Original Paper

The Influence of Ageing, Sex and Cause of Death on the Myocardial Tissue Collagen Fibrillary Component Behaviour

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ABSTRACT: Aim. The morphology and the function of human body are influenced by two main factors: the passing of the years and the increasing diversity of diseases with their subsequent lesions. The morphological structures of the heart are not exempt from the synergistic influence of these two major factors. The authors' goal was to compare the variations of interstitial mature collagen fibers (COLF) amount-% between the different main regions of the heart wall during patients´ ageing, on one hand, and, depending on patients' cause of death, on the other hand. Methods. Samples of myocardial tissue with no evident lesion, coming from 95 patients with different ages (0-24 years-AP_01, 25-44 years-AP_02, 45-64 years-AP_03 and >64 years-AP_04) deceased in hospital with different causes of death (vascular diseases-V_P, non-vascular diseases-NV_P, and suspect/violent cause of death-S/V_Dth) and autopsied, were collected from five epicardium-to-endocardium cross sections (left ventricle anterior wall-LV_AW, lateral wall-LV_LW and posterior wall-LV_PW, interventricular septum-IVS and right ventricle wall-RVW). Further, they were histologically processed and stained with Picro Syrius Red technique. COLF proportion was assessed, on virtual slides, with a a computer program designed specifically for this purpose. For statistical comparison of data, Kruskal-Wallis and Pearson correlation tests were used. Results. The COLF proportion had the smallest values in elderly and they increased markedly towards the younger periods of age. Then, the COLF proportion had the highest values in the RV_W (excepting the AP_04 period of life), and the lowest values in the IVS. In LV_W, COLF proportion had a general increasing trend from anterior to posterior regions in AP_01 and AP_03 periods of life and in males. In turn, it had decreasing trend from anterior to posterior regions in AP_02 and AP_04 periods of life and in females. The COLF proportion had the highest values in NV_P group in all cardiac main regions and the lowest values in V_P group (excepting LV), in both men and women. It had also the highest values in the RV_W and the lowest ones in IVS in all three diseases groups. In LV, COLF proportion had the highest values in NV_P group but the smallest ones in S-V_Dth group, in all main heart regions of this group. The COLF proportion had higher values in males than in females in all heart wall main regions in NV_P group and S-V_Dth group but vice versa in V_P group. Conclusions.

KEYWORDS: *Myocardial collagen fibers, morphometry, age, sex, cause of death.*

Introduction

Myocardial tissue is one of the fundamental components of the heart which has a complex structure. One of the important compartments of this structure is the interstitial space which, although very narrow, constitutes between 10% and 15% of the volume of the myocardium and in turn harbors a wide variety of cellular and noncellular elements. Among these, fibroblasts and their main products, collagen fibers occupy a central position [1-5].

Although they appear sparse and scattered, they represent the predominant non-cardiomyocyte cell type in the adult mammalian heart [6,7].

Collagen fibers, together with elastic fibers form a scaffolding network that support and maintain the architecture of the myocardial tissue as well as the transmission of forces throughout the entire heart wall. Its main levels of organization are: epimisium, perimisium and endomisium [8-10].

With the passage of time and the interaction with more and more diseases, myocardial tissue in general, as all other tissues, and its interstitial space in particular undergo complex morphological and functional changes that were gathered together under the label of senescent heart phenotype [11-13].

One morphological mark of the myocardial tissue ageing is the proliferation of the interstitial fibroblasts, whereas the functional cell population (myocardocytes) undergo a gradual reduction [14,15].

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Further, this event is accompanied by a collagen decompensation, consisting of the increase in type I collagen fibers amounts (responsible for tensile strength), the decrease in type II collagen fibers amounts (responsible for distensibility) and the appearance of bridges between collagen fibers resulting in a reticular structure [16-18].

The final result is the development of interstitial fibrosis which produces, on one hand, disorganization of the myocardial tissue structure, and, on the other hand, the establishment of cardiac wall rigidity, especially in the LV wall and impaired ventricular mechanical function [16-22].

Material and Methods

The base of study consisted of a series of 94 patients died either in the hospital or in sudden/violent/suspect conditions and were subjected to necropsy examination.

The variables taken into consideration were: Basic clinical data (Sex, Age, Cause of death) and Morphological data, represented by interstitial collagen proportion from the surface of myocardial tissue (%)-COLF.

Clinical data were split into subgroups using specially designed stratification scales as follows:

- **Scale for Sex** included two groups: Male (M) and Female (F)
- **Scale for Age** included four main life periods: first period, between 0-24 years, including Childhood and Adolescence (AP 01); second period, between 25-44 years, named Young Adult (AP 02); third period, between 45-64 years, named Mature Adult (AP 03); fourth period, >65 years, named Elderly (AP 04).
- **Scale for the Cause of death** included: first group, of vascular diseases (V_P), second group, of non-vascular diseases (NV_P) and third group of suspect/violent causes of death (S/V_Dth) which was considered also as control group (CG).

The working method was the clinical-statistical one, based on mixed, analytical and descriptive researches and consisted of a several steps algorithm.

Sampling of myocardial tissue

Five epicardium-to-endocardium cross sections at the level of the third transverse section through the heart, numbered from the apex to the base were sampled, being careful that these sections do not contain, as far as possible, visible lesions of the heart wall. The sampling areas and their labels are presented in Table 1 and illustrated in Figure 1.

Table 1. Heart wall main regions.

Sample	Description	Label
1	Anterior wall of the left ventricle	01 LV_AW
2	Lateral wall of the left ventricle	01 LV_LW
3	Posterior wall of the left ventricle	01 LV_PW
4	Interventricular septum	02 IVS/SIV
5	Right ventricle wall	03 RV

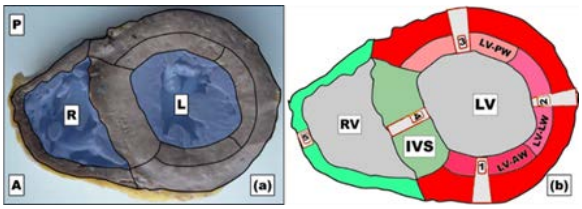

Figure 1. (a) Third transverse section through the heart from apex to base-view from the base; (b) Sites of study material collection (Modified after [23,24]).
Legend: A=Anterior; IVS=Interventricular Septum; L=Left; LV=Left Ventricle; LV-AW=LV Anterior Wall; LV-LW=LV Lateral Wall; LV-PW=LV Posterior Wall; P=Posterior; R=Right; RV=Right Ventricle

Histological processing

Formation of primary blocks. The tissue fragments obtained in the first step were fixed in 10% buffered formalin at neutral pH and embedded in paraffin.

