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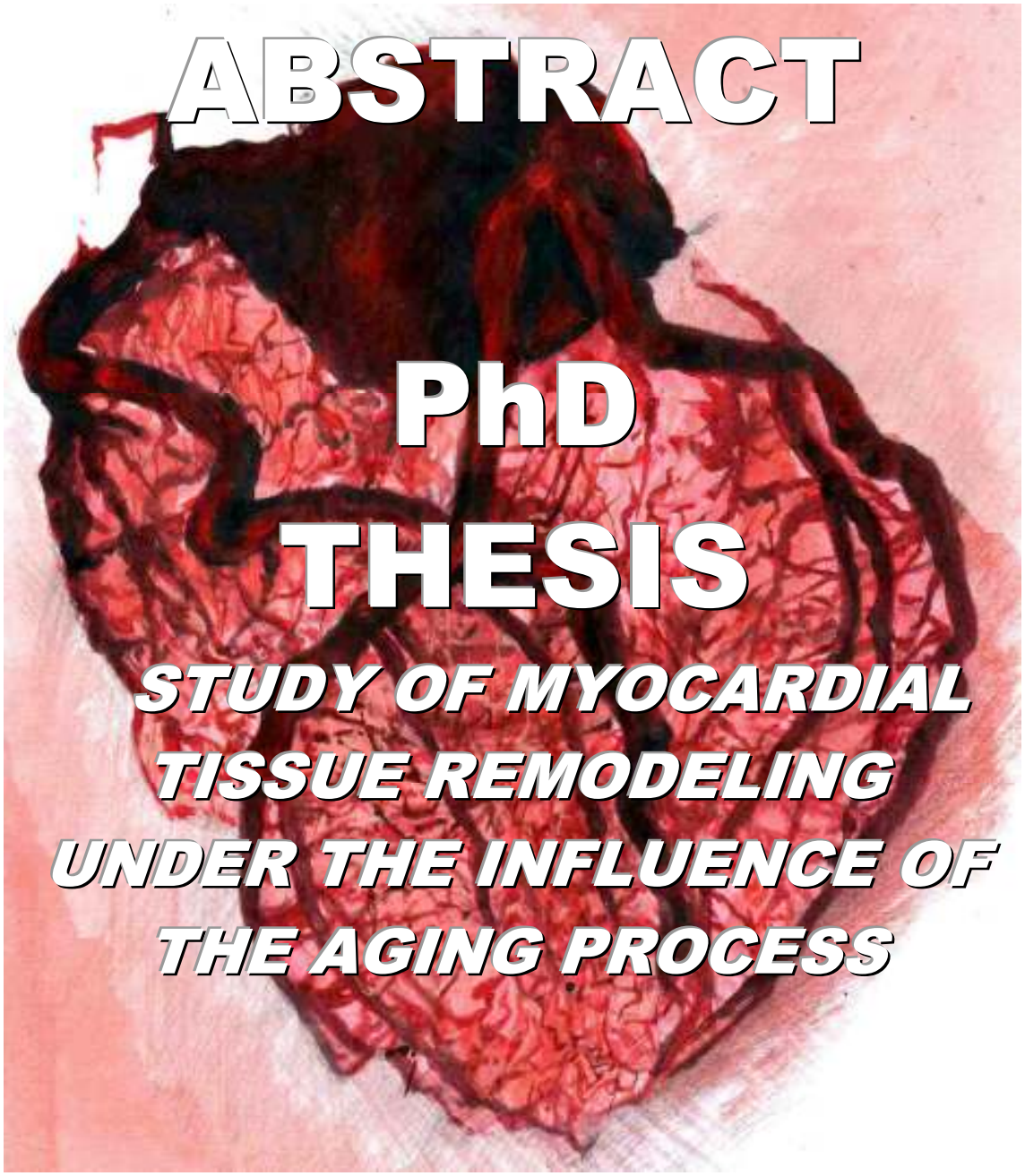

ABSTRACT

PhD THESIS

STUDY OF MYOCARDIAL TISSUE REMODELING UNDER THE INFLUENCE OF THE AGING PROCESS

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PHD CONTENTS

KEY WORD

Ageing, Miocard, Morphology

INTRODUCTION

Due to the spectacular growth in life expectancy over the past century, cardiovascular diseases have become one of the major causes of death in industrialized countries, despite the unprecedented advances in the diagnosis and treatment of these disorders.

