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Final Master’s Thesis

MORPHOLOGIC PATTERNS OF LEFT VENTRICLE REMODELING IN ISCHEMIC HEART DISEASE

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SUMMARY

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*Morphologic patterns of left ventricle remodeling in ischemic heart disease*

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**Consultant:** prof. dr. Dalia Pangonytė, MD

**Aim of study:** to determine the changes of left cardiac ventricular weight and geometry (endocardial surface area) for the patients diagnosed with ischemic heart disease.

**Objectives:**
1. To determine the changes of left ventricular weight;
2. To determine changes of left ventricular endocardial surface area;
3. To evaluate the correlation patterns between left ventricular weight and endocardial surface area;
4. To evaluate the correlation patterns between left cardiac ventricular organometric parameters and atherosclerotic stenosis lesions.

72 men who were ≥25 years old and died from IHD (mean age of men was 48.6 ± 8.2 years old). Selected men were divided into groups: IHD with no HF (n=18), IHD with HF of I-II NYHA functional class (n=22) and control group (n=32).

**Methods.** 72 men who were ≥25 years old and died from IHD (mean age of men was 48.6 ± 8.2 years old). Selected men were divided into groups: IHD with no HF (n=18), IHD with HF of I-II NYHA functional class (n=22) and control group (n=32). Morphologic macroscopic investigation of the deceased heart was performed at the Laboratory of Cardiac Pathology of Cardiology Institute according to modified WHO recommendations on methods of fatal IHD examination. Weight of left cardiac ventricle and interventricular septum, endocardial surface area of left cardiac ventricle were measured, and atherosclerotic stenosis index was calculated.

**Results.** Weight of free wall of left cardiac ventricle in IHD group with HF of I-II functional NYHA class (149.83 ± 29.58 g) was increased compared to IHD without HF group and control group (p<0.001). Mean overall endocardial surface area of left cardiac ventricle in IHD group with HF of I-II functional NYHA class (90.35 ± 22.91 cm²) was increased compared to both IHD group with no HF and control group (p<0.001). Linear regression pattern was detected between the weight and endocardial surface area of free wall of left cardiac ventricle (r>0.7, p<0.05). Correlation was detected between the weight of free wall of left cardiac ventricle and atherosclerotic stenosis index both in IHD group without HF and IHD with HF of I-II functional NYHA class (r=0.280, p=0.003 and r=0.402, p=0.02, respectively).

**Conclusions:**
1. Left cardiac ventricular remodeling process is initiated when there are no clinical symptoms of HF and continues in HF.
2. First structural transformation in myocardial tissue is already induced before HF as a result of exposure to injuring factor (IHD), and continues to progress in HF.
3. Shifts of cardiac ventricular geometry already start before the symptoms of HF at significant lengths, and continue as a less active process in HF.
4. Not only reconstruction of cardiac muscle tissue is initiated by injuring factor, but also the geometry itself shifts at a similar level as a part of complex remodeling process.
5. Injuring factor (IHD) initiates the cellular reconstruction of affected myocardial tissue that manifests on macroscopic level even before HF becomes symptomatic, and has a cumulative effect on failing cardiac muscle when the diagnosis of HF is established.
CONFLICT OF INTEREST

Author of master thesis declares no conflict of interest.

PERMISSION OF ETHICS COMMITTEE

Research of master thesis is approved by ethics committee (no. of permission BEC-MF-78; issued on 2017-11-21).
ABBREVIATION LIST

ANOVA – analysis of variance
cm – centimeters
cm² – square centimeters
DNA – deoxyribonucleic acid
ECM – extracellular matrix
etc. – et cetera
EU – European Union
fig. – figure
g – grams
HF – heart failure
IHD – ischemic heart disease
MI – myocardial infarction
mm – millimeters
MMP – matrix metalloproteinase
NYHA – New York heart association
p – level of significance
PGE2 – prostaglandin E2
PGI2 – prostacyclin
PIIINP – N-terminal type III collagen peptide
RAAS – renin – angiotensin – aldosterone system
ROS – reactive oxygen species
TIMP – inhibitors of matrix metalloproteinases
WHO – World Health Organization
INTRODUCTION

Age-standardized mortality from ischemic heart disease (IHD) in Lithuania is one of the highest among the European Union (EU) countries. In 2010, it was 436 per 100 000 males and 239 per 100 000 females (compared with the EU averages of 113 for males and 56 for females) [1]. IHD resulting from either acute myocardial infarction (MI) or chronic ischemia accounts for most cases of heart failure (HF). HF is a major public health issue which has increased significantly over the past decades with a current prevalence of over 23 million worldwide. Different studies estimate the overall prevalence of HF in the population to be about 2–3% [1,2].