Formation of microarray blocks. Two mm thick paraffin "slides" that included myocardial tissue from the pericardium to the endocardium were cut in the five primary paraffin blocks of each case and transferred in specially created molds [25,26] with five numbered slots from top to bottom, with the end facing the pericardium on the right (Figure 2).

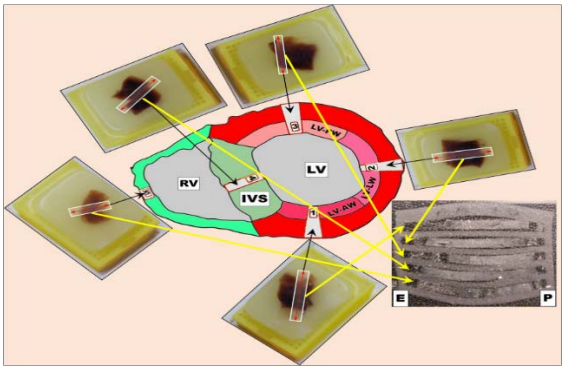

Figure 2. The algorithm of sampling.
Legend: A=Anterior; IVS=Interventricular Septum; L=Left; LV=Left Ventricle; LV-AW=LV Anterior Wall; LV-LW=LV Lateral Wall; LV-PW=LV Posterior Wall; P=Posterior; R=Right; RV=Right Ventricle.

The molds were inserted in secondary paraffin blocks which, finally were sectioned at 2mm, obtaining two serial sections: both passed on a regular histological slide and stained with Hematoxilin-Eosin and Picro Syrius Red staining technique, respectively.

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Quantitative assessment

Slide digitization and Image acquisition. The glass slides were converted to virtual slides with a Leica Aperio AT2 scanner, using the X40 objective. A region of interest (ROI) was selected from each of the five topographic heart wall fragments using the dedicated Aperio Image Scope program [v12.3.2.8013] (Figure 3).

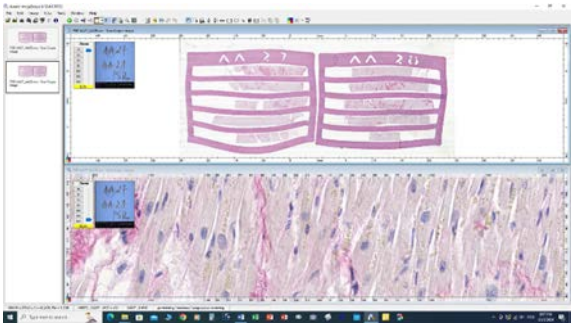

Figure 3. Aperio ImageScope virtual slide viewing software window [v12.3.2.8013].
(a) The whole virtual slide; (b) ROI selection.

All selected fields from each of the digital slides were exported as RGB-JPEG images and stored in a single directory.

Staining Normalization. An image considered empirically representative in terms of staining was selected and used as a staining reference for the normalization process.

Then, to reduce the variability in staining/brightness between images, each of the images selected from the digitized slides was normalized with respect to the reference image chosen using a custom color transfer algorithm designed and presented previously [27] that was applied to the LAB color space on each channel.

Morphological determinations. The acquired ROIs were evaluated and morphometric determinations were performed using the MATLAB programming environment (MathWorks, USA) using the functions incorporated in its "Image Processing Toolbox" menu. Image analysis was performed on the entire image without additional selection of a region.

Colored images were segmented into three color clusters. A K-means-based image segmentation algorithm (imsegkmeans) [28] was used (Figure 4):

- A **pale red colored cluster**-myocardial muscle cells
- A **red colored cluster**-collagen fibers
- A **white colored cluster**-tissue material between myocardial cells.

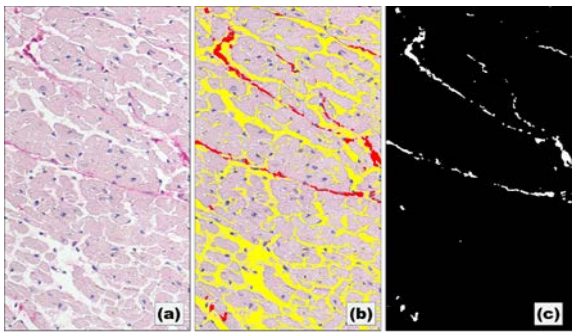

Figure 4. Color image processing of Picro Syrius Red technique stained slides.
(a) Initial image; (b) Segmentation into three clusters; (c) Final mask after segmentation into three clusters.

Quantification and Results. The workflow iterated over all image files in the input directory and generated per-image overlays and a summary spreadsheet. For each image, the following numerical and non-numerical parameters were recorded:

- Image Name
- Pixel Size-PxL=0.27 μ
- Pixel Area PxA=PxL X PxL=0.27 X 0.27=0.0729 μ^2
- Total Number of Pixels in the Entire Image of Photographed Tissue-Image Area in Pixels (fixed) IAPx=1,273,182/1,303,306 Pixels (depending on the resolution of the computer monitor on which the image analysis program with which the digital images were taken was installed).
- Number of pixels colored pale red, representing myocardial muscle fibers-RPPx_PSR
- Number of pixels colored red, representing collagen fibers-RPx_PSR
- Number of pixels colored white representing tissue material between myocardial fibers-WPx_PSR

Finally, for each image, the percentage occupied by collagen fibrillar structures in the entire image was calculated according to the formula: 100 X RPx_PSR/IAPx.