Of these, myocardial infarction is a major cause of mortality and morbidity worldwide. Myocardial infarction may be a minor event in a chronic disease can even be detected, but can also be a major event leading to severe haemodynamic deterioration or even sudden death. Installing cause ischemic myocardial necrosis may be the first manifestation of coronary artery disease or may occur repeatedly diagnosed patients with established disease (Thygesen et al. 2012).

As it was said in a report on existing policies and capacities for integrated prevention and control of noncommunicable diseases, including cancer conducted in 2006 in the 2006-2007 Biennial Collaborative Agreement between the World Health Organization and the Ministry of Public Health in Romania, in our country, the percentage of deaths associated with cardiovascular events ranks first removable, with a percentage of 62% (Mark 2007).

Myocardial tissue and especially the left ventricle undergoes structural changes along with age, being the most obvious wall thickening (Ganau et al 1995), and an increased mass-volume ratio with a discrete volume reduction procedures (Cheng et al 2009).

This pattern of morphological remodeling is associated with an increased risk of cardiovascular disease (Cheng et al 2009; Bauml 2010) and contribute to the installation of systolic heart failure (Mosterd et al 1999) diastolic dysfunction (Kitzman 2000) or rhythm disorders (Manolio et al 1994) observed in people with age.

Personal motivation for choosing this research direction is explained by the observation, over time, in practice, a large number of patients with cardiovascular pathology, requiring a multidisciplinary approach. The challenges of this suffering so diagnosis and therapy related applied to improve quality of life are the ones that made me understand this subject.

STAGE OF KNOWLEDGE

The aging process of the heart is primarily characterized by an increase in left ventricular mass compared to chamber volume and a decrease in diastolic function (Swine 1992). Even in subjects with no apparent hypertension or other causes of the increase afterload, aging is associated with a small increase in the volume of the heart, suggesting a degree of left ventricular hypertrophy (Gerstenblith et al. 1977).

For an overview of cardiac physiological changes while exercising during the aging process, it was suggested that an aged heart behaves like a youth one on beta blocker therapy (Julius et al. 1976).

Cardiac alteration correlated with age is related to the lusitrop function, with delay of relaxation as a result of increased duration of contraction. Finally, the results are due to prolongation of action potential and active state than to the passive mechanism changes or to the myocardial catecholamine content (Lakatta et al. 1982).

The alterations listed in Table 1 may have considerable clinical importance as a possible functional substrate in elderly persons tendens to develop heart failure.

Table 1 Comparative Table of cardiac changes during life (Lakatta and Yin 1982)

Age-Associated Changes	Plausible Mechanisms	Possible Relation to Human Disease
Cardiac structural remodeling		
↑ LV wall thickness	↑ LV myocyte size with altered Ca^{2+} handling	Retarded early diastolic cardiac filling
	↓ Myocyte No. (necrotic and apoptotic death)	↑ Cardiac filling pressure
	Altered growth factor regulation	Lower threshold for dyspnea
	Focal matrix collagen deposition	↑ Likelihood of heart failure with relatively normal systolic function
		LVH
↑ Left atrial size	↑ Left atrial pressure/volume	↑ Prevalence of atrial fibrillation and other atrial arrhythmias
Cardiac functional changes		
Reduced threshold for cell Ca^{2+} overload	Changes in gene expression of proteins that regulate Ca^{2+} handling; increased ω6: ω3 polyunsaturated fatty acids ratio in cardiac membranes	Lower threshold for atrial and ventricular arrhythmia
		Increased myocyte death
		Increased fibrosis
		Reduced diastolic and systolic function
↓ Cardiovascular reserve	↑ Vascular load	Lower threshold for, and increased severity of heart failure
	↓ Intrinsic myocardial contractility	
	Ventricular-vascular load mismatch during stress	
	↑ Plasma levels of catecholamines	
	↓ βadrenergic modulation of heart rate myocardial contractility and vascular tone due to post-synaptic signaling deficits	
↓ Reduced physical activity	Learned lifestyle	Exaggerated age changes in some aspects of cardiac structure and function, eg, impaired LV ejection reserve capacity
	Frailty	Negative impact on atherosclerotic vascular disease, hypertension and heart failure