Furthermore, heart dysfunction is one of the most relevant clinical conditions in all healthcare system with significantly negative outcomes inducing high rates of mortality worldwide. Currently, a greater focus on targeting structural changes occurring in failing heart has grown, and efforts have expanded to therapeutically target the underlying cellular and molecular processes involved in the development of progressing heart muscle injury [3].

The anatomic basis for progressing cardiac dysfunction and, eventually, HF is a constant remodeling process which occurs as a result of any ongoing myocardium exposure to injuring factors. Remodeling is described as activation of cardiac genome, inducing molecular, cellular and interstitial structural shifts, clinically manifesting as significant changes of cardiac geometry including cardiac size, chamber volume and form, as well as functional changes after myocardial injury and the events of overload by increased pressure or blood volume.

Since pathogenesis mechanisms of heart dysfunction are complex and there is an increasing demand for scientific data about the relationship between geometrical changes of particular cardiac chamber parameters and heart dysfunction in IHD on anatomical basis, it is essential to have a more thorough knowledge on the morphometric shifts of cardiac ventricles in cases of heart dysfunction caused by IHD.

Identifying more scientifically-based key facts regarding the pathological processes in the heart, including ventricular remodeling on anatomical level at different stages of IHD, will ensure a presentation of fundamental, structure-based information which is required for an earlier diagnosis of failing heart muscle and more efficient, individualized treatment of this condition, decreasing heart disease-associated morbidity and mortality, as well as balancing expenses of health care system adequately.
AIMS AND OBJECTIVES

**Aim:** to determine the changes of left cardiac ventricular weight and geometry (endocardial surface area) for the patients diagnosed with ischemic heart disease.

**Objectives:**
1. To determine the changes of left cardiac ventricular weight.
2. To determine changes of left cardiac ventricular endocardial surface area.
3. To evaluate the correlation patterns between left cardiac ventricular weight and endocardial surface area.
4. To evaluate the correlation patterns between left cardiac ventricular organometric parameters and atherosclerotic stenosis lesions.
1. LITERATURE REVIEW

1.1. Pathophysiological mechanisms of ventricular remodeling process

Ventricular remodeling is a dynamic process that causes shifts in ventricular geometry by increasing ventricular chamber volume and inducing ventricular reconfiguration. These changes occur as a result of cellular and molecular alterations associated with shifts in cardiomyocytic shape, function and metabolism as well as reconfiguration of interstitial tissue that interacts with these major functional cells of myocardium [4]. Ventricular remodeling is induced and stimulated by constant, ongoing injury of myocardium which usually is a result of chronic ischemia process or acute ischemia attack [4].

Ventricular remodeling is already initiated at first days after acute ischemia episode, when necrosis focus is replaced by regenerating connective tissue that results in thinning of ventricular wall [4]. Myocardial injury which is associated with ventricular wall stress and, as a result, impaired cardiac hemodynamics, up-regulates a cascade of neuroendocrine, paracrine and autocrine processes that include activities of renin-angiotensin-aldosterone system (RAAS), adrenergic system, oxidative stress, inflammation-stimulating cytokines and vasoconstrictors, such as endothelin [4]:

a) Sympathetic nervous system, physiologically maintaining adrenergic stimulation-related processes, is activated in the early stages as a response to ongoing myocardial muscle injury. Decrease in cardiac output activates the sympathetic system. The inhibitory input from baroreceptors and mechanoreceptors decreases, and excitatory input increases. The result of these processes is a loss of heart rate variability and increase in peripheral vascular resistance. Moreover, increased release and decreased uptake of norepinephrine causes peripheral constriction. Increased sympathetic activation of the beta1-adrenergic receptor results in increased heart rate and force of myocardial contraction, and increased cardiac output. Stimulation of myocardial alpha1-adrenergic receptors, elicits a modest positive inotropic effect, as well as peripheral arterial vasoconstriction. As a result, myocardial energy requirements are augmented, therefore activation of the sympathetic nervous system provides short-term support that becomes maladaptive over the long term;

b) RAAS is activated as a result of prolonged myocardial tissue exposure to injuring factors. Decreased perfusion to the kidney provokes increased renin release, and, as a result, increased production of angiotensin II that has several important actions critical to maintaining short-term circulatory homeostasis. However, sustained expression of angiotensin II is maladaptive, leading to myocardial, renal and other organs fibrosis, as well as worsening neurohormonal activation by enhancing the release of norepinephrine from sympathetic nerve endings, and stimulating zona
glomerulosa of adrenal cortex to produce aldosterone. Prolonged increased expression of aldosterone may have negative effects on cardiovascular system by provoking hypertrophy and fibrosis in blood vessels and myocardium, therefore contributing to reduced vascular compliance and increased ventricular stiffness. In addition, aldosterone provokes endothelial cell dysfunction, baroreceptor dysfunction, and inhibits norepinephrine uptake [5]. In the setting of RAAS activation, the release of vasopressin and brain natriuretic peptide may serve as a significant counter-regulatory mechanism that maintains sodium and water homeostasis. However, for reasons that are not entirely clear, the renal effects of the natriuretic peptides appear to become significantly decreased with advancing heart failure, leaving the effects of RAAS unopposed;