The sets of continuous variables represented by the values of the myocardocytes proportions were compared using a Kruskal-Wallis test and Pearson correlation test.

Interpretation and graphical representation of results were realized 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, trial version.

Results

Presence in the main heart segments

Mature collagen fibers (MCFs) in the interstitium of uninjured myocardial tissue have a much lower proportion than that of functional myocardial cells. The first step of the assessment of the COLFs proportion in myocardial tissue was to determine it for each of the main anatomical segments of the heart wall (Figure 5).

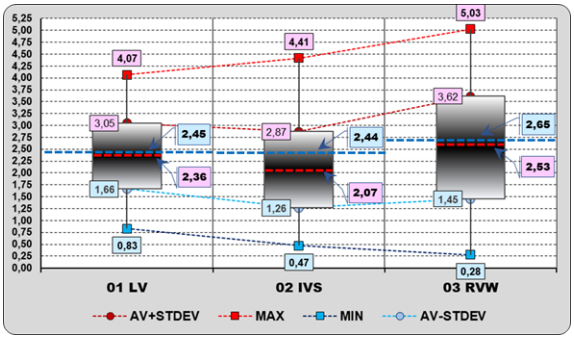

Figure 5. Distribution of the COLF proportion values in the main heart segments.

Thus, it was observed that the percentage occupied by COLFs of the myocardial tissue surface in areas without lesions also varied within very wide limits for all three main segments of the heart wall.

The largest range of variation, 4.75%, was found in the right ventricular (RV) wall, where both the highest maximum value (5.03%) and the lowest minimum value (0.28%) were recorded.

The narrowest range of variation (3.24%) was observed in the left ventricular (LV) wall and this is because here both the lowest maximum value (4.07%) and the highest minimum value (0.83%) were recorded.

However, the concentration ranges of most values were much narrower, two to two and a half times smaller than the dispersion ranges of all values, the narrowest being in the LV (1.39%) and the widest being in the RV (2.16%). They were determined by very small standard deviation (STDEV) values that ranged from 0.69% in the LV to 1.08% at the RV.

What is noteworthy, however, is the fact that these intervals were shifted towards the middle of the dispersion ranges because they were centered by COLF proportion mean values that varied between 2.07% in the IVS and 2.53% at the RV.

The COLF proportion decreased slightly from the LV to the IVS and then increased by almost one percent towards the RV, a variation confirmed as significant by the Kruskal-Wallis test (”p” value=0.008).

Variation by age

The percentage of COLF showed a similar pattern of evolution in each of the three main segments of the heart wall with advancing age. (Figure 6).

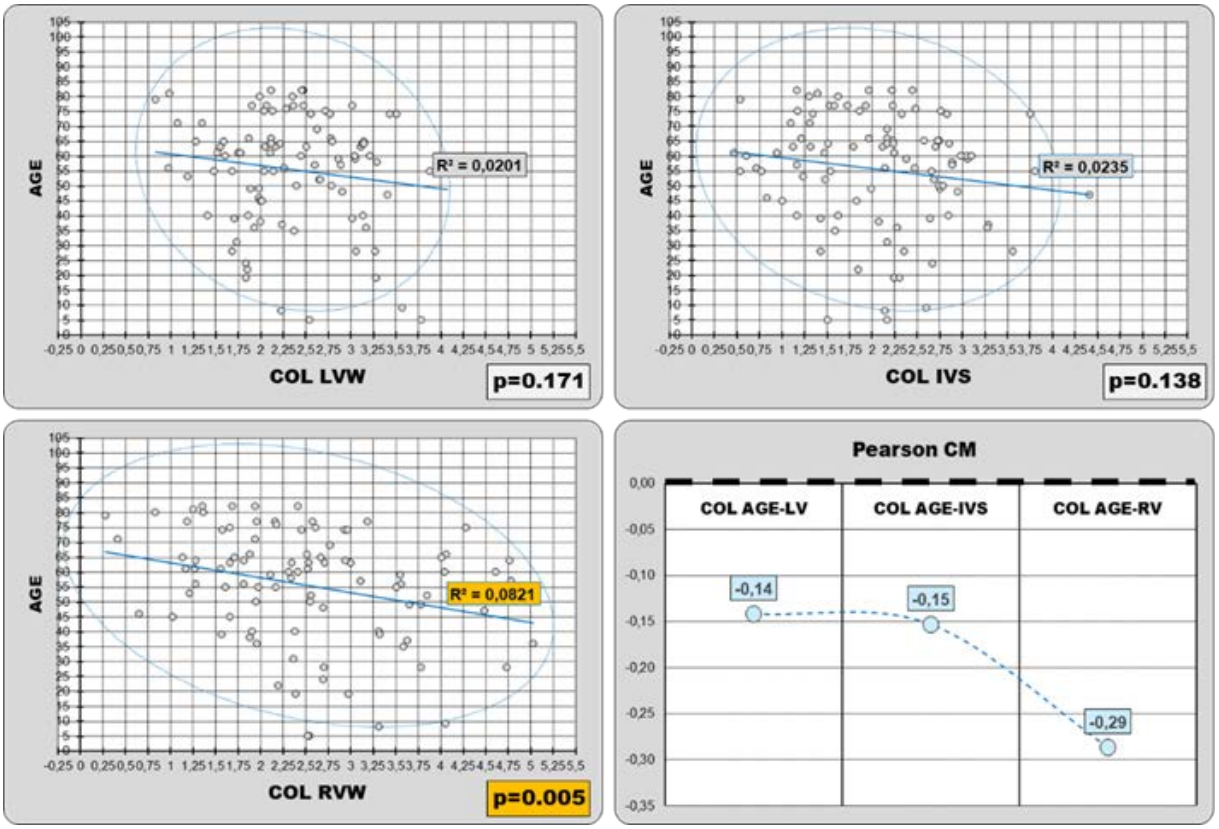

Figure 6. Variation in the percentage of COLF in each main heart segment according to age.