PERSONAL CONTRIBUTION

MATERIAL AND METHODES

Aim. The present study was designed as an analysis of correlations between determinants and favoring ischemic heart disease identified in the selected study group.

Type of study. The study had: a retrospective component concerned with group I patients who underwent a heart transplant and a prospective component covered by group II patients died in hospital and autopsied to determine the cause of death.

Materialul studiat. Group I included 118 cases transplanted for ischemic heart disease in Puerta de Hierro University Hospital Madrid between 1995-2012. Group II included 81 cases and was splited in: Group IIA - patients with cardiovascular (CV) cause of death și Group IIB - patients with non - cardiovascular cause (NCV) of death.

Sursele de date are represented: *For group I* by: Clinical observation documents of transplanted patients included in the study, Pathologic diagnosis documents and Human biological material which consisted of: Paraffin blocks of operated cases included in the study and Histological slides obtained in all cases included in the study.

For group II by: Clinical observation documents of dead and autopsied patients included in the study, necropsy protocols, and human biological material which consisted of: fragments of the heart taken during necropsy, paraffin blocks made of myocardial tissue fragments collected during necropsy and histological slides obtained in all cases included in the study.

Assessed parameters. *For group I* they were: Clinical parameters (Sex, Age), Cardiovascular risk factors, Status at admission, Morphological parameters (Ultrasound exploration, Anatomopathological Exploration). *For group II* they were: Clinical parameters (Sex, Age), Morphological parameters (The average thickness of the LV wall, The average thickness of the anterior wall of the left ventricle (LVAW), The average thickness of the lateral wall of the left ventricle (LVLW), The average thickness of the LV posterior wall (LVPW), The average thickness of the interventricular septum (SIV), The average thickness of the wall of the right ventricle).

For quantitative morphological determinations a morphometry module was developed with software environment MATLAB (Mathworks) In each field were made the following determinations: The percentage of fibrosis and the average diameter of the miocardial fibers.

CLINICAL PROFILE

The analysis of all clinical parameters in the two groups, allowed us to sketch the profiles that have emerged from bundling these parameters. In Table 2 are recorded the significant issues arising from the evaluation of clinical data from the two groups.

Group I (Spain)

For **Group I** of patients, tose who underwent heart transplantation, the analysis of clinical data has led to the shaping of a possible profile of the candidate to cardiac transplantation.

He is usually a man aged about 60 years, overweight, smoker or former smoker and alcohol consumer, not necessarily with dyslipidemia and usually without diabetes, known with coronary disease, with at least one heart attack in his history, the most often located in the left coronary anterior descending branch territory which,

Usually didn't benefit of a corrective procedure for the coronary occlusion, and usually without valvular involvement. When present, the valvular damage was localized mainly in the mitral valve.