c) Effects of vasoconstrictors: renal sympathetic stimulation also can lead to the non-osmotic release of vasopressin from the posterior pituitary, which reduces the excretion of free water and contributes to worsening peripheral vasoconstriction, as well as increased endothelin production. Neurohormones causing vasoconstriction, also activate counter-regulatory vasodilator effects, including release of natriuretic peptides, nitric oxide, bradykinin, apelin, vasodilating prostacyclin (PGI2) and prostaglandin E2 (PGE2). In addition to being a vasodilator, PGE2 enhances renal sodium excretion and modulates the antidiuretic action of vasopressin. Under normal circumstances, the continuous release nitric oxide from the endothelium counteracts vasoconstriction and allows for appropriate vasodilatory responses. As heart dysfunction advances endothelial cell–mediated vasodilatory effect is lost, and these processes contribute to the excessive peripheral arterial vasoconstriction and formation of advanced heart failure (HF).

Furthermore, in cultured cardiac myocytes reactive oxygen species (ROS) stimulate myocyte hypertrophy, re-expression of fetal gene programs, and apoptosis. ROS also can modulate fibroblast proliferation and collagen synthesis, as well as triggering increased matrix metalloproteinase (MMP) activation. ROS also can affect the peripheral vasculature in heart failure by decreasing the bioavailability of nitric oxide.

In conclusion, these multiple counteracting systems are able to restore cardiovascular function to a homeostatic condition, and patient remains asymptomatic for a period of time until these compensatory reactions are exhausted. The function deterioration is usually not stopped by initial compensatory mechanisms. With time, sustained activation of these systems may lead to secondary ventricular damage, with worsening ventricular remodeling process leading to cardiac decompensation. As a result of these changes, patients undergo the transition from asymptomatic to symptomatic heart failure [5].
1.2. Cardiomyocyte and interstitial tissue morphology alterations in ventricular remodeling

Although the complex changes that occur in the heart during ventricular remodeling have traditionally been described in anatomic terms, the process of ventricular remodeling also has an important impact on the biology of cardiac myocyte on changes in the volume of myocyte and non-myocyte components of the myocardium, and, eventually, on the geometry and architecture of the left ventricular chamber.

Numerous studies have suggested that failing human cardiac myocytes undergo a number of important changes that might be expected to lead to a progressive loss of contractile function, including decreased alpha-myosin heavy chain gene expression and increase in beta-myosin heavy chain expression, progressive loss of myofilaments in cardiac myocytes, alterations in cytoskeletal proteins, alterations in excitation-contraction coupling and desensitization of beta-adrenergic signaling. However, the most important and studied alteration of cardiac myocyte is the hypertrophy.

Two basic patterns of cardiac hypertrophy occur:

a) In pressure overload hypertrophy (for example, aortic valve orifice stenosis, systemic or pulmonary arterial hypertension), the increase in systolic wall pressure leads to addition of sarcomeres, an increase in myocyte cross-sectional area, and increased ventricular wall thickening. This pattern of remodeling is “concentric” hypertrophy;

b) In volume overload hypertrophy (for example, aortic or pulmonary valve regurgitation), increased diastolic wall stress leads an increase in myocyte length with the addition of sarcomeres in series, causing increased ventricular dilation. This pattern of remodeling is “eccentric” hypertrophy or a “dilated” phenotype.

Cardiac myocyte hypertrophy also leads to changes in the biologic phenotype of cardiac myocyte that are secondary to reactivation of fetal genes normally not expressed in postnatal period of life. Reactivation of these fetal genes is accompanied by decreased expression of genes that are normally expressed in the adult heart. Activation of the fetal gene program may contribute to the contractile dysfunction that develops in failing cardiac myocyte [6]. The stimuli for the genetic reprogramming of cardiac myocyte include mechanical stretch/strain of cardiac myocyte, neurohormones (norepinephrine, angiotensin II), inflammatory cytokines (tumor necrosis factor, interleukin-6), growth factors, and ROS).

