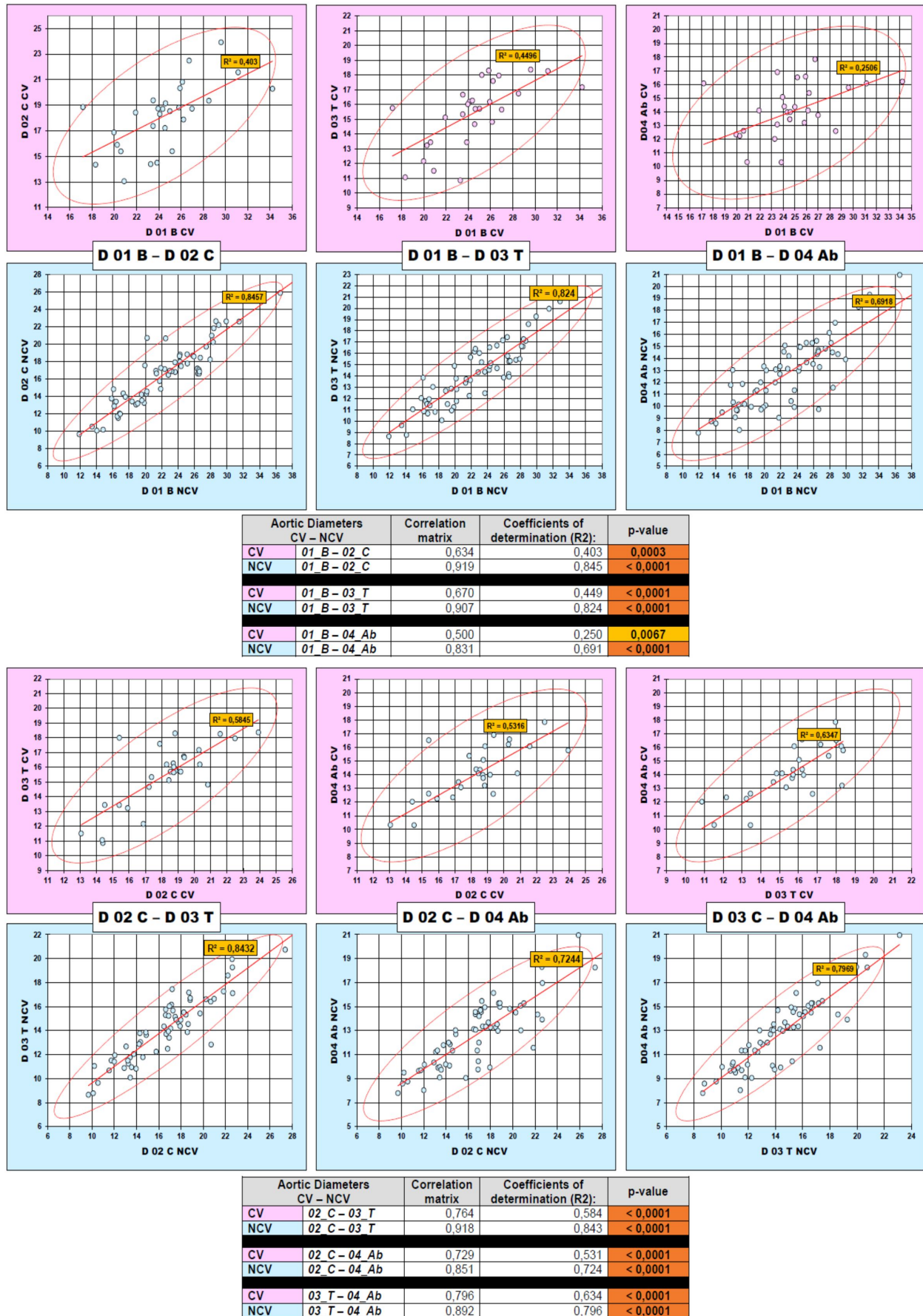


Figure 10 – Pearson's correlation test between diameters' values of different aortic levels of measurement in CV and NCV groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; D: Diameter; NCV: Noncardiovascular.



### Differences related to patients' sex

#### Comparison between sexes in each group

The analysis of aortic diameter's mean values in each aortic region, in men as compared with women showed us that, in both groups, the aortic diameters had a continuous decreasing trend in both men and women CV group from the aortic base toward the abdominal region (Figure 11 – upper part graphs). This relationship was validated by the Pearson's correlation test as a very significant one (Figure 11 – center table and lower part graphs). Another interesting observation was that, in each aortic region, the mean value of the aortic diameter was higher in men than in women both in the CV group and in the NCV group (Figure 11 – upper part graphs).

#### Comparison between groups in each sex

The analysis of aortic diameter's mean values in each aortic region, in CV group as compared with NCV group revealed that, in both sexes, the aortic diameters had a continuous decreasing trend in both CV and NCV groups from the aortic base toward the abdominal region (Figure 12 – upper part graphs). This relationship was validated by the Pearson's correlation test as a very significant one (Figure 12 – center table and lower part graphs). Another interesting observation was that, in each aortic region, the mean value of the aortic diameter was higher in CV group than

in NCV group both in men and women (Figure 12 – upper part graphs).

### Tunica intima

The second step in our analysis was the assessment of the *tunica intima* thickness values evolutions in the two groups according to the cause of death in each of the four aortic regions.

#### Individual values' variations

##### CV group

In CV group, the highest value was observed at the aortic abdominal level and the lowest value was observed at the base level. Ranges of values variation enlarged in general their amplitude from aortic base level toward abdominal region. Both range limits increased from one region to the other. The same trend was present also in intervals gathering most values (defined by the STDEV around AVs) which, in all regions, were obviously narrower than the ranges of variation (Figure 13 – upper left graph; Figure 14 – upper part).

##### NCV group

In NCV group, the situation was almost similar, but highest values of the ranges of variation in every aortic region were greater than those in the CV group and lowest values were smaller than their counterparts, resulting in larger ranges of variation of values than in CV group.

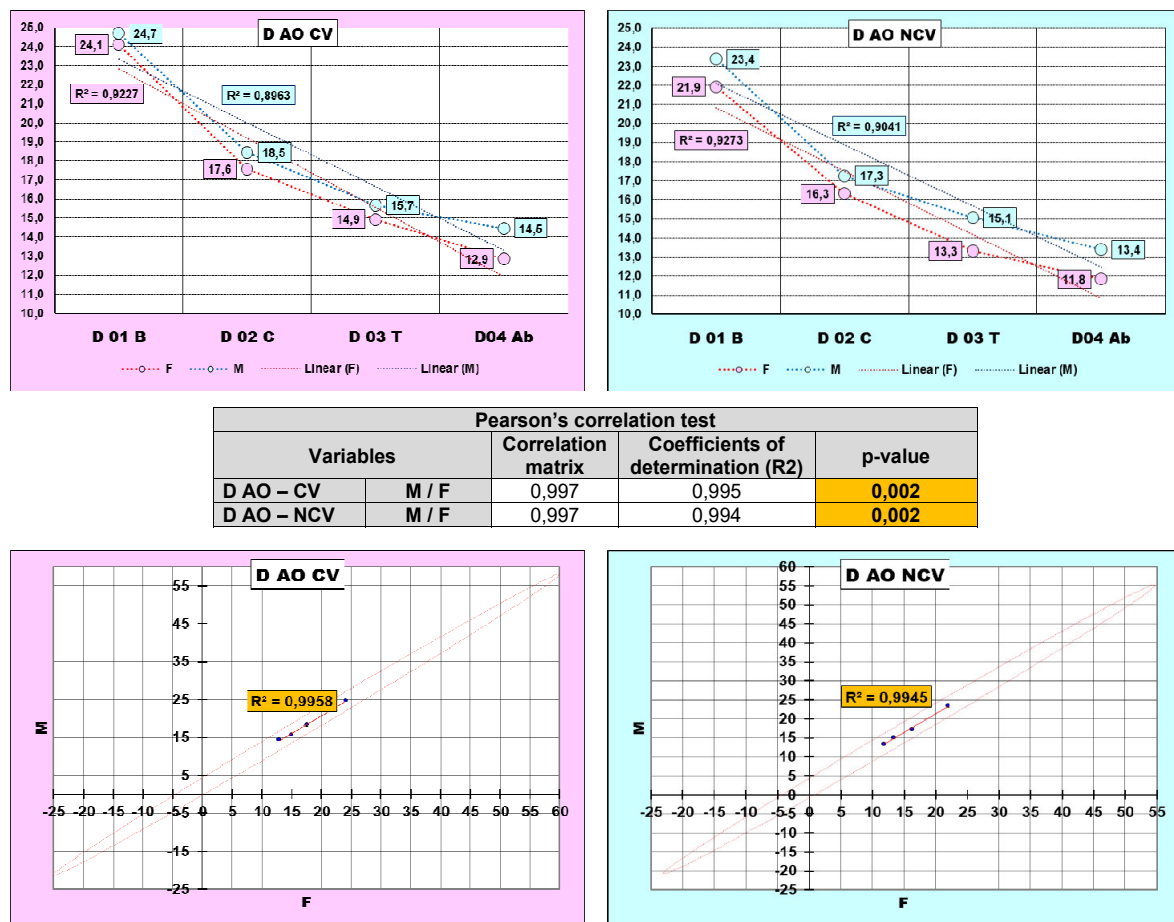


Figure 11 – Comparative study of aortic diameters' mean values between men and women in the two groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; AO: Aorta; CV: Cardiovascular; D: Diameter; F: Female; M: Male; NCV: Noncardiovascular.