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Thus, a constant descending trend was observed in the LV wall and IVS (the Pearson test R coefficient was negative at both segments and discretely lower in the IVS-0.15 vs-0.14 and the "p" values were much higher than the reference value "0.05"). In the RV, the decrease in the amount of COLF with advancing age became evident, with the negative R coefficient of the Pearson test drastically reducing to-0.29 and the "p" value being even 0.005 (Figure 6 Bottom Right).

When dividing the group of subjects into subgroups according to life period, it was observed that the amount of COLF undergoes similar variations from one age period to another in each of the three main segments of the heart wall (Figure 7).

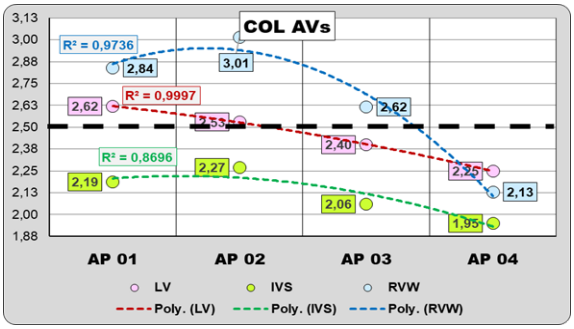

Figure 7. Variation of mean values of the percentage of COLF in each of the main heart segments according to life periods.

In general, percentage values for COLF were located on either side of the half of the dispersion interval of the values (7-black dotted line).

Thus, the values for the LV and RV were above this threshold in the first two periods of life, while the values for all segments of the heart wall in the last period of life were below this threshold.

The percentage of COLF presented the highest values at the RV in all life periods, except for the third age compared to the rest of the segments and the lowest values compared to the rest of the segments at the IVS in all life periods.

The general trend was of a slight increase in the percentage from Adolescence to Young Adult, followed by a steady decrease after 44 years until senescence.

The exception was the LV, where the phenomenon of decreasing the amount of COLF.

Variation in the two sexes

Comparing the values of the amount of COLF in the different segments of the heart wall in the two sexes highlighted the fact that, in both men and women, the amount of COLF had a similar evolution, namely, after an obvious decrease from the LV to the IVS, an increase followed at the RV to values higher than those recorded at the LV wall (Figure 8).

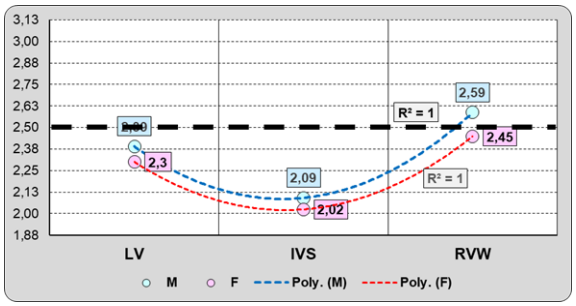

Figure 8. Variation in mean values of the percentage of COLF in each of the main heart segments in the two sexes.

It should also be noted that, except for the value recorded in men in the RV, the rest of the values were below the threshold of half the dispersion interval of all values (Figure 4-black dotted line). It can also be highlighted that the percentage of COLF in men were always higher than those in women (Figure 8).

Evolution with age in both sexes

Comparative analysis of the evolution of the collagen fiber percentage (COLF) within the myocardial tissue at different topographical levels of the heart wall in the two sexes showed that the COLF percentage decreased with age in all three main segments of the heart wall in both men and women (the Pearson test R coefficient was negative).

This decrease was a trend in the first two main segments of the heart wall (LV IVS) in both men and women because the Pearson test “p” value was greater than the reference value “0.5” (Figure 5-Left and Middle). It became significant in the RV in both sexes because the Pearson test “p” value became less than the reference value “0.5” (Figure 9-Right).

However, distinct variations could be observed in the two sexes along the heart wall.

Thus, in men, the decreasing of the percentage of COLF became more pronounced with advancing age from one segment to another, a fact demonstrated by the values of the Pearson test coefficient R (Figure 10-dotted line with blue values, associated with the blue scale on the left),

In women, the trend was first reduced in the IVS compared to the LV, and then significantly increased in the RV compared to the other segments, a fact also demonstrated by the values of the Pearson test coefficient R (Figure 10-dotted line with pink values, associated with the red scale on the Right).

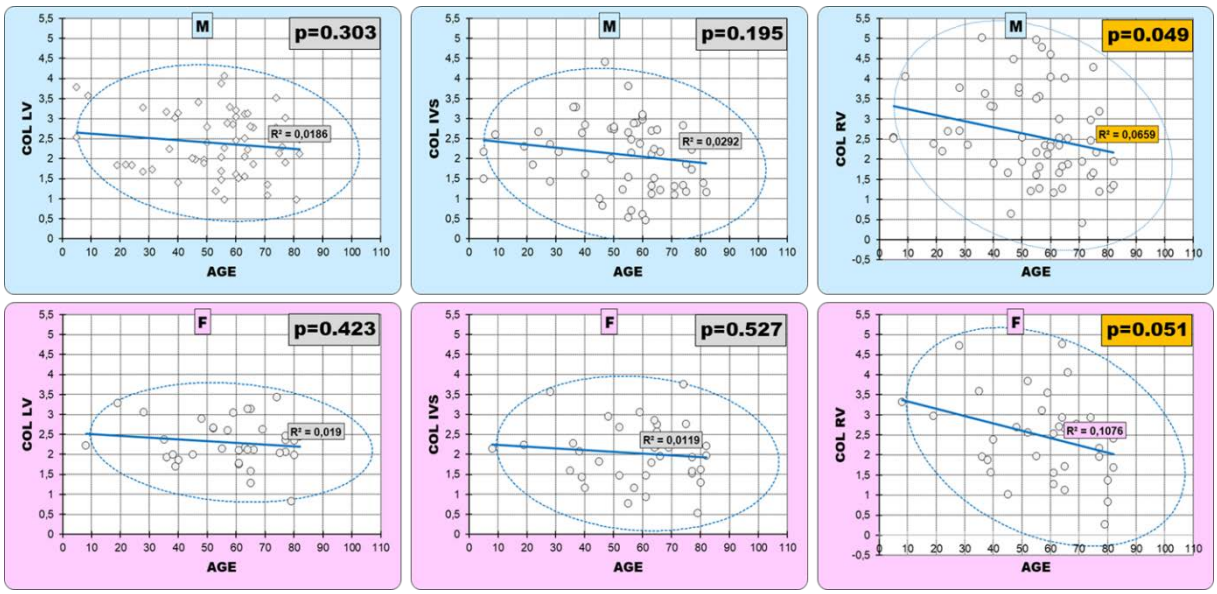

Figure 9. Graphical expression of the Pearson Correlation Test between the amount of COLF and Age. Legend: Men=Blue Graphs; Women=Pink Graphs.