The early stage of cardiac myocyte hypertrophy is characterized morphologically by hyperplasia of myofibrils and mitochondria, as well as, hypertrophy of mitochondria and enlargement of nuclei. At this stage, cardiac myocytes are larger than normal, but with preservation of cellular organization. As hypertrophy continues, there is an increase in the number of mitochondria, as well as, the addition of new contractile elements in localized areas of the cell. Cells with a long term
hypertrophy show more obvious disruptions in cellular organization, such as markedly enlarged nuclei with highly lobulated membranes, accompanied by the displacement of adjacent myofibrils with loss of the normal registration of the Z-bands. The late stage of hypertrophy is characterized by myocytolysis with marked disruption of Z-bands and severe disruption of normal parallel arrangement of the sarcomeres, accompanied by dilation and increased tortuosity of T-tubules.

Alterations observed in remodeling of myocardium may be classified into those that occur within increasing:

a) Volume of cardiac myocytes;

b) Volume and composition of the extracellular matrix.

With respect to the changes that occur in cardiac myocyte component of myocardium, increasing evidence suggests that progressive myocyte loss through necrosis, apoptosis, or autophagocytosis, may contribute to progressive cardiac dysfunction in ventricular remodeling [7]:

- Necrosis of cardiac myocyte occurs in IHD, myocardial injury, toxin exposure, infection, and inflammation. In contrast with apoptosis, the rupture of cellular membranes within cell necrosis releases intracellular contents, which evoke an intense inflammatory reaction, leading granulocytes, macrophages, and collagen-secreting fibroblasts into the area of myocardial tissue injury. The final outcome of irreversible myocardial injury is a substitutional fibrosis, or scar, which may alter the structural and functional properties of the myocardium;

- Apoptosis, or programmed cell death, is an evolutionarily conserved process that allows multicellular organisms to selectively remove cells through a highly regulated program of cell suicide. Apoptosis pathway is activated by extrinsic factors utilizing cellular surface receptors or intrinsic factors, such as inadequate nutrition, hypoxia, oxidative stress, nutrient stress, protein toxic stress, deoxyribonucleic acid (DNA) damage, chemical and physical toxins. Under pathologic circumstances, such as acute ischemia and/or in dilated cardiomyopathy, the apoptotic program can be triggered inappropriately, resulting in inadvertent cell death that can lead to organ failure. In contrast with the cell swelling that characterizes necrosis, during apoptosis the cell shrinks and eventually breaks up into small, membrane-surrounded fragments. The dying cell is engulfed by macrophages, which prevents the release of the reactive intracellular contents, thereby preventing an inflammatory reaction. The exact physiologic significance and consequence(s) of apoptosis in human heart failure is so far difficult to determine because of the lack of knowledge with respect to the actual rate of cardiac myocyte apoptosis in failing human heart;

- Autophagy refers to the homeostatic cellular process of sequestering organelles, proteins, and lipids in an autophagosome, where the contents are subsequently delivered to the lysosome for degradation. In contrast with necrosis and apoptosis, autophagy is primarily a survival mechanism that regulates the quality and abundance of intracellular proteins and organelles. When autophagy involves
the total destruction of the cell, it is referred to as autophagy. Recent studies have demonstrated the existence of autophagy in hypertrophied, failing, and hibernating myocardium.

Although the distinction between necrosis and apoptosis is obvious in certain circumstances, the dividing line between these two conditions often is less clear in the failing heart. Indeed, similar mechanisms can operate in both types of cell death. Therefore, instead of the existence of distinct types of cell death in heart failure, a more likely scenario is a continuum of cell death responses that contribute to progressive myocyte loss and disease progression.

Changes within the extracellular matrix (ECM) constitute the second important myocardial adaptation that occurs during cardiac remodeling. The myocardial ECM consists of basement membrane, fibrillar collagen network, proteoglycans and glycosaminoglycans, as well as specialized proteins such as matricellular proteins. The major fibrillar collagens in the heart are type I and III, with a ratio of type I to type III of approximately 1.3 to 1.9:1. The organization of myocardial fibrillar type I and type III collagen ensures the structural integrity of surrounding myocytes and is essential for maintaining alignment of myofibrils within the myocyte through the interaction of collagen and integrins with cytoskeletal proteins [8,9].

Matricellular proteins are a class of non-structural ECM proteins exerting regulatory functions, most likely through their interactions with cell surface receptors, the structural proteins, and soluble extracellular factors, such as growth factors and cytokines. Osteopontin, a matricellular protein which is likely to have a role in regulatory functions is upregulated in cases of heart failure and, can potentially be a marker of disease progression and severity [8,9].

During cardiac remodeling there are important changes in the ECM, including changes in fibrillar collagen synthesis/degradation and in the degree of collagen cross-linking, as well as loss of collagen structures that connect the individual cardiac myocytes. Markers of collagen turnover have been shown to be increased in patients with dilated cardiomyopathy compared with age-matched control subjects [9].