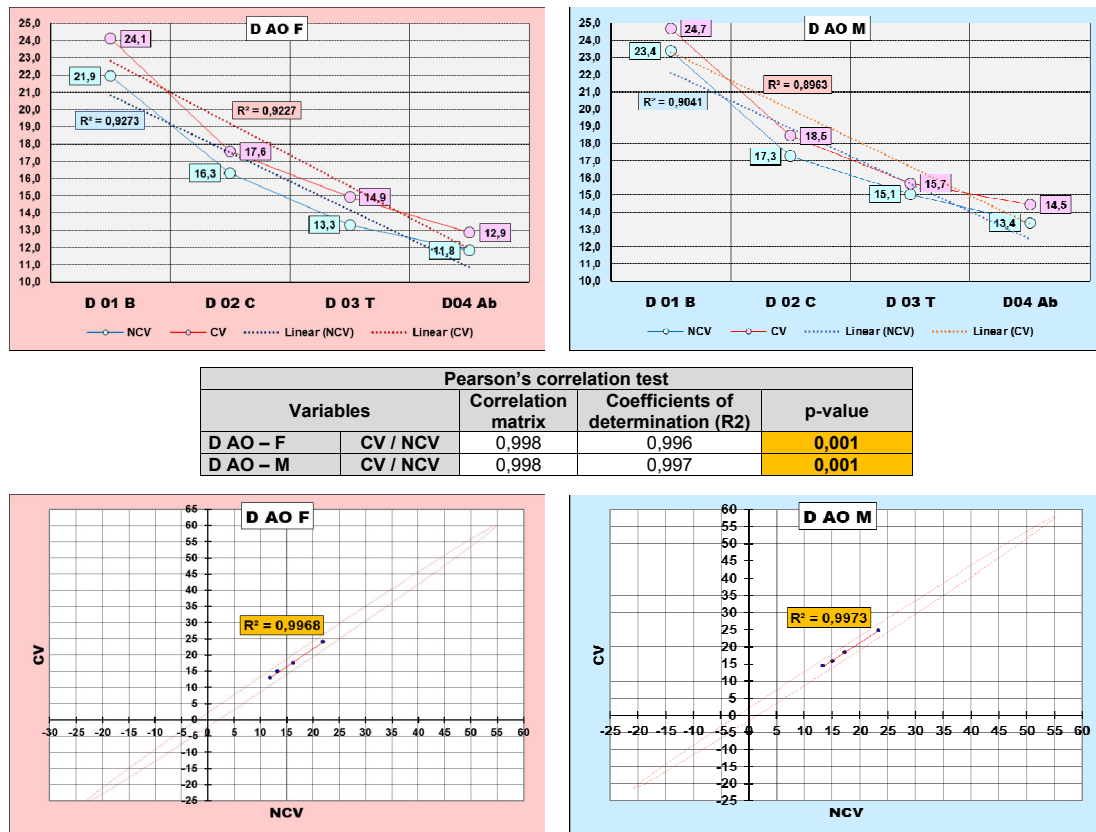


Figure 12 – Comparative study of aortic diameters' mean values between the two groups in men and women. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; AO: Aorta; CV: Cardiovascular; D: Diameter; F: Female; M: Male; NCV: Noncardiovascular.

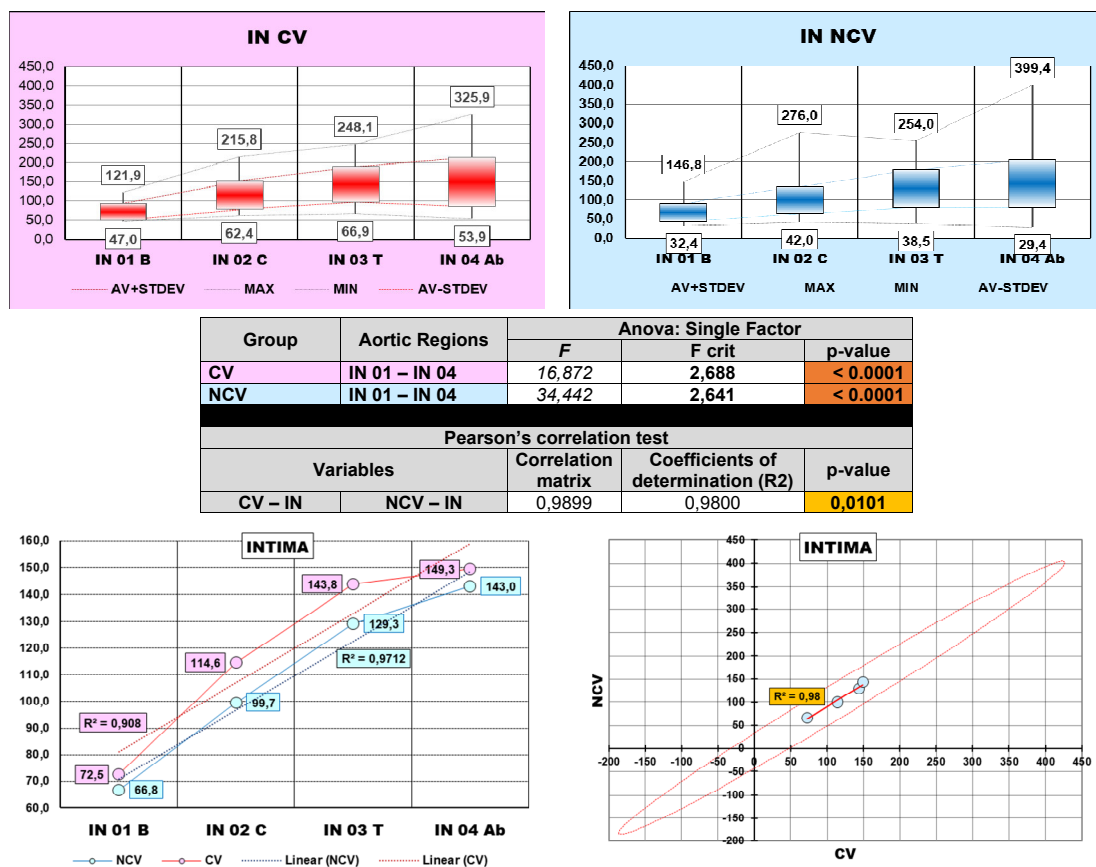
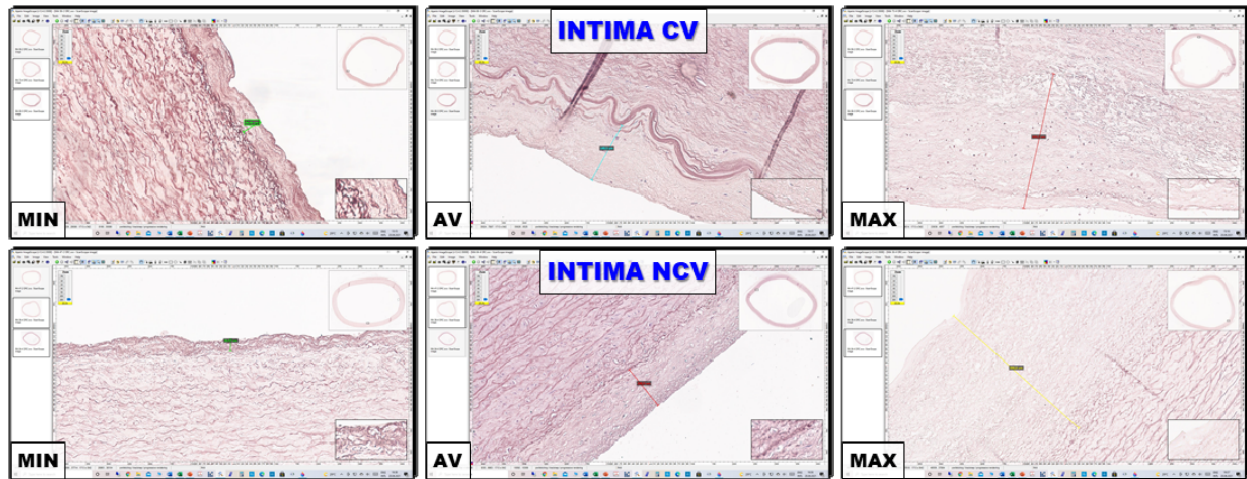


Figure 13 – Comparative study of intima layers between CV and NCV groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; ANOVA: Analysis of variance; AV: Mean value; CV: Cardiovascular; IN: Intima; MAX: Highest value; MIN: Lowest value; NCV: Noncardiovascular; STDEV: Standard deviation.

Intervals gathering most values (defined by the STDEV around AVs) had the same increasing trend in amplitude from the aortic origin toward abdominal region and they were almost similar in general with their counterparts in CV group (Figure 13 – upper right graph; Figure 14 – lower part).

These continuous increasing trends of intima thickness from the aortic base towards the abdominal region in both groups of cases were statistically validated by the ANOVA single factor test (Figure 13 – center table, upper part).