Table 2: Comparative Sinopsis of the clinical profiles of the groups

Parameter	Group I	Group IIA	Group IIB
Sex	**M/F10,8**	M/F = 1,6	M/F = 1,39
Age (years)	56,4 (17-68)	63,9 (45-82)	52,7 (8-82)
BMI (body mass index)	Overweight		
Risk Factors			
Smoking	Smoker/Ex smoker		
Alcohol	Consumer		
Dyslipidemia	NonD ?		
Diabetes Mellitus	Non DM		
Cardiac Profile			
Old Infarctions	At least one Mainly Anterior descending branch of LC		
Prostetists/revascularisation	Rarely, Usually Bypass		
Valvular involvement	Usually No		
Status at admission			
Cardiac failure Grade	NYHA 3-4		
Cardiogenic Shock	Rarely		
Associated Conditions	Pulmonary hypertension Arterial hypertension		
EKG Exploration			
QRS Complex	Usually normal		
Arrhythmias	Prezent 2/3 of cases Usually solitary		
US Exploration			
Kinetics Disturbances	Prezent. More frequently associated		
VEF	Reduced for both venricles		

On admission, patients came with a high degree of heart failure, rarely with cardiogenic shock, but with either rhythm or conduction disturbances rarely associated, with a QRS complex usually within normal limits in terms of duration and amplitude. In turn, the ultrasound examination identified the presence of cardiac walls kinetic disorders, often associated with a low ventricular ejection fraction mainly of the left ventricle

Group II (Craiova)

The analysis of the only two clinical parameters revealed that, although dead patients in both subgroups were rather men than women in the subgroup with cardiovascular cause of death men percentage was higher and with a higher mean age.

MORPHOLOGICAL STUDY

The analysis of all morphological parameters in the two groups, allowed us to sketch the profiles that have emerged from bundling these parameters..

LOTUL I (SPANIA)

For **Group I** of patients, tose who underwent heart transplantation, the analysis of clinical data has led to the shaping of a possible morphological profile of the removed heart.

The hearts had, usually a weight and dimensions (longitudinal and transversal diameters) over the limits accepted as normal.

The values of cardiac walls thickness were higher than values considered as normal in most of the cases, with a special mention for the right ventricle wall which, in a significant percentage of cases, had a thickness higher than 1 cm (Table 3).

Table 3: Values of cardiac wall mean thickness in different segments in Group I
la nivelul diferitelor segmente ale cordului la Lotul I

Group	LV	IVS	RV
Group I (Spain)	16,8	10,9	**9**

In one third of cases, cardiac walls presented areas of dilatation, usually unicameral, mainly in the left ventricle. Aneurismal dilatations were rare, appearing on scar areas after extended infarctions.

Coronarian tree harbored in practically all patients atheromatous lesions, complicated in a significant number of cases, extended to at least two of te main arterial branches and realising in most of the cases an occlusion of more than 70% of the arterial lumen.

Acute ischaemic attacks at the admission were not so frequent but when existed, they were placed in the left coronary territory.

The microscopic analysis of the miocardial tissue revealed the signs of a chronic ischaemic distress, represented by miocardosclerosis who represented in some cases 20% of the miocardial tissue.

Analiza morfologică microscopică a ţesutului miocardic a scos în evidenţă semnele unei suferinţe ischemice cronice, tradusă printr-un proces de miocardoscleroză (MCS) care a ajuns în unele cazuri să reprezinte 20% din ţesutul miocardic.

Miocardial fibers diameter (MFD) ranged usually between the limits considered as normal but with a trend of values aggregation towards the inferior limit of the normal values variation range.

GROUP II (CRAIOVA)

For the group II of patients, computer assisted morphological analysis revealed two interesting aspects.

On one hand, despite the cause of death, the anterior segment of left ventricle wall was the thinnest, the thickness increasing towards the posterior segment and having the biggest values in the interventricular septum (Table 4).

Table 4: Mean values of the LV and IVS walls thickness in the three groups

Group	LV	LV-Ant	LV-Lat	LV-Post	IVS
Entire Group	12.96	11.97	12.82	13.51	13.88
GroupIIA - CV	12.69	11.73	12.28	13.44	13.41
Grooup IIB - NCV	13.08	12.08	13.08	13.54	14.1

On the other hand, mean values of different cardiac wall segments, including those of right ventricle wall were bigger in group IIB than in group IIA (Tables 4 and 5).