A more detailed analysis of the evolution of the amount of COLF over life periods confirmed, on the one hand, the general trend of similar evolution according to sex of the amount of COLF within the myocardial tissue without lesion, but, on the other hand, nuanced this trend in each of the sexes.

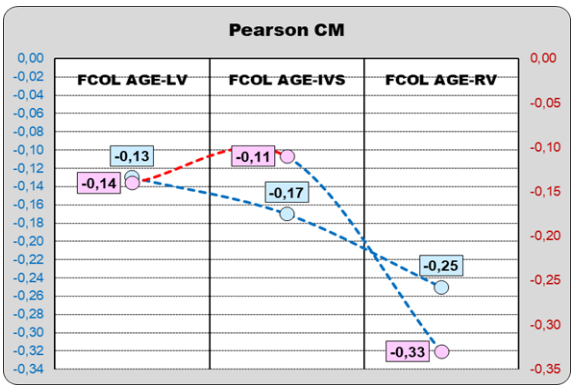

Figure 10. Evolution of Pearson's R coefficient values. Men=Blue Scale (Left); Women=Red Scale (Right).

A first observation is that, in each of the three segments, the percentages of COLF were almost always discretely higher in men than in women.

A second observation is that the general stratification of the values observed in each of the three segments of the heart wall was similar to that recorded for the entire group (Figure 7), respecting in both sexes the reversal in the third age between the values for the LV and those for the RV (Figure 11).

A third observation is that the developments were not linear across all three main segments of the heart wall throughout life.

Thus, in men, if in the LV wall the reduction in the amount of COLF was continuous from adolescence to old age, in the other two segments-IVS and RV-a slight increase could be observed from adolescence to adult age, and then the amount of COLF was reduced until old age to values lower than those in adolescence (Figure 11-Left).

In women, on the other hand, although the general trend was one of decrease, the evolution was different in each of the three segments. Thus, in the LV, the amount of COLF recorded oscillating values throughout life, namely it decreased towards the young adult period, returned towards the mature adult period to then decrease at the third age, but to higher values than in the young adult.

At the IVS, the percentage of COLF decreased continuously until the mature adult period, to return in old age but below the values of the adolescence period. Finally, in the RV, there was a continuous reduction of COLF from adolescence to old age (Figure 11-Right).

Comparing the evolution of the amount of COLF throughout life in the two sexes, it was found that the ways in which they evolved in each segment of the heart wall were different. Thus, in LV, the amount of COLF was constantly reduced in men throughout life, while in women, the evolution was decreasing but with oscillations.

In the IVS, in men, the percentage of COLF increased towards the adult life periods to then decrease towards old age, while in women, it decreased towards the adult life periods to return to old age.

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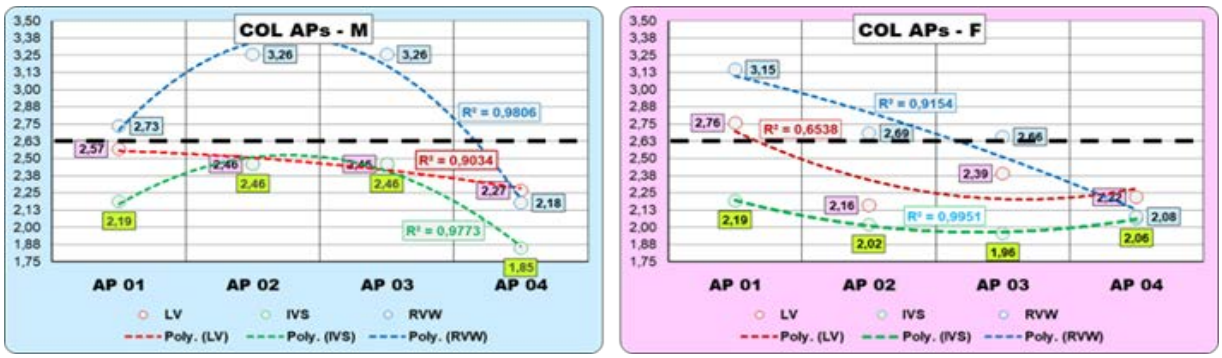

Figure 11. Variation of the average values of the percentage of COLF in each of the main heart segments according to the periods of life in the two sexes.

Variation by cause of death

Finally, the comparative analysis of the evolution of the percentage of COLF along the three main segments of the heart wall in the different types of pathological conditions that led to the death of the patients revealed several significant aspects.

The first is that, except for the values of the percentage of COLF in the RV from the group of patients who died due to nonvascular diseases, the rest of the values were below the threshold of half of the dispersion interval of the values (Figure 12-black dotted line).

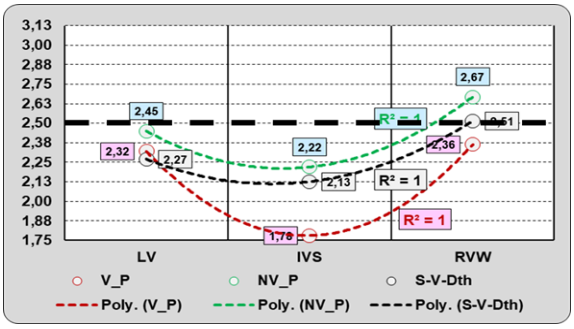

Figure 12. Variation in mean values of the percentage of COLF in each heart segment according to the cause of death.