**Figure 14 – Illustration of highest, mean, and lowest values of intima's thickness determinations in the two groups. AV: Mean value; CV: Cardiovascular; MAX: Highest value; MIN: Lowest value; NCV: Noncardiovascular.**

#### Average values' variations

Average values of each aortic region diameters had a clear descending trend from the aortic base towards the abdominal region in both groups of cases. An interesting observation was that, in each region, the mean value of the intima thickness was higher in the CV group than in the NCV group (Figure 13 – lower left graph). This direct correlation was validated by the Pearson's correlation test as very high significant (Figure 13 – lower right graph and center table, lower part).

#### Comparisons between regions

##### Base region–cross region

The relationship between the intima thickness value evolution in the two neighbor aortic regions is a direct one, meaning that, when the intima of the base region is thickening, the same thing is happening in the cross region. The phenomenon is similar in both CV and NCV groups. However, the correlation is more obvious in the CV group, its slope being more elevated (Figure 15 – upper left graphs) and its correlation matrix being higher than that of the NCV group –  $0.524 > 0.253$  (Figure 15 – upper table, upper rows).

##### Base region–thoracic region

The relationship between the intima thickness value evolution in the base region as compared with thoracic region presented a tendency to a direct correlation, meaning that, when the intima of the base region is thickening, the intima of the thoracic region has the tendency to follow it. The trend is similar in both CV and NCV groups but is clearer in NCV group, its correlation matrix being higher than that of the CV group –  $0.198 > 0.123$  (Figure 15 – upper table, middle rows). The slope is also slightly more elevated in NCV group (Figure 15 – upper center graphs). However, the relationship was not validated

from statistical point of view,  $p$ -values of Pearson's correlation test being  $> 0.05$  in both groups (Figure 15 – upper table, middle rows).

##### Base region–abdominal region

The relationship between the intima thickness value evolution in the base region as compared with abdominal region revealed some particularities. Thus, in CV group, the intima thickness value of the base was in an inverse relation with the intima thickness value of the abdominal region, meaning that when the values of one increase, the values of the other decrease and vice versa (Figure 15 – upper right graphs). This is expressed by the negative value of the correlation matrix:  $-0.376$ . The relationship was also validated by the Pearson's correlation test (Figure 15 – upper table, lower rows). In turn, in the NCV group, there was no relationship between the evolution of intimal thickness in the two regions (Figure 15 – upper right graphs), meaning that they are evolving independently, fact confirmed by the Pearson's correlation test (Figure 15 – upper table, lower rows).

##### Cross region–thoracic region

The relationship between the intimal thickness value evolution in the cross region as compared with thoracic region is a direct one, meaning that, when the intimal layer of the cross region is thickening, the same thing is happening in the wall of the thoracic aorta. The relationship has similar behavior in both CV and NCV groups, with parallel slopes in both groups (Figure 15 – lower left graphs) and almost equal correlation matrix –  $0.363$  vs.  $0.351$  (Figure 15 – lower table, upper rows).

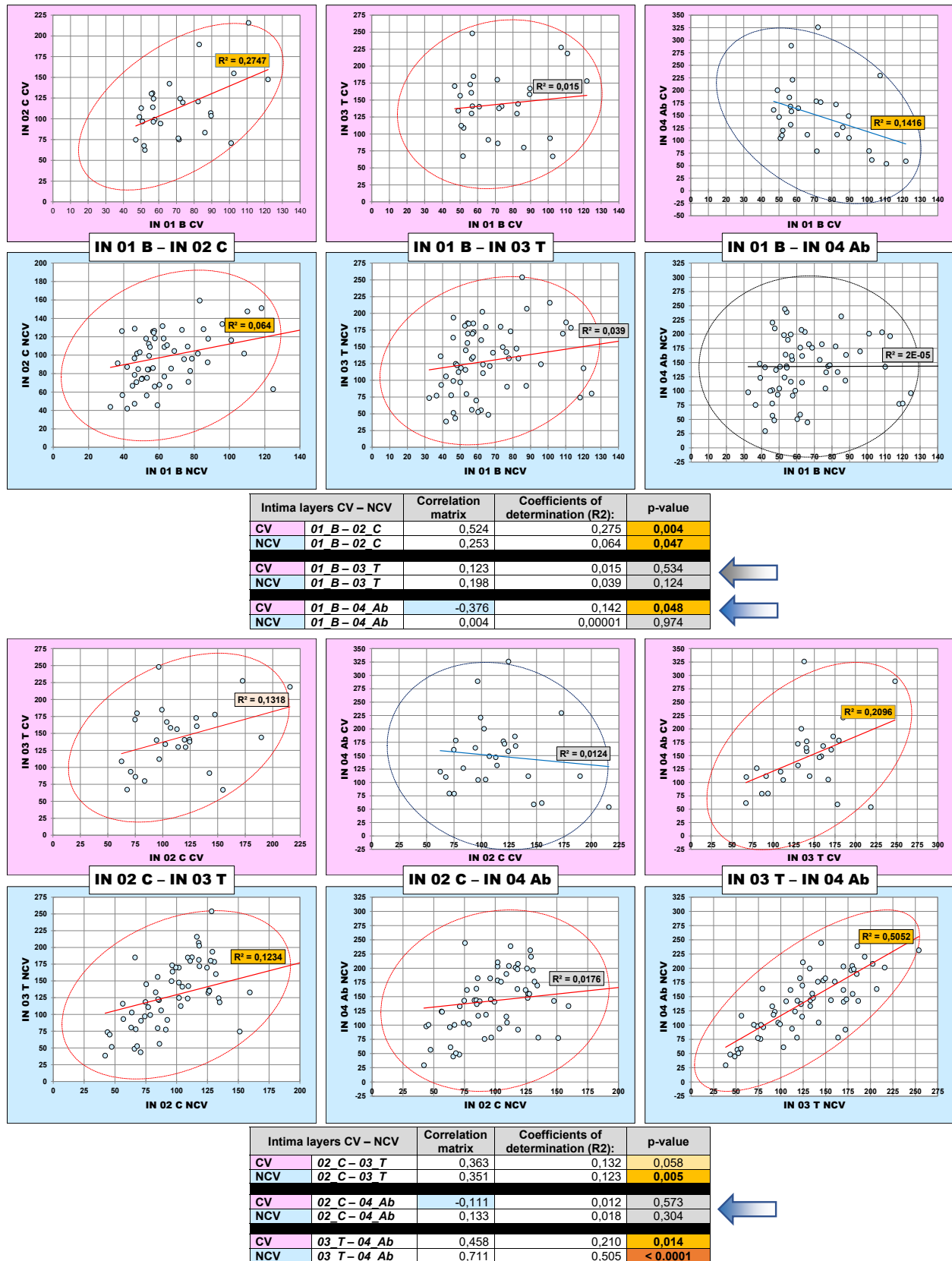
##### Cross region–abdominal region

The relationship between the intimal thickness value evolution in the cross region as compared with abdominal



region revealed also a particular situation. Thus, in CV group, the intima thickness value of the cross region expressed a tendency to an inverse relation with the intima thickness value of the abdominal region, meaning that

when the values of one increase, the values of the other tend to decrease and vice versa (Figure 15 – lower center graphs). This is expressed by the negative value of the correlation matrix: -0.111.



**Figure 15 – Pearson's correlation test between Intima thicknesses of different Aortic levels of measurement in CV and NCV groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; ANOVA: Analysis of variance; AV: Mean value; CV: Cardiovascular; IN: Intima; NCV: Noncardiovascular.**

The relationship was, however, not validated by the Pearson's correlation test (Figure 15 – lower table, middle rows).

In turn, in NCV group, the relationship between the intima thickness value evolution in the cross region as compared with abdominal region presented only a tendency to a direct correlation, meaning that, when the intima of the cross region is thickening, the intima of the abdominal region has the tendency to follow it (Figure 15 – lower center graphs).

However, the relationship was not validated from statistical point of view,  $p$ -values of Pearson's correlation test being  $>0.05$  in both groups (Figure 15 – lower table, middle rows).

### Thoracic region–abdominal region

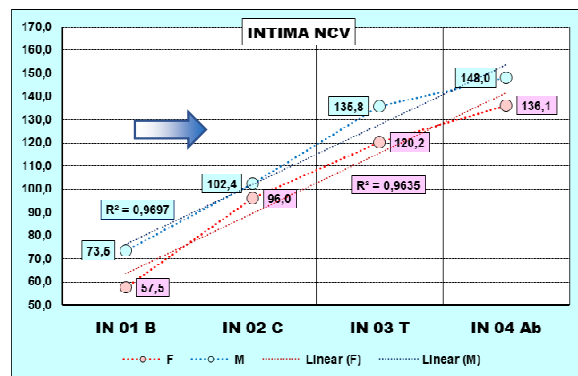
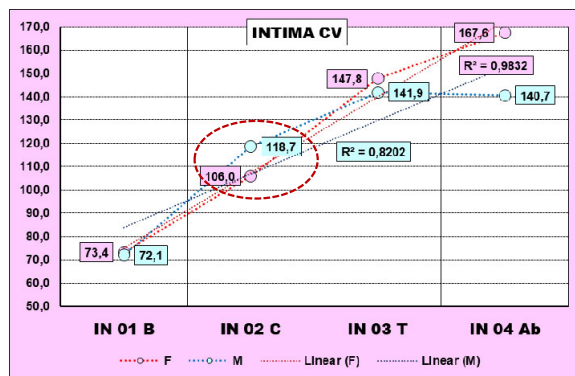
The relationship between the intimal thickness value evolution in the thoracic region as compared with abdominal region is, finally a direct one too. It means that, when the intimal thickness of the thoracic region is increasing the intimal thickness of the abdominal region will have the same behavior. Although the trend is similar in both groups, it is a little bit clearer in NCV group, where the correlation matrix is higher than that of the CV group –

$0.714 > 0.458$  (Figure 15 – lower table, lower rows), and the slope seem to be more elevated (Figure 15 – lower right graphs). In both groups, Pearson's correlation test validated the observed trends (Figure 15 – lower table, lower rows).