Table 5: Mean values of the RV wall thickness in the three groups

Group	Entire Group	GroupIIA - CV	Grooup IIB - NCV
RVWTh	4,48	4,35	**4,53**

In Table 6 are summarised the significant aspects resulted from the morphological analysis.

Table 6: Comparative Sinopsis of the morphological profiles of the groups

Parameter		Group I	Group IIA	Group IIB
Macroscopic Morphology				
Cord Weight		Usually over normal limits		
Dimensions		Usually increased		
Dilatations		Significant - 1/3, Usually unicameral - LV		
Aneurism		Rarely, on scars of extended infarctions		
Wall Dimensions	*LV*	2/3 > 15 mm	Bigger in NCV group	
	IVS	Ovăr 50% > 10 mm	Bigger in NCV group	
	RV	40% >10 mm	Bigger in NCV group	
Microscopic Morphology				
Coronary Involvement	***Oclusion Type***	Atheromatosis 40% complicated		
	Oclusion Grade	Usually Grade 4 (>70%)		
	Extension	At least 2 principal truncks		
Recent Infarctions		Rarely (< 20%) LC teritory		
Miocard Evaluation	***MCS***	Sclerosis: 11 - 21% from tissue area		
	MFD	Normal with decreasing trend		

CORELAȚII CLINICO-MORFOLOGICE

În urma analizei corelațiilor între parametrii morfologici evaluați la fiecare din cele două loturi, dar și a comparării evoluției unora dintre parametri la cele două loturi s-au conturat s-au conturat câteva observații.

The analysis of correlations between morphological parameters evaluated at each of the two groups, but also of the comparison of the evolution of some of the parameters in the two groups were outlined some observations.

Group I (Spain)

La Lotul I cu pacienți supuși transplantului cardiac, prima corelație verificată statistic a fost cea între greutatea și dimensiunile cordului. Evaluarea statistică a demostrat că greutatea și dimensiunile cordului s-au modificat paralel, în sensul că mărirea greutății cordului s-a însoțit de o mărire a diametrelor sale principale.

In **Group I**, with patients undergoing heart transplantation, the first statistically verified correlation was that between weight and size of the heart. Statistical evaluation demonstrated that heart weight and dimensions have changed in parallel, meaning that the increase in heart weight was accompanied by an increase in its main diameters.

Miocardosclerosis seems to have an inverse relationship with body mass index, meaning that people with low BMI would present a higher degree of myocardial sclerosis but the correlation was not statistically validated.

The evolution of myocardial interstitial spaces fibrosis process does not seem to influence the main morphological macroscopic parameters ie the heart weight and main diameters the cardiac ventricles wall thickness as well as the principal functional parameter ie ejection fraction although there is a very discreet decreasing trend of ejection fraction values in cases with a greater percentage of miocardosclerosis.

There was however an exception ie the degree of miocardosclerosis in the IVS which seems to directly influence the size of the septum, fact supported by the statistical evaluation.

The mean value of the myocardial muscle fibers diameter showed the same inverse trend of correlation with BMI as the miocardoscelrosis process, meaning that people with low BMI would present higher values of MFD but, as for miocardosclerosis, statistical evaluation did not validate this trend.

In turn, MFD changes seem to directly influence the main morphological macroscopic parameters of the heart, ie the weight and the main diameters, especially longitudinal diameter as well as cardiac wall thickness in the ventricles.

Ejection fraction, the main functional parameter, showed more pronunced decreasing trend at bigger values of MFD as compared with miocardosclerosis, but, as for miocardosclerosis, statistical evaluation did not validate this trend

As in miocardosclerosis, IVS was an exception as meaning that MDF changes had noinfluence on its size this time.