The second interesting aspect is that the amount of COLF had, in all three groups of deceased patients, a pattern of evolution along the segments of the heart wall similar to that found in the groups of men and women. Thus, in all three groups of patients, there was a decrease in the percentage of COLF from the LV wall to the IVS, followed by a significant increase towards the RV where values higher than those in the LV were recorded (Figure 12).

It should also be noted that the highest values of the amount of COLF were, in all segments of the heart wall, in patients who died due to nonvascular diseases and the lowest values were observed in patients who died due to vascular diseases, the values of patients in the control group being somewhere in the middle.

Evolution by causes of death in both sexes

Comparing how the amount of COLF evolves along the main segments of the heart wall in men and women in the three groups of patients with different causes of death showed several differences both from the evolutions recorded for the entire group and between the two sexes.

Thus, the general stratification of the values observed in the three segments of the heart wall was similar to that recorded for the entire group in men over time; in women, the amount of COLF in the group of patients who died from violent/suspicious causes were almost always higher than those in the other groups of patients.

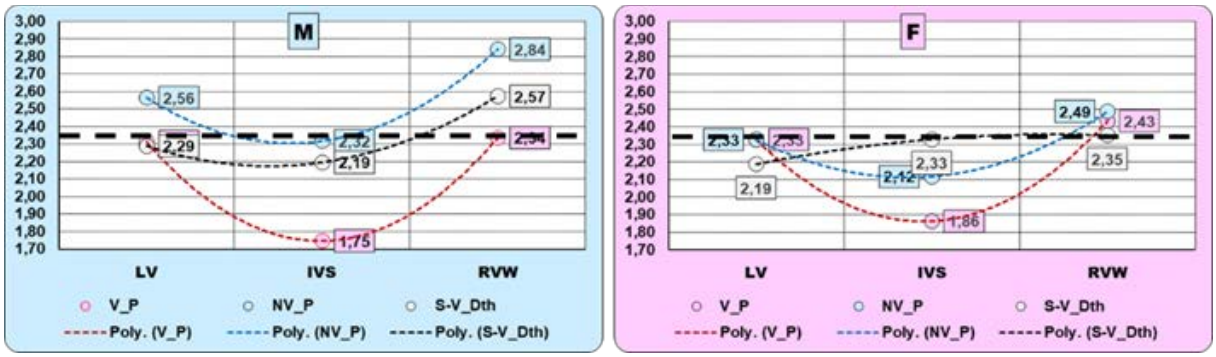

Figure 13. Variation of mean values of the percentage of COLF in each heart segment according to the cause of death in the two sexes.

Another observation is that, in general, in both men and women, the values of the amount of COLF are located below the threshold of the half-range value of their dispersion interval (Figure 13-black dotted line in both graphs) with the exception of the values in the group of patients who died from nonvascular causes in which the vast majority of the values recorded in men are located above the threshold of the half-range value (Figure 14-Left) while the vast majority of the values recorded in women are located above this threshold (Figure 13-Right).

Comparative evolution by segments

The way in which the percentage values of COLF in the different segments of the heart wall evolved in relation to each other was very closely parallel and direct in both men and women, a behavior also confirmed by the statistical apparatus.

Thus, the "p" values of the Pearson correlation test between the values of the amount of COLF in the heart wall segments taken two by two in both men and women were much lower than the reference value "0.05", namely<0.0001 (Figure 14).

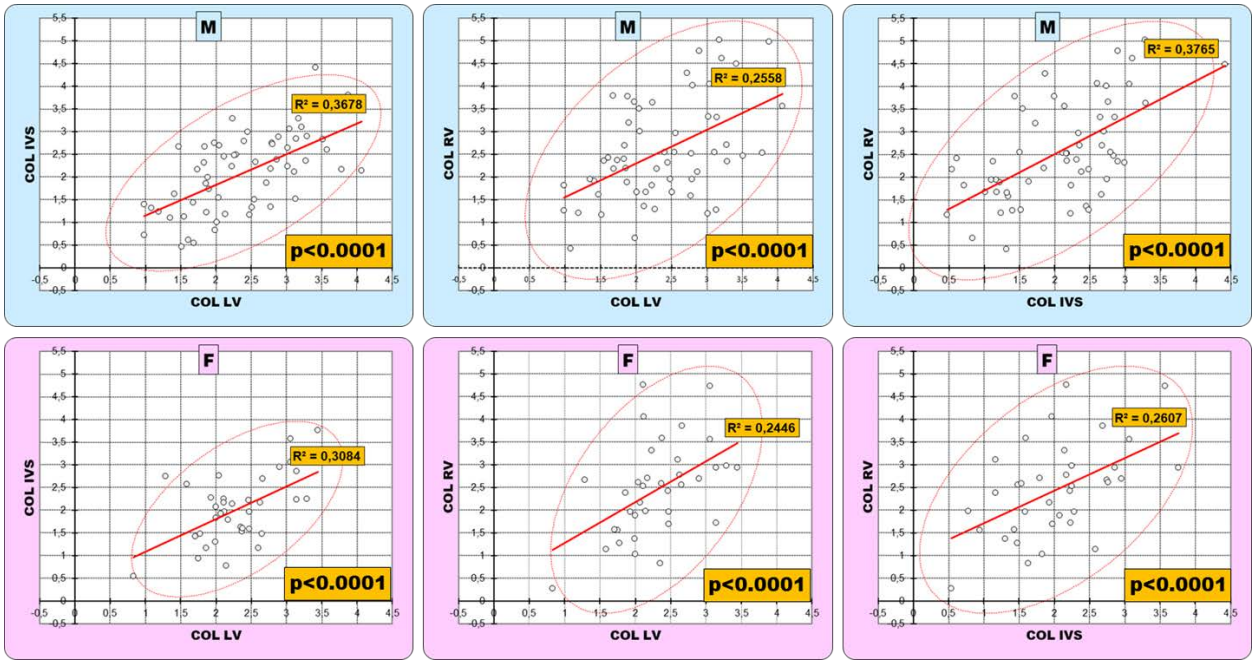

Figure 14. Graphical expression of the Pearson Correlation Test between the percentages of COLF in the cardiac wall segments; Men=Blue Graphs; Women=Pink Graphs.