### Differences related to patients' sex

#### Comparison between sexes in each group

The analysis of intimal thickness's mean values in each aortic region, in men as compared with women showed us that, in both groups, the intimal thickness had a continuous increasing trend in both men and women from the aortic base toward the abdominal region (Figure 16 – upper part graphs). This relationship was validated by the Pearson's correlation test as a very significant one only for the NCV group. In CV group,  $p$ -values of the Pearson's correlation test were very close but still  $>0.05$  (Figure 16 – center table and lower part graphs). There were, however, some particular aspects to be mentioned. Thus, in CV group intimal thickness mean values were higher in women than in men in all aortic regions excepting the cross region where intima was thicker in men. In turn, in the NCV group, intimal intima was thicker in men than in women at all levels of measurement (Figure 16 – upper part graphs).



Pearson's correlation test			
Variables		Correlation matrix	Coefficients of determination (R <sup>2</sup> )
INTIMA – CV	M / F	0.935	0.875
INTIMA – NCV	M / F	0.991	0.982
			p-value
			0.064
			0.008

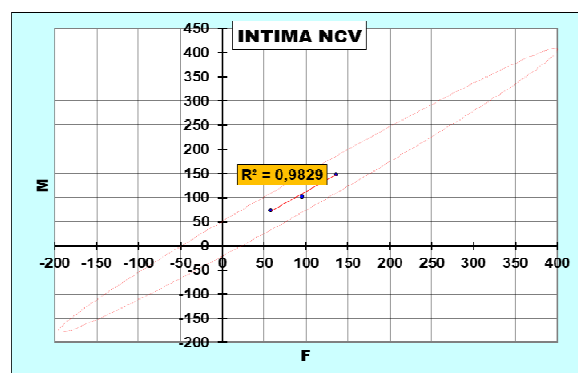
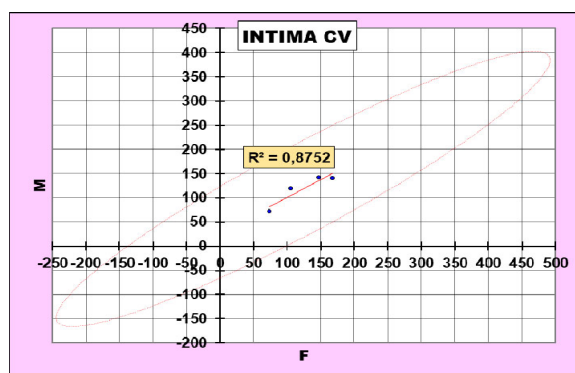


Figure 16 – Comparative study of intima thicknesses' mean values between men and women in the two groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; F: Female; IN: Intima; M: Male; NCV: Noncardiovascular.

Comparison between groups in each sex. The analysis of intimal thickness's mean values in each aortic region, in CV group as compared with NCV group revealed that, in both sexes, the intimal thickness had a continuous

increasing trend in both CV and NCV groups from the aortic base toward the abdominal region (Figure 17 – upper part graphs).

This relationship was validated by the Pearson's

correlation test as a very significant one (Figure 17 – center table and lower part graphs).

There was, however, a particular aspect to be mentioned, here too. Thus, whereas in women, intimal thickness mean

values were higher in CV group than in NCV group in all aortic regions, in men, intimal thickness mean values were higher (slightly, is true) at the extremities of the aorta, namely in base and abdominal regions (Figure 17 – upper part graphs).

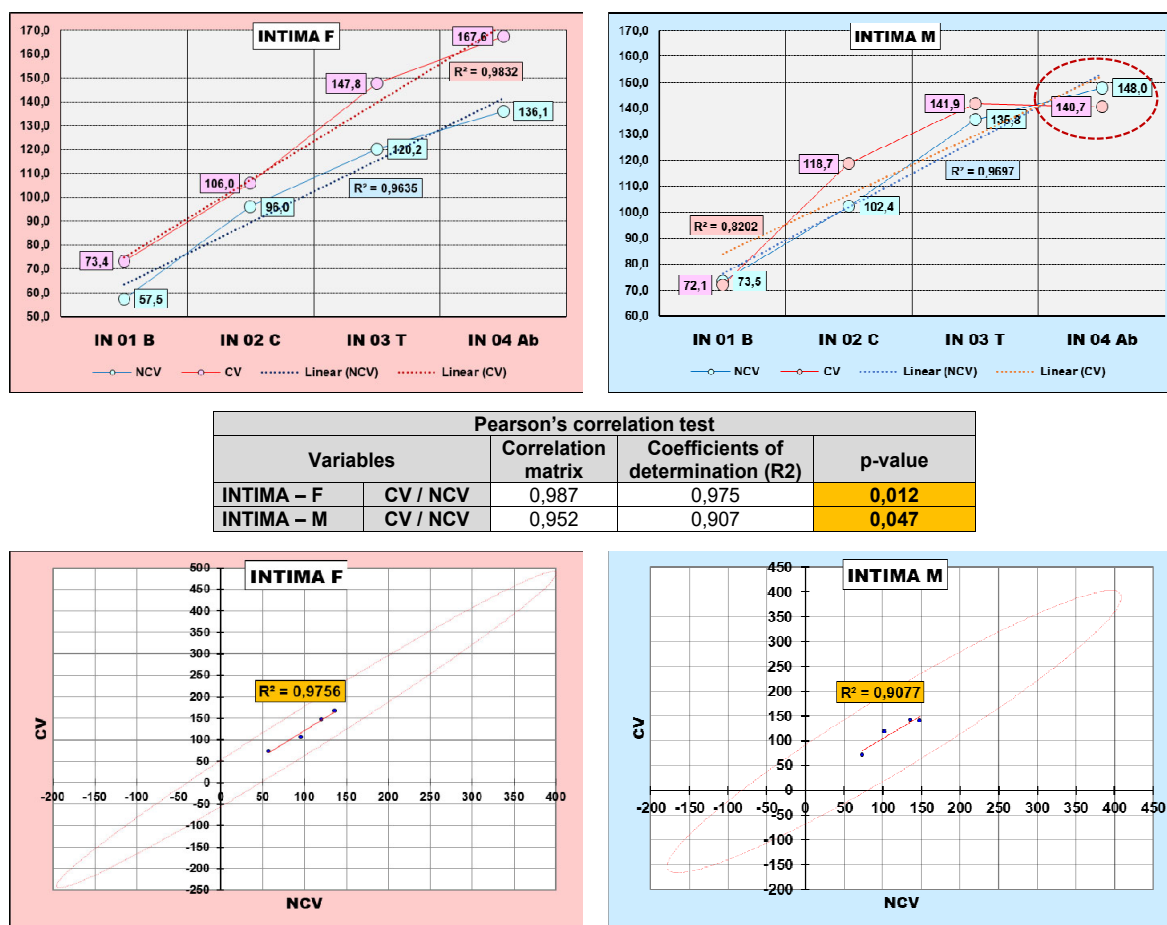


Figure 17 – Comparative study of intima thicknesses' mean values between the two groups in men and women. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; F: Female; IN: Intima; M: Male; NCV: Noncardiovascular.

## Tunica media

The third step in our analysis was the assessment of the *tunica media* thickness values evolutions in the two groups according to the cause of death in each of the four aortic regions.

### Individual values' variations

#### CV group

In CV group, the highest value was observed at the aortic base level and the lowest value was observed at the abdominal level. Ranges of values variation reduced slightly their amplitude from aortic base level toward cross region then remained almost constant. High range limit decreased from one region to the other whereas lowest range limit increased towards cross region then decreased constantly towards abdominal region where it reached a value lower than in the base region. Intervals gathering most values (defined by the STDEV around AVs) were, in all regions, obviously narrower than the ranges of variation but they reduced just a little bit their amplitude from base region to abdominal region (Figure 18 – upper left graph).

#### NCV group

In NCV group, the situation was almost similar, but highest values of the ranges of variation in every aortic region were greater than those in the CV group and lowest values were smaller than their counterparts, resulting in larger ranges of variation of values than in CV group. Intervals gathering most values (defined by the STDEV around AVs) were also narrower than the ranges of values variation but they were larger in general than their counterparts in CV group. However, they had no significant variation in amplitude from the aortic origin toward abdominal region (Figure 18 – upper right graph). These continuous decreasing trends of media thickness from the aortic base towards the abdominal region in both groups of cases were statistically validated by the ANOVA single factor test (Figure 18 – center table, upper part).