Group II (Craiova)

In group II, evaluarea corelațiilor dintre **genul pacienților** decedați și grosimile principalelor segmente ale peretelui cardiac a arătat două patternuri diferite și anume:

In **Group II**, the assessment of correlations between gender of deceased patients and the thickness of the cardiac wall main segments showed two different patterns, namely:

- In the LV wall and septal wall, mean thickness was higher in women than in men in the group of patients who died because of cardiovascular diseases and higher in men than in women in the group of patients died because of noncardiovascular diseases
- In the right ventricle wall, the mean thickness was higher in men than in women in both subgroups

The assessment of correlations between gender of deceased patients and the thickness of the cardiac wall main segments showed no correlation statistically validated between age and morphological changes of the cardiac wall in none of the two subgroups.

However, even if not validated statistically, some individual profiles of correlation with age for each segment of the cardiac wall were outlined. Thus:

- In the left ventricular wall, there was an increasing trend of the mean thickness in both subgroups
- In the right ventricular wall, the thickness had a decreasing trend with age in subgroup IIA and an increasing trend in IIB subroup.
- In the interventricular septum, the profile was opposite of what happened in the RV as meaning that septal thickness had an encreasing trend with age in subgroup IIA and a decreasing trend in group IIB.

On the microscopic level, MDF reducing showed no clear trend of correlation with the increased degree of miocardosclerosis, statistical evaluation showing rather a lack of influence of morphological changes of the myocardial muscle fiber on the miocardosclerosis phenomenon.

COMPARISONS BETWEEN GROUPS

Age of the patients

Comparative analysis of the distribution of patients by age group shows that the vast majority of patients undergoing cardiac transplantation (83%) were aged between 50 and 70 years, more than half of them being aged between 60 and 70 years. Interestingly, unlike the Romanian groups, no patient undergoing transplant had over 70 years of age. In turn, in the Romanian group of patients who died from cardiovascular diseases, patients aged over 70 years have been a significant contingent of nearly one third of patients. In the Romanian group of patients who died by noncardiovascular diseases, age distribution was more even, patients with age between 20 and 60 years accountting for almost half of the patients.

Gender of the patients

The comparison between gender distribution in all studied groups revealed that there was a predominance of men in all groups but Spanish patients in group undergoing heart transplantation were almost exclusively men.

Dimensions of cardiac walls

Left Ventricle Wall. Comparative analysis of the LV wall mean thickness in groups under study revealed an increased value in patients undergoing transplantation as compared with our study. The explanation may reside in the particularities of Spanish group mentioned above and summarised in Table 6. On the other hand, determination in Romanian group were made on tissue fixed in formalin, which determines a reduction of the tissue dimensions.

IVS Wall. In the interventricular septum a smaller mean value in the group of cords replaced by transplant as compared with our study subgroups could be observed which could mean that, in the interventricular septum, functional decompensation is expressed mainly by myocardial fiber elongation than by its diameter increasing. Thus, the mean value of the IVS thickness in the group with patients dead by noncardiovascular diseases could be taken as reference.

Right Ventricle Wall. The right ventricular wall situation is similar to that found in the left ventricle. However, the difference between Spanish group and ours is striking. The explanation may be also like in left ventricle

CONCLUSIONS

El a condus la câteva concluzii care pot avea importanţă în abordarea viitoare a a înțelegerii fenomenelor de îmbătrânire a țesutului miocardic în condiții normale dar și patologice

Our study has led to several conclusions that can be important in the future approach of understanding aging phenomena in the myocardial tissue in both normal and pathological conditions.

GROUP I

Cazurile din lotul de pacienți supuși transplantului cardiac au prezentat profiluri clinic și morfologic al cordului particulare care au stat la baza alterării funcționale severe ce a impus înlocuirea cordului.