The degree of direct correlation was higher in men, with the values of the Pearson test coefficient R being higher than the corresponding ones in women (Figure 15-Blue dotted line, associated with the Blue scale on the Left), being lower between LV and RV and more pronounced between LV and IVS and between IVS and RV.

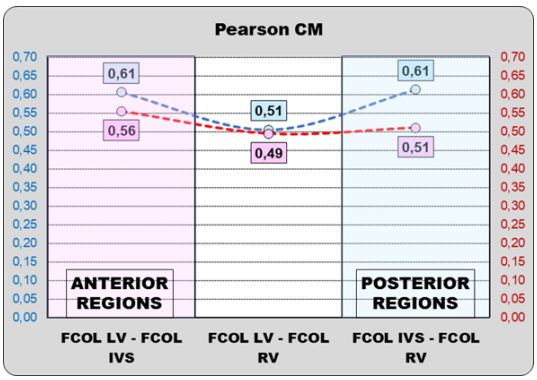

Figure 15. Evolution of the values of the Pearson R Coefficient test compared in men and women.

In women, the values of the Pearson test coefficient R were clearly reduced when we reported LV to RV and less so when we reported IVS to RV (Figure 15-Red dotted line, associated with the Red scale on the Right).

Discussion

Synthesizing observations on the behavior of mature collagen fibers in the myocardial interstitium over time, our research showed that the percentage of interstitial collagen fibers was lowest in the elderly and increased significantly towards the early periods of life.

The percentage of interstitial collagen fibers had the highest values in the right ventricular wall except during the senescence period and the lowest values in the interventricular septum.

Regarding the behavior of collagen fibers in the myocardial interstitium in the different segments of the heart wall in people who died

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from different causes, our research showed that the highest percentage of interstitial collagen fibers was found in the group of patients who died from nonvascular causes in all regions of the heart wall and the lowest percentage in the group of patients who died from vascular causes, except for the left ventricular wall, in both men and women.

Also, regardless of the cause of death, the percentage of interstitial collagen fibers had the highest values in the right ventricular wall and the lowest values in the intraventricular septum.

Synthesizing the observations on the behavior of mature collagen fibers in the myocardial interstitium over time compared to the two sexes, it was observed that, in the left ventricular wall, the percentage of interstitial collagen fibers had a general trend of increase from anterior to posterior during childhood and adolescence, and during mature adulthood in men, and a decreasing trend from anterior to posterior during young adulthood and during senescence in women.

Regarding the behavior of mature collagen fibers in the myocardial interstitium in the different segments of the heart wall in people who died from different causes, the amount of interstitial collagen fibers was higher in men than in women in all regions of the heart wall in the group of patients who died from nonvascular causes and in the group of people who died from suspicious/violent causes, and higher in women than in men in all regions of the heart wall in the group of patients who died from vascular causes.

Conclusions

Regarding remodeling with age, the interstitial collagen fiber compartment showed a process of remodeling in quantity along regions of the heart wall with age with a pattern of general decreasing trend in both men and women, but with variations across different regions.

Regarding remodeling in the groups of deceased patients from the different causes of death, the amount of interstitial collagen fibers showed the same distribution pattern in all three groups of deceased subjects and, in each of them, along the regions of the heart wall, with higher values in men than in women, except for the left ventricle.

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Author Contributions

Conceptualization: L.E.C, C.F, and I.E.P; Methodology: L.E.C and I.E.P; Investigation: L.E.C and R.M.P; Data analysis, M-S Ș; Manuscript writing and initial draft preparation, L.E.C and R.M.P; Manuscript review and editing, C.F, and I.E.P; Supervision, C.F, and I.E.P. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that they have no competing of interests.

Institutional Review Board

The study was conducted according to the guidelines of the Declaration of Helsinki. All patients' relatives signed an informed consent agreement for the necropsy. The study and the protocols utilized therein were approved by The Universitary and Scientific Ethics and Deontology Commission through the Advisory Report No. 70/15.06.2018.

Consent Statement

All patients' relatives signed a written informed consent agreement for the necropsy.

Data Availability

All data presented in the manuscript are available from the authors upon request.

References

1. Gartner LP, Hiatt JL. Color Textbook of Histology. Third Edition, Sauders, p 175, 2014
2. Junqueira LC Carneiro J. Basic Histology-Text and Atlas. 11h Edition, The McGraw-Hill Companies, 2007
3. Berry Gerald J. Billingham Margaret E. Normal Heart, In: Mills, Stacey E. Histology for Pathologists, 3rd Edition, 2007 Lippincott Williams & Wilkins, p527-544
4. Kuehnel W. Color Atlas of Cytology, Histology, and Microscopic Anatomy-4th edition, revised and enlarged 2003, Thieme, p172-177
5. Gersch C, Dewald O, Zoerlein M, Michael LH, Entman ML, Frangogiannis NG. Mast cells and macrophages in normal C57/BL/6 mice. Histochem Cell Biol. 2002, 118(1):41-49. doi: 10.1007/s00418-002-0425-z. PMID: 12122446.
6. Moore-Morris T, Cattaneo P, Puceat M, Evans SM. Origins of cardiac fibroblasts. J Mol Cell Cardiol. 2016, 91:1-5. doi: 10.1016/j.yjmcc.2015.12.031. PMID: 26748307; PMCID: PMC4764439
7. Zeisberg EM, Kalluri R. Origins of cardiac fibroblasts. Circ Res. 2010, 107(11):1304-1132. doi: 10.1161/CIRCRESAHA.110.231910. PMID: 21106947; PMCID: PMC3098499.
8. Shirwany A, Weber KT. Extracellular matrix remodeling in hypertensive heart disease. J Am Coll Cardiol. 2006, 48(1):97-98. doi: 10.1016/j.jacc.2006.04.004. PMID: 16814654.
9. Berk BC, Fujiwara K, Lehoux S. ECM remodeling in hypertensive heart disease. J Clin Invest. 2007, 117(3):568-575. doi: 10.1172/JCI31044. PMID: 17332884; PMCID: PMC1804378.