### Average values' variations

Average values of media thickness in each aortic region had a clear descending trend from the aortic base towards the abdominal region in both groups of cases. An interesting observation was that, in each region, the mean value of the media thickness was higher in the CV



group than in the NCV group excepting the base region where media thickness men value was higher in NCV group than in the CV group (Figure 18 – lower left graph; Figure 19). Because of this isolated shift, Pearson's

correlation test almost validated this direct relationship from statistical point of view (Figure 18 – lower right graph and center table, lower part).

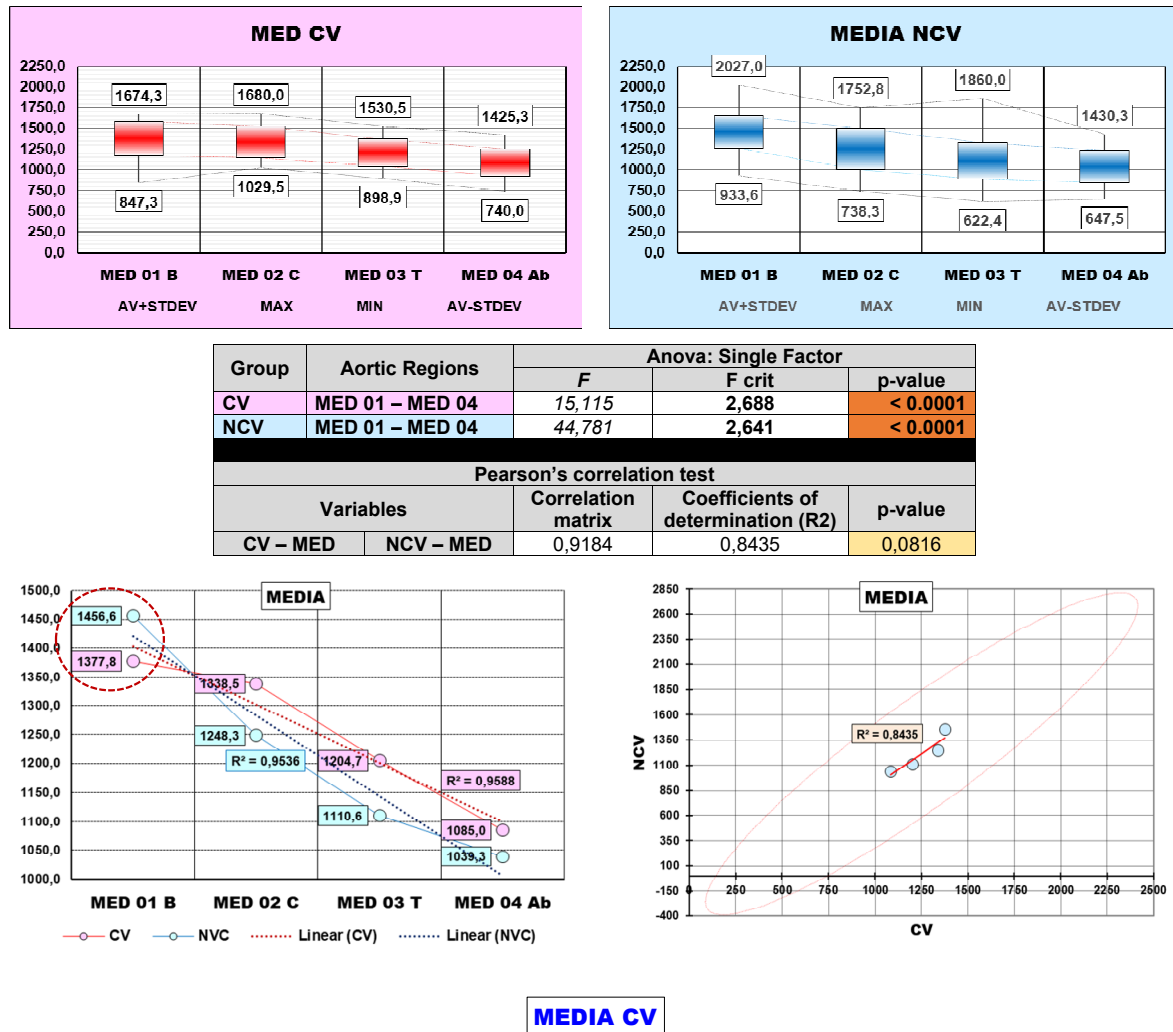


Figure 18 – Comparative study of intima layers between CV and NCV groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; ANOVA: Analysis of variance; AV: Mean value; CV: Cardiovascular; IN: Intima; MAX: Highest value; MED: Media; MIN: Lowest value; NCV: Noncardiovascular; STDEV: Standard deviation.

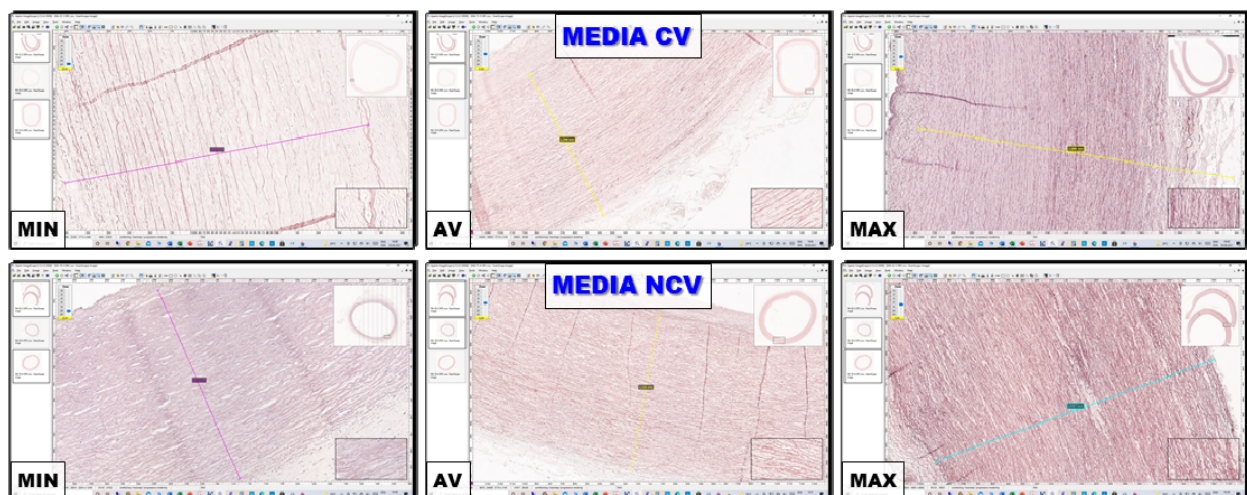


Figure 19 – Illustration of highest, mean, and lowest values of media's thickness determinations in the two groups. AV: Mean value; CV: Cardiovascular; MAX: Highest value; MIN: Lowest value; NCV: Noncardiovascular.

### Comparisons between regions

#### Base region–cross region

The relationship between the media thickness value evolution in the two neighbor aortic regions is a direct one, meaning that, if the media of the base region is thickening, the same thing is happening with the media of

the cross region. The phenomenon is almost the same in both CV and NCV groups. However, the correlation is slightly more obvious in the CV group, its slope being more elevated (Figure 20 – upper left graphs) and its correlation matrix being higher than that of the NCV group –  $0.577 > 0.472$  (Figure 20 – upper table, upper rows).