Recently, it has been suggested that a family of collagenolytic enzymes becomes activated within the failing myocardium. Collectively, these collagenolytic enzymes have been referred to as matrix metalloproteinases (MMPs). Conceptually, progressive activation of MMPs might be expected to lead to progressive degradation of ECM, which would in turn lead to mural realignment. However, degradation of matrix is also controlled by glycoproteins tissue inhibitors of MMP (TIMPs). Alterations in ECM that occur during ventricle remodeling are likely to be characterized by periods of ongoing structural protein degradation and deposition throughout the process of ventricular remodeling [9].

In patients with idiopathic or ischemic dilated cardiomyopathy, serum N-terminal type III collagen peptide (PIIINP) levels have been shown to be independent predictors of mortality [10].
Moreover, it is becoming increasingly apparent that the three-dimensional organization of the extracellular matrix plays an important role in regulating cardiac structure and function in HF [11].

Studies in failing human myocardium have shown a quantitative increase in collagen types I, III, VI, and IV along with fibronectin, laminin, and vimentin, and a decrease in the ratio of type I collagen to type III collagen in patients with IHD [11]. Moreover, clinical studies point to a progressive loss of cross-linking of collagen in the failing heart, as well as loss of connectivity of the collagen network with individual myocytes, which would be expected to result in profound alterations in left ventricular structure and function. Furthermore, loss of cross-linking of the fibrillary collagen has been associated with progressive ventricular dilation after myocardial injury.

Alternatively, collagen accumulation can occur as a result of microscopic scarring (replacement fibrosis), that develops in response to cardiac myocyte cell necrosis. This scarring or “replacement fibrosis” is an adaptation to the loss of parenchyma and is therefore critical to preserve the structural integrity of the heart.

In conclusion, ventricular myocardium remodeling process manifests in microscopic alterations of cardiac myocytes increasing their size and intracellular structure according to the type of stress these functional cells are exposed to. Moreover, rearrangements of extracellular matrix which contributes towards sustaining adequate-to-workload myocardial tissue structure and function, also plays an important role in reconfiguration of cardiac ventricular geometry when myocardial tissue is exposed to environmental, external and internal injuring factors.

1.3. Geometric and functional shifts of cardiac ventricle in remodeling process

Shifts in biology of cardiac myocyte in failing myocardium are largely responsible for the progressive ventricular dilation and, simultaneously, dysfunction that occurs during cardiac remodeling. As discussed further on, many of the structural changes that accompany ventricular remodeling may contribute to worsening HF. Indeed, one of the first observations with respect to the abnormal geometry of remodeled ventricle is the consistent finding that the remodeled heart was not only larger, but also more spherical in shape [11, 12, 13]. Outcomes of changes in cardiac ventricular shape from an ellipse to a more spherical shape causes increase in wall stress of ventricle, thereby creating a de novo energy burden for the heart. Also, as a result of ventricular dilation an increase in afterload is provoked, which will increase mechanical energy loss of the ventricle, which exacerbates the underlying problems with energy utilization in failing ventricle.

In addition to increased cardiac ventricle end-diastolic volume, ventricular wall thinning also occurs as the ventricle begins to remodel. Increase in wall thinning along with the increase in afterload
created by ventricular dilation leads to a functional “afterload mismatch” that may further contribute to a decrease in forward cardiac output [13].

Increased cardiac ventricular wall stress also can lead to sustained expression of stretch-activated genes (angiotensin II, endothelin, and tumor necrosis factor) and/or stretch activation of hypertrophic signaling pathways. Moreover, the high end-diastolic wall stress might be expected to lead to episodic hypoperfusion of subendocardium, and, as a result, worsening of ventricular function, as well as increased oxidative stress and activation of families of genes that are sensitive to free radical generation (tumor necrosis factor and interleukin-1β).

Another important mechanical problem that results from progressive ventricular dilation is that the papillary muscles are pulled apart, resulting in incompetence of atrioventricular valve and development of “functional atrioventricular regurgitation”. In addition to the loss of forward blood flow, atrioventricular regurgitation results in further hemodynamic volume overloading of the ventricle. Taken together, the mechanical burdens caused by ventricular remodeling might be expected to lead to increased ventricular dilation, decreased forward cardiac output, and increased overall hemodynamic overloading [13].

Thus, alterations in remodeling ventricle may develop a self-amplifying situation, in which worsening neurohormonal activation occurs in response to the inability of the remodeled ventricle to respond appropriately to these compensatory mechanisms. Moreover, at some point it is predictable that the cumulative effects of end-organ changes that occur within injured ventricle may progress to the point where no amount of neurohormonal stimulation can maintain cardiovascular homeostasis, at which point HF may progress independently of the neurohormonal status of the patient.
2. RESEARCH METHODOLOGY AND METHODS

Object of research: morphologic changes of left cardiac ventricle weight and endocardial surface area in IHD with no HF and with HF of I-II functional NYHA class in the context of IHD.