10. Weber KT. Cardiac interstitium in health and disease: the fibrillar collagen network. J Am Coll Cardiol. 1989, 13(7):1637-1652. doi: 10.1016/0735-1097(89)90360-4. PMID: 2656824.
11. Ruiz-Meana M, Bou-Teen D, Ferdinandy P, Gyongyosi M, Pesce M, Perrino C, Schulz R, Sluijter JPG, Tocchetti CG, Thum T, Madonna R. Cardiomyocyte ageing and cardioprotection: consensus document from the ESC working groups cell biology of the heart and myocardial function. Cardiovasc Res. 2020, 116(11):1835-1849. https://doi.org/10.1093/cvr/cvaa132. PMID: 32384145
12. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, Sinha M, Dall'Osso C, Khong D, Shadrach JL, Miller CM, Singer BS, Stewart A, Psychogios N, Gerszten RE, Hartigan AJ, Kim MJ, Serwold T, Wagers AJ, Lee RT. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell. 2013, 9;153(4):828-39. https://doi.org/10.1016/j.cell.2013.04.015. PMID: 23663781; PMCID: PMC3677132.
13. Biernacka A, Frangogiannis NG. Aging and Cardiac Fibrosis. Aging Dis. 2011, 2(2):158-173. PMID: 21837283; PMCID: PMC3153299.
14. Mirza M, Strunets A, Shen WK, Jahangir A. Mechanisms of arrhythmias and conduction disorders in older adults. Clin Geriatr Med. 2012, 28(4):555-573. doi: 10.1016/j.cger.2012.08.005. PMID: 23101571; PMCID: PMC3610528
15. Frangogiannis NG. Cardiac fibrosis: Cell biological mechanisms, molecular pathways and therapeutic opportunities. Mol Aspects Med. 2019, 65:70-99. doi: 10.1016/j.mam.2018.07.001. PMID: 30056242
16. Horn MA, Trafford AW. Aging and the cardiac collagen matrix: Novel mediators of fibrotic remodelling. J Mol Cell Cardiol. 2016, 93:175-185. doi: 10.1016/j.yjmcc.2015.11.005. PMID: 26578393; PMCID: PMC4945757.
17. Lindsey ML, Goshorn DK, Squires CE, Escobar GP, Hendrick JW, Mingoia JT, Sweterlitsch SE, Spinale FG. Age-dependent changes in myocardial matrix metalloproteinase/tissue inhibitor of metalloproteinase profiles and fibroblast function. Cardiovasc Res. 2005, 66(2):410-419. doi: 10.1016/j.cardiores.2004.11.029. PMID: 15820210.
18. Mendes AB, Ferro M, Rodrigues B, Souza MR, Araujo RC, Souza RR. Quantification of left ventricular myocardial collagen system in children, young adults, and the elderly. Medicina (B Aires). 201, 72(3):216-220. PMID: 22763158.
19. Murtha LA, Morten M, Schuliga MJ, Mabotuwana NS, Hardy SA, Waters DW, Burgess JK, Ngo DT, Sverdlov AL, Knight DA, Boyle AJ. The Role of Pathological Aging in Cardiac and Pulmonary Fibrosis. Aging Dis. 2019, 10(2):419-428. doi: 10.14336/AD.2018.0601. PMID: 31011486; PMCID: PMC6457057
20. Fajemiroye JO, da Cunha LC, Saavedra-Rodríguez R, Rodrigues KL, Naves LM, Mourão AA, da Silva EF, Williams NEE, Martins JLR, Sousa RB, Rebelo ACS, Reis AADS, Santos RDS, Ferreira-Neto ML, Pedrino GR. Aging-Induced Biological Changes and Cardiovascular Diseases. Biomed Res Int. 2018, 2018:7156435. doi: 10.1155/2018/7156435. PMID: 29984246; PMCID: PMC6015721.
21. Sundararajan S, Babu S, Das SD. Comparison of localized versus systemic levels of Matrix metalloproteinases (MMPs), its tissue inhibitors (TIMPs) and cytokines in tuberculous and non-tuberculous pleuritis patients. Hum Immunol. 2012, 73(10):985-91. doi: 10.1016/j.humimm.2012.07.042. PMID: 22820625; PMCID: PMC3511911
22. Gazoti Debessa CR, Mesiano Maifrino LB, Rodrigues de Souza R (2001). Age related changes of the collagen network of the human heart. Mech Ageing Dev, 122(10):1049-58
23. Mirea OC, Ancuţa AA, Şerbănescu MS, Postolache P, Uscatu CD, Marin C, Pleşea IE, Chiuţu LC. Analysis of aortic size in subjects died due to cardiovascular and non-cardiovascular events: a necropsy study. Rom J Morphol Embryol, 2014, 55(3 Suppl):1105-1109. PMID: 25607392
24. Szalay D, Frołow M. Aortic Aneurysms. In: Jaeschke R, Gajewski P, O'Byrne P (eds)., McMaster Textbook of Internal Medicine. Kraków: Medycyna Praktyczna. https://empendium.com/mcmtextbook/chapter/B31.II.2.22.
25. Gologan, D.; Ștefan, A.E.; Militaru, M.; Sanda, A.C.; Arjan, S.; Mușat, S.; Leavitt, M.O.; Stan, R. Quality Assurance and Cost Reduction in Histopathology Laboratories Using Tissue Microarrays. Vet. Sci. 2023, 10, 280. https://doi.org/10.3390/vetsci10040280.
26. Stefan AE, Gologan D, Nitescu DA, Musat S, Stan R, Sanda CA, Arjan S, Militaru M. Pre-processed TMA biomimetic material-applications and benefits. Rev Rom Med Vet, 33(1): 26-32
27. Şerbănescu MS, Pleşea IE. A hardware approach for histological and histopathological digital image stain normalization. Rom J Morphol Embryol, 2015, 56(2 Suppl):735-741. PMID: 26429166
28. Imsegkmeans function. In MATLAB, MathWorks.com (https://www.mathworks.com/help/images/ref/imsegkmeans.html).

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