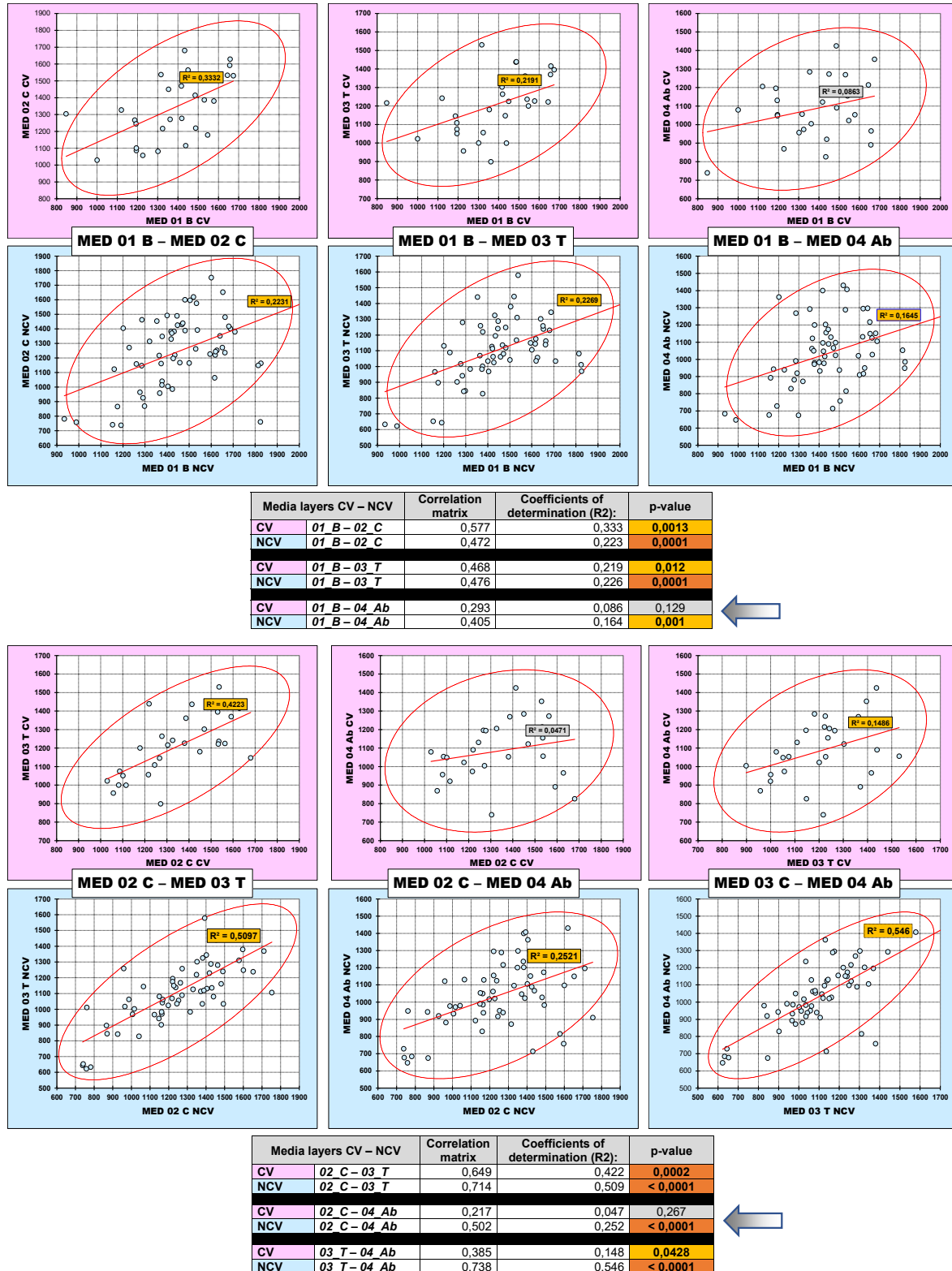


Figure 20 – Pearson's correlation test between media thicknesses of different aortic levels of measurement in CV and NCV groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; MED: Media; NCV: Noncardiovascular.

### Base region–thoracic region

The relationship between the media thickness value evolution in the base region as compared with thoracic region is also a direct one, meaning that, when the media of the base region is thickening, the media of the thoracic region is thickening too. The trend is similar in both CV and NCV groups, with correlation matrix almost equal –  $0.468 < 0.476$  (Figure 20 – upper table, middle rows) and slopes almost parallel (Figure 20 – upper center graphs).

### Base region–abdominal region

The relationship between the media thickness value evolution in the base region as compared with abdominal region is somehow particular. In the CV group, there was only a tendency to a direct correlation, meaning that, when the media of the base region is thickening, the media of the abdominal region has the tendency to thicken (the  $p$ -value of the Pearson's correlation test was  $>0.05$ ). In turn, in the NCV group, there is a statistically validated direct relationship between the media thickness value evolution in the base region as compared with abdominal region (the  $p$ -value of the Pearson's correlation test was  $<0.05$ ). Although the trend is similar in both groups, it is clearer in NCV group, where the correlation matrix is

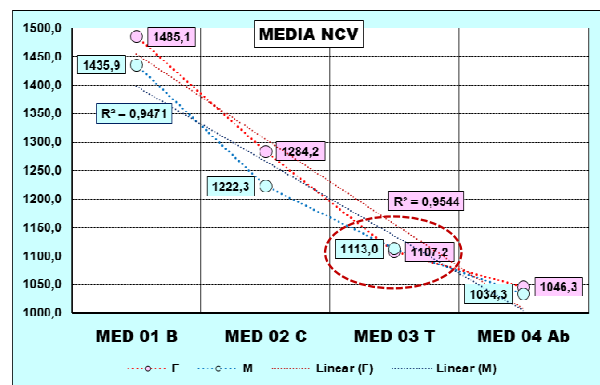
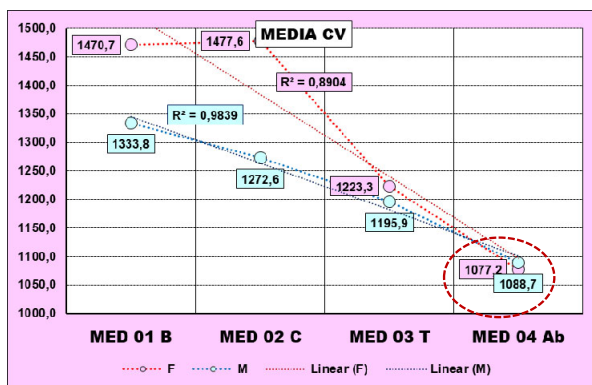
higher than that of the CV group –  $0.405 > 0.293$  (Figure 20 – upper table, lower rows) and where the slope is more elevated (Figure 20 – upper right graphs).

### Cross region–thoracic region

The relationship between the media thickness value evolution in the cross region as compared with thoracic region is a direct one, meaning that, when the media of the cross region is thickening, the same thing is happening in the thoracic region. The relationship has similar behavior in both CV and NCV groups, with slopes almost parallel (Figure 20 – lower left graphs) and correlation matrix almost equal –  $0.649$  vs.  $0.714$  (Figure 20 – lower table, upper rows).

### Cross region–abdominal region

The relationship between the media thickness value evolution in the base region as compared with abdominal region is also somehow particular. In the CV group, there is only a tendency to a direct correlation, meaning that, when the media of the cross region is thickening, the media of the abdominal region has the tendency to thicken (the  $p$ -value of the Pearson's correlation test was  $>0.05$ ).



Pearson's correlation test				
Variables		Correlation matrix	Coefficients of determination (R2)	p-value
MEDIA – CV	M / F	0,959	0,921	0,040
MEDIA – NCV	M / F	0,993	0,986	0,007

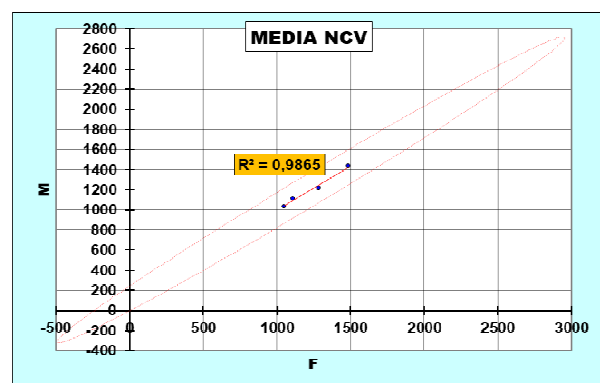
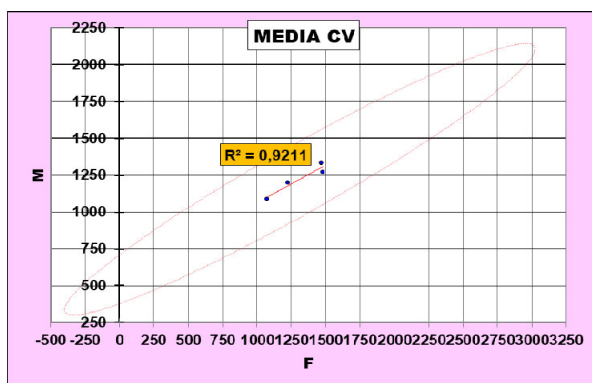


Figure 21 – Comparative study of media thicknesses' mean values between men and women in the two groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; F: Female; MED: Media; M: Male; NCV: Noncardiovascular.

In turn, in the NCV group, there is a statistically validated direct relationship between the media thickness value evolution in the cross region as compared with

abdominal region (the  $p$ -value of the Pearson's correlation test was  $<0.05$ ). Although the trend is similar in both groups, it is clearer in NCV group, where the correlation



matrix is higher than that of the CV group –  $0.502 > 0.217$  (Figure 20 – lower table, middle rows) and where the slope is more elevated (Figure 20 – lower center graphs).

### Thoracic region–abdominal region

The relationship between the media thickness value evolution in the thoracic region as compared with abdominal region is, finally, a direct one too. It means that, when the media of the thoracic region is thickening the media of the abdominal region has the same behavior. Although the trend is similar in both groups, it is a clearer in NCV group, where the correlation matrix is higher than that of the CV group –  $0.738 > 0.385$  (Figure 20 – lower table, lower rows) and the slope clearly more elevated (Figure 20 – lower right graphs).