2.1. Characteristics of selected group for study

72 men who were ≥25 years old and died from IHD (mean age of men was 48.6 ± 8.2 years old). They were not diagnosed with any other condition that can induce myocardial structure changes: systemic arterial hypertension, cardiomyopathy, congenital and acquired heart valve diseases, diabetes mellitus, pulmonary diseases. Myocardial fibrosis-modulating treatment was not applied for selected men.

Selected men were divided into two groups [14; 15]:

1) First group – IHD with no HF group consisted of men who died within 6 hours from the first symptoms in the first episode of acute cardiac attack, outside the hospital, IHD was diagnosed when a selected man was alive or during post-mortem examination, no scars after MI were detected during autopsy, only acute ischemic injuries lasting for <12 hours were found when examining the removed heart, and mean age of men was 46.3±5.9 years old (n=18);

2) Second group – IHD with HF of I-II NYHA functional class group consisted of men who died within 6 hours from the first symptoms of relapsed episode of acute cardiac attack, outside the hospital, IHD was diagnosed when a selected man was alive or during post-mortem examination, acute ischemic injuries, lasting for <12 hours were found, scars of previous MI were detected, and mean age of men was 49.7±7.8 years old (n=22).

32 men (mean age was 46.0±10.8 years old) who died at the same period of time due to external circumstances and acute diseases not associated with cardiac pathology were included into control group (n=32).

2.2. Methods of cardiac ventricles investigation

Morphologic macroscopic investigation of the deceased heart and myocardium was performed at the Cardiac pathology laboratory of Cardiology Institute according to the modified World Health Organization (WHO) recommendations on methods regarding fatal IHD examination and applying
recommended definitions [14]. During post-mortem examination a heart was separated from the large blood vessels avoiding atrial injury: a heart was lifted up and cava vein, pulmonary trunk as well as aorta were separated at 2-3 cm distance from semilunar valves (fig. 1).

![Heart after separation from large arteries: anterior (left) and posterior (right) views](image1.jpg)

**Fig. 1.** Heart after separation from large arteries: anterior (left) and posterior (right) views

All the blood clots were removed from cardiac chambers. Epicardium coronary arteries and their larger branches were separated, and the heart was weighed. Left cardiac ventricular wall thickness was measured in supraapical area. Also, left and right cardiac ventricular inflow tracts (distance from bicuspidal or tricuspidal valve annular ring to cardiac apical region, respectively) and outflow tracts (from cardiac apical region to the base of posterior aortic valve or right pulmonary valve, respectively) were measured (fig. 2).

![Opening of right cardiac ventricular chamber and removing the blood clots](image2.jpg)

**Fig. 2.** Opening of right cardiac ventricular chamber and removing the blood clots
Later, interventricular septum was separated from right and left cardiac ventricles, and interatrial septum was separated from cardiac atria. The heart was separated into six fragments: free walls of left and right ventricles, interventricular septum, free walls of left and right atria and interatrial septum. All the epicardial fat tissue was removed from these fragments and they were weighed (fig. 3).

**Fig. 3.** Cardiac chambers after separation procedure: left ventricular free wall (left), interventricular septum (right)

Free walls of left ventricle and interventricular septum were attached to the glass plate and contours of these fragments were copied onto the glass plate and recopied on paper sheets. Endocardial surface area was measured by applying computerized visual planimetry. A ratio of ventricular weight and endocardial surface area reflecting relative weight was calculated to evaluate the proportions of ventricular geometry shifts in different groups of selected men.

### 2.2.1. Methods of epicardial coronary arteries investigation

Epicardial coronary arteries that were separated from the heart were fixed by 10% neutral formalin solution and cut in 5 mm transverse sections. Epicardial coronary arteries were separated into 15 segments according to American association of cardiology recommendations with a purpose to identify the exact localization of a particular stenosis level caused by atherosclerotic plaque: right
coronary artery – 1, 2, 3, 4 segments; left coronary artery: trunk of left coronary artery – 5, anterior interventricular branch – 6, 7, 8, 9, 10 segments; circumferential branch – 11, 12, 13, 14, 15 [16].