Patients undergoing heart transplantation showed particular clinical and morphological profiles of the heart which were the basis for severe functional alterations that imposed heart replacement

The clinical profile included:

- Patient, usually a man aged about 60 years, overweight, smoker and alcohol consumer, known with coronary artery disease, with at least one heart attack in history, most often located in the territory of the anterior descending branch of the left coronary artery, which, more often didn't benefit of corrective procedure for the coronary occlusion
- The patient, hospitalized for a high degree of heart failure, with either rhythm are or conduction disturbances, with abnormal cardiac wall kinetic and with a low left ventricular ejection fraction

The morphological profile included:

Myocardial tissue with signs of chronic ischemic distress, expressed by an extensive miocardosclerosis up to 20% of myocardial tissue in some cases, and signs of myocardial fiber functional overtraining, expressed by thinner fibers, with values of the mean diameter placed at the lower limit of the considered normal values range.

- Cord with weight and main dimensions and at each of the main segments of the cardiac wall bigger than normal and quite frequently, with expansion areas in the cardiac walls
- A special mention must be done for the right ventricle wall which in a significant percentage of cases had an impressive thickness, exceeding 1 cm
- Coronary tree atheromatous affected by atheromatous lesions, usually complicated, extended across at least two of the main arterial trunks and producing an over 70% occlusion of the arterial lumen in most of the cases, occlusion which, sometimes, led even to acute ischemic accidents, usually in the left coronary artery territory, which led to hospitalization for the transplant
- Myocardial tissue with signs of chronic ischemic distress, expressed by an extensive miocardosclerosis up to 20% of myocardial tissue in some cases, and signs of myocardial fiber functional overtraining, expressed by thinner fibers, with values of the mean diameter placed at the lower limit of the considered normal values range.

Quantitative analysis of myocardial interstitial process of fibrosis revealed the direct participation of the fibrilogenetic process only in the remodeling of the IVS dimensions. For the rest, the interstitial fibrosis was not the one responsible for the changes in the size of the main segments of the cardiac wall and heart weight.

Quantitative analysis of myocardial fibers dimensions revealed their participation in the change of major morphological macroscopic parameters of the heart both

by increasing their diameter, in the first step, and by their elongation in the second step of adaptation mechanisms in order to take over the functional effort especially determined by the acute ischemic accidents which destroy fraction of miocardial working capital, fractions replaced by the nonfucntional fibrillar collagen tissue.

Quantitative analysis of myocardial fibers dimensions revealed their participation in the change of major morphological macroscopic parameters of the heart both by increasing their diameter, in the first step, and by their elongation in the second step of adaptation mechanisms in order to take over the functional effort especially determined by the acute ischemic accidents which destroy fraction of miocardial working capital, fractions replaced by the nonfucntional fibrillar collagen tissue.

It seems that changes of myocardial fibers do not participate in the remodeling of IVS where, as mentioned above, the main role seems to belong to the sclerosis process.

LOTUL II

Patients in our group showed also particular clinical and morphological profiles that should be highlighted

Clinical profile included a patient, usually a man aged over 60 years in the group of patients died of cardiovascular causes, more close to the average age of the group with heart transplant and aged around 50 years the group of patients who died by causes non cardiovascular

Quantitative analysis of the main segments of the cardiac wall dimensions in deceased patients of our study revealed two interesting aspects:

- Overall, the mean thickness of different segments of the cardiac walls (LV, IVS, RV) were bigger in the group of patients who died because of noncardiovascular disease than in the group of patients who died because of cardiovascular disease
- Irrespective the cause of death type, the anterior segment of the left ventricular wall was the thinnest, the thickness increasing towards the posterior segment of the left venticular wall and having the highest values in the IVS wall

The results of this study can be a starting point for developing futher research on morphological changes of the cardiac tissue related with the age and the presence or not of its damage both in the number of cases included in the study as well as in the morphologic and clinical parameters investigated. An additional argument to continue these studies is their reduced number, somehow inexplicablle in the literature.

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