### Differences related to patients' sex

#### Comparison between sexes in each group

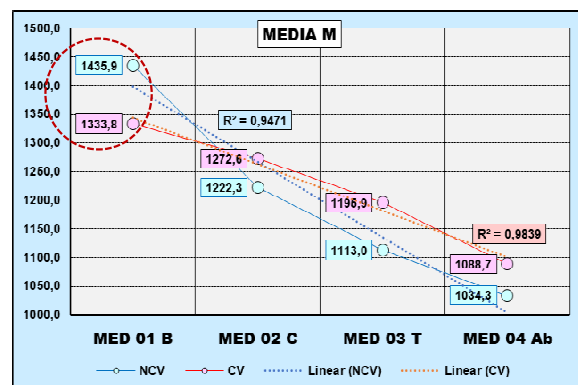
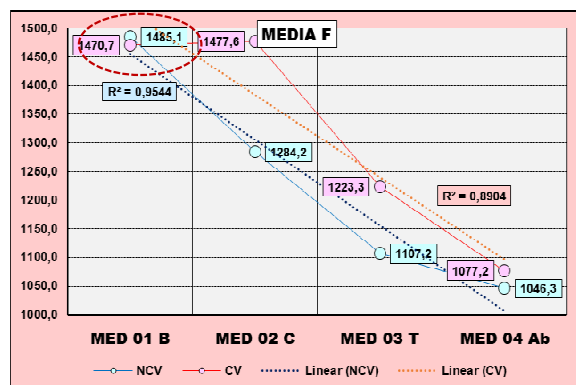
The analysis of aortic media's thickness mean values in each aortic region, in men as compared with women showed us that, in both groups, the media thickness had a continuous decreasing trend in both men and women from the aortic base toward the abdominal region (Figure 21 – upper part graphs). This relationship was validated by the Pearson's correlation test as a very significant one (Figure 21 – center table and lower part graphs). However, there is an interesting observation to be mentioned. In each

aortic region, the mean value of the media thickness was higher in women than in men both in the CV group and in the NCV group with two exceptions: in abdominal region of the CV group and in the thoracic region of the NCV group, media thickness mean values were shifted, being slightly higher in men than in women (Figure 21 – upper part graphs).

#### Comparison between groups in each sex

The analysis of media's thickness mean values in each aortic region, in CV group as compared with NCV group revealed that, in both sexes, the media thickness had a continuous decreasing trend in both CV and NCV groups from the aortic base toward the abdominal region (Figure 22 – upper part graphs). However, this relationship was not validated by the Pearson's correlation test in females, and it was almost validated in males (Figure 22 – center table and lower part graphs).

This lack of validation was determined, probably by a particularity of the evolutionary trends in CV group as compared with NCV group both in men and women. Thus, in each aortic region, the mean value of the media thickness was higher in women than in men both in the CV group and in the NCV group with two exceptions: in base regions of both sexes, media thickness mean values were shifted, being higher in men than in women (Figure 22 – upper part graphs).



Pearson's correlation test				
Variables		Correlation matrix	Coefficients of determination (R²)	p-value
MEDIA – F	CV / NCV	0,890	0,792	0,109
MEDIA – M	CV / NCV	0,938	0,880	0,061

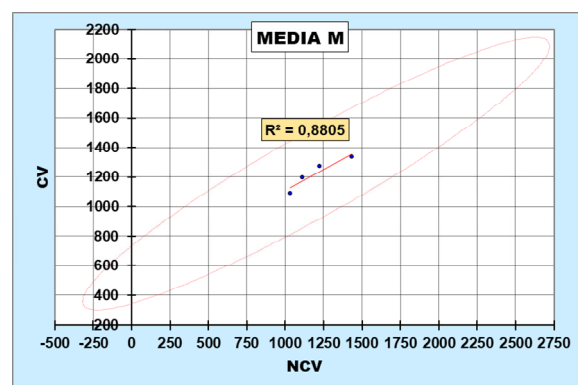
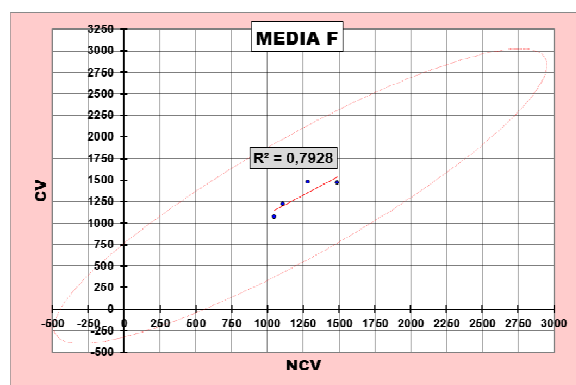


Figure 22 – Comparative study of media thicknesses' mean values between the two groups in men and women. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; F: Female; MED: Media; M: Male; NCV: Noncardiovascular.

## Discussions

The existence of some variations in the rate of aortic enlargement both in length and the breadth, both between men and women and from decade to decade, process that continues well in the elderly period of life is a reality [20]. In our study we tried to investigate one of the multitudes of these aspects meaning the changes of the dimensions of one of the most important arteries both along its topographical segments but also in relation with the patient's cause of death.

### Causes of death

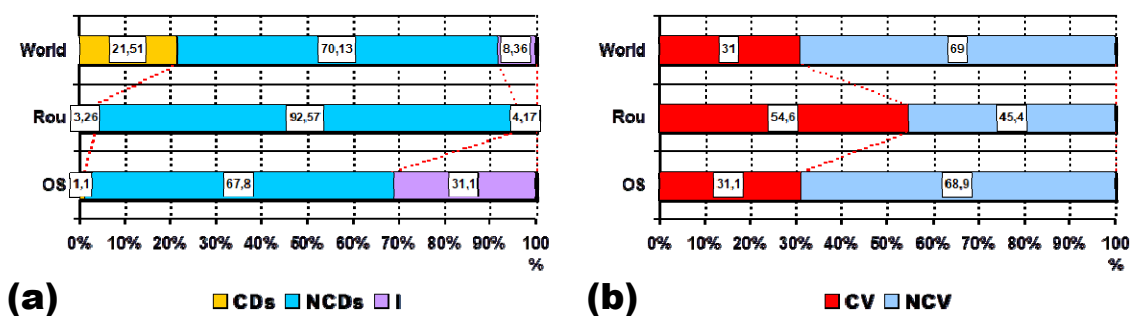
Causes of death are quite numerous and extremely diverse. International groups of experts organized all this "jungle" of entities, defining main categories, group, and subgroups [39]. From all cause of death, CV diseases are by far and for some time, the number one cause of

morbidity and mortality all over the world but specially in the western world [40, 44].

We tried to compare our data concerning the cause of death with official data from the period we gathered our study group using two classifications.

The first was the international classification of causes of death by categories. The composition of our group, with only one case of CD and numerous injuries could be explained on one hand, by the specific of our Medical Institution, which had not a Department of Infectious Diseases and, on the other hand, because our Prosecture Department was used also by the local Institute of Forensic Medicine and so we had access to younger people, usually died in car accidents or following aggressions (Figure 23a).

The second was our classification in CV diseases and NCV diseases. Following this classification, our group fitted to the situation reported for the entire world and not with that reported officially for our country (Figure 23b).



**Figure 23 – Comparison of causes of death distribution with other data: (a) Comparison using International Classification of causes of death [29]; (b) Comparison using our classification – data are from [29].** CDs: Communicable diseases; CV: Cardiovascular; I: Injuries; NCDs: Noncommunicable diseases; NCV: Noncardiovascular; OS: Our study; Rou: Romania.

### Diameters

It is well known that elastic arteries, including aorta, tend to be large-diameter vessels close to the heart [45].

In our study, aortic diameter decreased continuously and constantly from the aortic origin till the aortic end.

Aorta was larger in any of its segments in men than in women, irrespective their cause of death and in people who died of CV diseases than in those died of any other cause irrespective their sex.

The correlation degree between changes in the values of the diameters of different segments of the aorta is much stronger and more stable along the aorta in NCV group than in CV group. Whereas in NCV group this degree of correlation between the changes in the enlargement of different aortic segments has a slight trend to decrease towards the aortic end, in CV group, the degree of correlation has an increasing trend towards the aortic end (Figure 24).

Therefore, the difference in the remodeling of the aortic diameter between the two groups is smaller towards the abdominal region.

The degree of correlation has the lowest value in both groups when compared the extremities of the aorta (base region with abdominal region), being weaker in CV group.

### Intima

Due to its small thickness in young arteries, the intima is usually neglected when considering the different layer

contributions to the global mechanical resistance of the vessel wall [45]. Moreover, there are studies that are proving that arterial wall thickness is depending on intimal layer changes [46].

Therefore, one of the goals of our study was to assess intima's thickness along different topographical regions of the aorta.

We observed that intima thickness increased continuously and constantly from the aortic origin till the aortic end. Intima thickness was larger especially in women died of CV diseases as compared with those died of any other cause whereas in men there were some shifts at the extremities of the aorta, intima becoming thicker in those died from any other cause of death than in those died of CV diseases.

Another particularity was that intima was thicker in any aortic segment in men than in women died of NCV diseases whereas in the group died of CV diseases, intima was in general thicker in women than in men.

The analysis of the graph representing the correlation degree between changes in the values of the intimal thickness of different segments of the aorta is rather complicated than in case of aortic diameters.

Thus, in both groups the degree of correlation is very fluctuating and not stable not only within each group but also when compare the two groups. Thus, in some aortic regions, the correlation degree is stronger in CV group than in NCV group whereas in other regions the situation is reversed.