Atherosclerotic stenosis degree (area of coronary artery lumen occupied by atherosclerotic plaque was calculated), localization and extent was determined. Atherosclerotic stenosis degree was evaluated from 0 to 5 points: 0 points – no stenosis, 1 point – stenosis <25%, 2 points – stenosis is 25 – 50%, 3 points – stenosis is 50 – 75%, 4 points – 75 – 90%, 5 points – ≥90% [17]. Coronary artery stenosis of 50% was assumed as mild injury, 50 – 57% stenosis – as moderate injury, and ≥75% stenosis – as severe injury. A general parameter of atherosclerotic injury – stenosis index – was calculated as a sum of atherosclerotic injury points of all 15 segments of coronary arteries. This parameter indicates the extent of stenosis-causing atherosclerosis.

2.2.2. Methods of myocardium investigation

After fixation with neutral 10% formalin solution multiple transverse sections of left ventricle and interventricular septum fragments by 5 mm intervals were cut from the cardiac base towards apical region. These transverse cuts, or histotopograms, were examined visually and focal ischemic injuries (necrosis, connective tissue proliferation etc.) were evaluated (fig. 4 and 5). Necrosis or substitute fibrosis foci more than 0,5 cm in diameter were evaluated as MI or scar after MI, respectively.

Fig. 4. Multiple transverse cuts of free wall of left cardiac ventricle (fixed by neutral 10% formalin solution)
The effect of scar after MI for remodelling process were evaluated by calculating volume and percentage volume of injured myocardium and comparing it with overall volume of ventricular myocardium applying computerized planimetry method.

2.3. Statistical analysis methods

Initial statistical analysis of morphometric data group was performed when checking hypothesis $H_0$ by $\chi^2$ and *Kolmogorov – Smirnov* criteria [18] with a purpose to evaluate the type of morphometric data distribution. There was no statistical evidence to disprove these criteria ($p>0.05$), therefore $H_0$ hypothesis was confirmed, meaning that measures of mean value and variance of morphometric data parameters were calculated assuming the data was of normal distribution [18].

More detail statistical analysis of morphometric data was performed by calculating descriptive parameters. Calculated values were presented as mean value with standard deviation in IHD with no HF, IHD with HF of I-II NYHA functional class, and control groups.

Multiple variance test (ANOVA) method was applied to check probability if all confidence intervals contain the true difference between control, IHD with no HF, IHD with HF of I-II NYHA functional class groups. Correlations between analysed morphometric parameters were calculated and determined by applying regressive analysis. Statistical significance was $p<0.05$ [18].
3. RESULTS

Parameters of left cardiac ventricle and interventricular septum weight and endocardial surface area in IHD group with no HF and IHD group with HF of I-II functional NYHA class were analysed, and all obtained organometric parameters were compared to the same parameters in control group.

When evaluating left ventricular parameters, free wall of left cardiac ventricle in IHD group with no HF weighed more compared to the control group (128.78 ± 19.84 g and 104.25 ± 12.76 g, respectively, p<0.001). Weight of free wall of left cardiac ventricle in IHD group with HF of I-II functional NYHA class (149.83 ± 29.58 g) was increased compared to the same parameter in IHD without HF group and control group (p<0.001) (fig. 6).

After weighing the interventricular septum, a calculated mean weight of interventricular septum in IHD group with no HF was significantly increased compared to control group (70.74 ± 12.48 g and 57.78 ± 7.61 g, respectively, p<0.001). A mean weight of interventricular septum in IHD with HF of I-II functional NYHA group (77.28 ± 14.28 g) was significantly increased compared to both IHD group with no HF and control group (p<0.001) (fig. 7).

**Fig. 6.** Weight of free wall of left ventricle in different groups (mean±standart deviation): *p<0.001 – between IHD with no HF group and control group, **p<0.001 – between IHD with HF group and IHD with no HF group
Fig. 7. Weight of interventricular septum in different groups (mean±standard deviation):
*p<0.001 – between IHD with no HF group and control group, **p<0.001 – between IHD with HF group and IHD with no HF group

Fig. 8. Endocardial surface area of free wall of left cardiac ventricle (mean±standard deviation):
*p<0.001 – between IHD with no HF group and control group, **p<0.001 – between IHD with HF group and IHD with no HF group
After more detail examination of free wall of left cardiac ventricle and interventricular septum, endocardial surface area of these cardiac segments was calculated. The mean endocardial surface area of free wall of left ventricle was significantly increased in IHD group with no HF compared to control group (50.45 ± 12.34 cm² and 37.49 ± 7.77 cm², respectively, p<0.001). The mean endocardial surface area of free wall of left ventricle in IHD group with HF of I-II functional NYHA class (60.27 ± 16.01 cm²) was significantly increased compared to IHD with no HF and control groups (p<0.001) (fig. 8).

Also, interventricular septum was examined by calculating mean endocardial surface area of left ventricular side. The mean endocardial surface area of interventricular septum was significantly increased in IHD group with no HF compared to control group (26.61 ± 7.75 cm² and 19.11 ± 4.1 cm², respectively, p<0.001). The mean endocardial surface area of interventricular septum in IHD group with HF of I-II functional NYHA class (30.07 ± 8.81 cm²) was significantly increased compared to both IHD with no HF and control groups (p<0.001) (fig. 9).