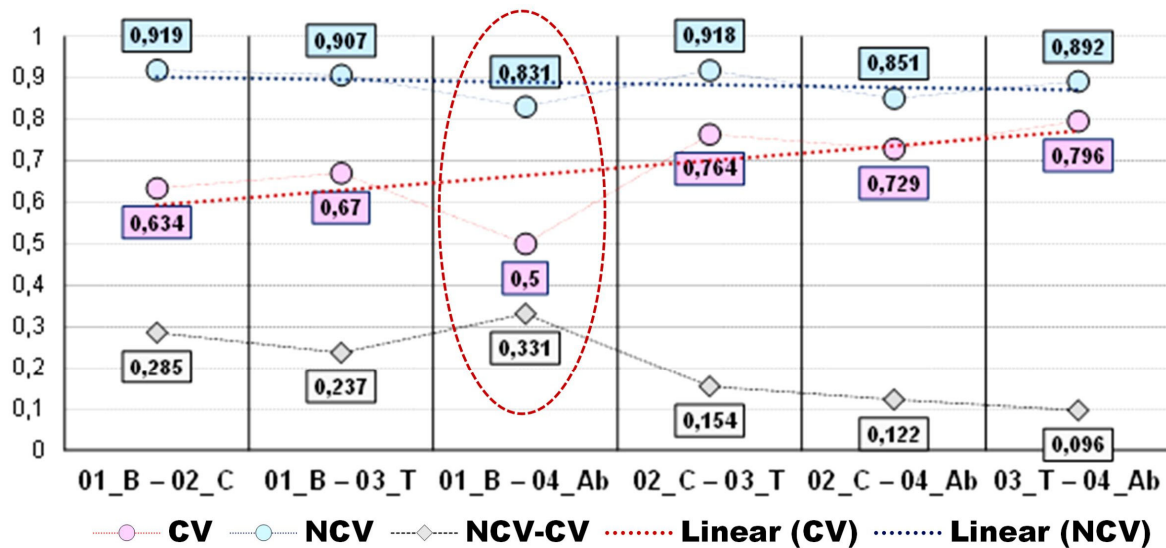


Figure 24 – Comparison between correlation matrix values and their difference in the two groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; NCV: Noncardiovascular.

However, whereas in the NCV group the degree of correlation between intimal thickness of different aortic regions has a general ascending trend towards the terminal regions, in the CV group, this trend is opposite, meaning slowly descending. In other words, whereas, in NCV group, the intima tends to thicken towards the terminal regions, in CV group, the variation in thickness of the

intimal layer seems to be independent of the topographical position.

Therefore, the difference in the remodeling of the intimal thickness between the two groups is extremely variable, pleading for no relationship of intimal changes neither with the aortic regions nor with the cause of death (Figure 25).

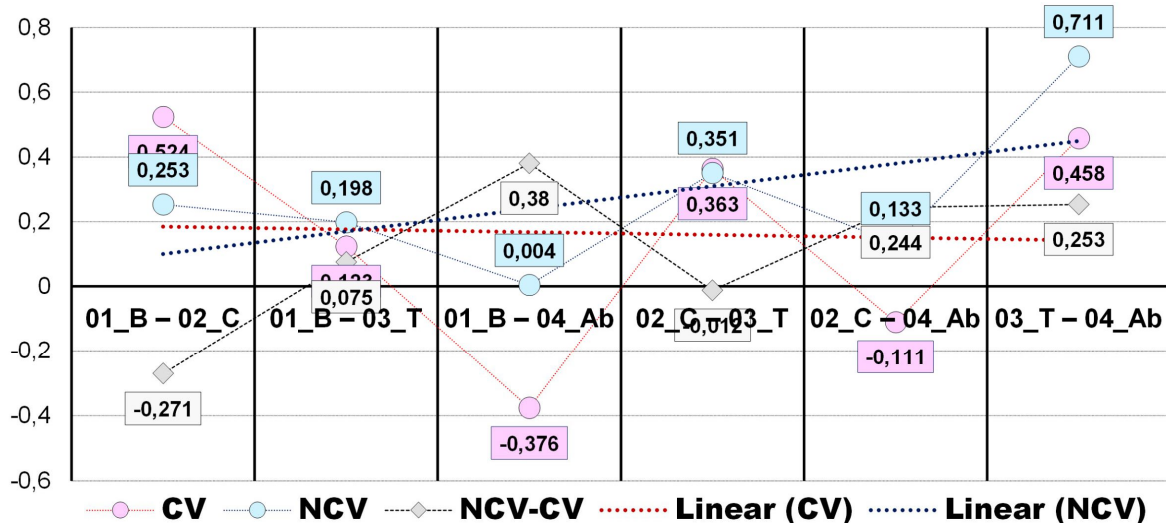


Figure 25 – Comparison between correlation matrix values and their difference in the two groups. 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; NCV: Noncardiovascular.

## Media

*Tunica media* was proved to be affected by numerous morphological changes during diseases and aging, one of them being the process of thickening [12].

Therefore, another goal of our study was to assess media's thickness along different topographical regions of the aorta. We observed that media thickness decreased continuously and constantly from the aortic origin till the aortic end.

Media was thicker in almost all of its segments in CV group than in NCV excepting the base region where people

died of NCV cause of death proved to have thicker media layer. It was also larger in people who died of CV diseases than in those died of any other cause irrespective their sex excepting the base region in both females and males, where the media was thicker in NCV group. Finally, women had larger media layers than men in both CV and NCV groups, excepting thoracic region in NCV group and abdominal region in CV group where medial layer was slightly thicker in men than in women. The correlation degree between changes in the values of the media thickness of different segments of the aorta was from a little to significant stronger along the aorta in NCV group than in

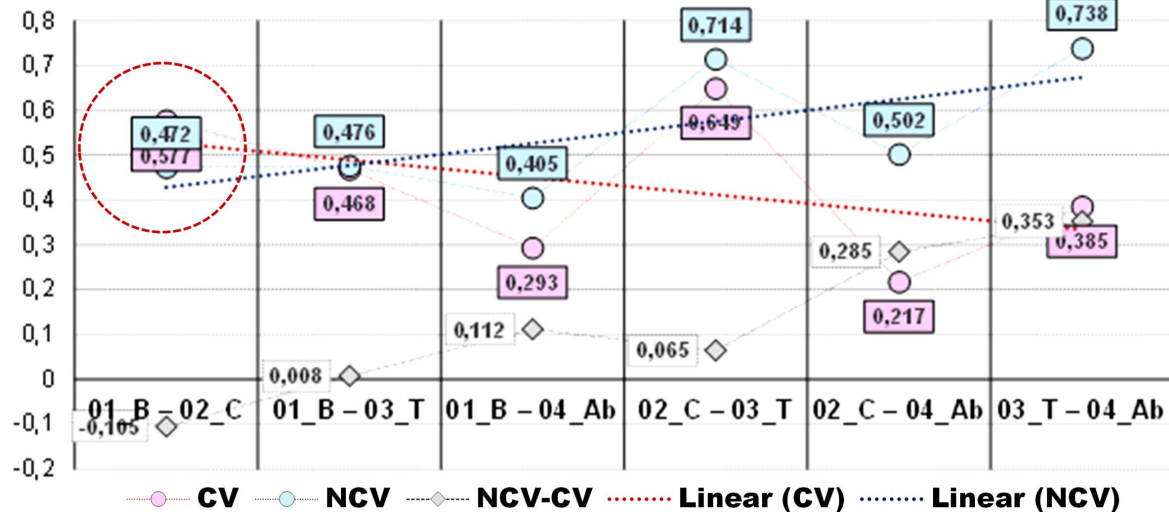


CV group. However, in both group this correlation degree fluctuated along the aorta.

There was, however, a difference between the evolution of the correlation degree between changes in the values of the media thickness of different segments of the aorta, namely, whereas in the NCV group the correlation trend between different regions was ascending towards the

terminal regions of the aorta, in the CV group this trend, despite the fluctuations, was a descending one.

This divergent evolution was reflected by the increasing trend of the difference between the correlation matrix values toward the terminal regions of the aorta. Not to forget to mention that, at the aortic origin, the correlation degree was stronger in CV group than in NCV group (Figure 26).



**Figure 26 – Comparison between correlation matrix values and their difference in the two groups.** 01 B: Aortic base level; 02 C: Aortic cross level; 03 T: Thoracic level; 04 Ab: Abdominal level; CV: Cardiovascular; NCV: Noncardiovascular.

## Conclusions

In both groups defined according to the cause of death, aortic diameters and media thickness decreased along the aortic length from the proximal regions toward the distal ones whereas intima thickness had an opposite behavior, increasing from the proximal toward distal regions. Aortic diameters and intima thickness were larger in CV group, than in NCV group in all aortic regions, whereas media thickness was slightly smaller in CV group than in NCV group only in the base region of the aorta. In women, aortic diameters, intima thickness and media thickness were larger in CV group than in the NCV group along the aortic length with one exception for the latter who had larger values in NCV group in the abdominal area. In men, aortic diameters were larger in CV group than in the NCV group along the aortic length whereas intima thickness and media thickness have each one exception to the rule, with larger values in NCV group than in CV group, the former in the abdominal region and the latter in the base region. In CV group, aortic diameters are larger in men than in women, while intima thickness and media thickness are larger in women than in men with one exception for each of them: larger values in men in cross area for the former and in in abdominal area for the latter. In NCV group, aortic diameters and intima thickness are larger in men than in women, along the aortic length whereas media thickness is larger in women than in men with one exception, the thoracic area. Concerning the differences between the two groups in the remodeling of the three aortic parameters: (i) for the aortic diameters, these differences are reducing towards the distal regions of the aorta; (ii) for the intimal thickness, the differences are extremely variable; (iii) for the media thickness the differences are increasing towards the distal regions of the aorta.

## 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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