When examining overall endocardial area of left cardiac ventricle, a mean endocardial surface area of all left ventricle was calculated summing up endocardial surface area of free wall of left cardiac ventricle and endocardial surface area of left ventricular side of interventricular septum. The mean overall endocardial surface area of left cardiac ventricle in IHD group with no HF was significantly

![Graph showing endocardial surface area](image-url)

**Fig. 9.** Endocardial surface area of interventricular septum (mean±standart deviation): *p<0.001 –* between IHD with no HF group and control group, **p<0.001 –* between IHD with HF group and IHD with no HF group.
**Fig. 10.** Overall endocardial surface area of left cardiac ventricle (mean±standard deviation): *p<0.001* – between IHD with no HF group and control group, **p<0.001** – between IHD with HF group and IHD with no HF group

**Fig 11.** A linear regression pattern of relative weight of left ventricle increase in IHD groups without and with HF of I-II functional NYHA class (p<0.001)
increased compared to control group (77.06 ± 18.61 cm² and 56.61 ± 11.04 cm², respectively, p<0.001). The mean overall endocardial surface area of left cardiac ventricle in IHD group with HF of I-II functional NYHA class (90.35 ± 22.91 cm²) was significantly increased compared to both IHD group with no HF and control group (p<0.001) (fig. 10).

**Fig. 12.** Regression pattern of the weight of left cardiac ventricle and atherosclerotic stenosis index (r=0.28, p=0.003) in IHD group without HF

**Fig. 13.** Regression pattern of the weight of left cardiac ventricle and atherosclerotic stenosis index (r=0.402, p=0.02) in IHD group with HF of I-II functional NYHA class
A relative weight parameter (ratio of weight and endocardial surface area) was calculated, and it was determined that ratio of left cardiac ventricle and endocardial surface area remains the same both in IHD with no HF group and control group (2.73±0.75 and 2.88±0.61, respectively, p>0.05). A relative weight of left cardiac ventricle was 2.79±0.65, and there was no statistically significant difference of this parameter compared to the same parameter in IHD with no HF and control groups (p>0.05). A statistically significant linear regression pattern was detected between the weight and endocardial surface area of free wall of left cardiac ventricle (r>0.7, p<0.05). These calculated parameters demonstrated a regularly constant increase of weight and endocardial surface area of left ventricle in IHD without and with HF of I-II functional NYHA class (fig. 11).

A significant correlation was detected between the weight of free wall of left cardiac ventricle and atherosclerotic stenosis index both in IHD group without HF and IHD with HF of I-II functional NYHA class (r=0.280, p=0.003 and r=0.402, p=0.02, respectively, fig. 12 and 13).
5. DISCUSSION OF RESULTS

Principal function of the heart is to provide an appropriate blood amount to supply the tissues to fuel their metabolism both at rest and during activity [19]. When the heart is exposed to injuring effect of IHD, some morphologic changes already starts occurring in early stages of cardiac injury without any functional features of HF. When analysing the data obtained from weighing free wall of left cardiac chamber of selected men, the mean weight of left cardiac ventricle in IHD group without any HF is increased by 23.4% compared to control group (p<0.001), and results of the same parameter in IHD group with HF that represents I-II functional NYHA class is increased by 15.91% compared to IHD group without HF and even by 43.6% compared to control group (p<0.001). Similar patterns of macroscopic tissue reconfiguration are observed within the measurements of weight of interventricular septum in different groups of IHD. This parameter in IHD group with HF that represents I-II functional NYHA class is significantly increased by 9.24% compared to IHD without HF and by 33.74% compared to control group (p<0.001).

These features of morphometric left cardiac ventricle weight shifts suggest that initial macroscopic and microscopic changes in tissue structure are already induced before HF as a result of exposure to injuring factors such as IHD, and continues to progress, when the HF condition is confirmed. As a result of increased mechanical demands due to increased workload, new sarcomeres and contractile proteins are produced within cardiac myocytes, and these processes contributes towards significantly decreasing the energy demands of weakening myofibrils in hypertrophied cardiac myocytes. Since energy demand is negatively proportional to the tension of cardiac wall during systole, hypertrophy itself increases the efficiency of myocardial contraction and contributes towards saving the energy. Although, if there are factors that stimulate hypertrophy for a prolonged period of time, negative effects of hypertrophy manifest, especially those associated with inefficient cardiac muscle reconstruction, and a deficiency of energy becomes even more significant to this overloaded heart [20].

Moreover, as a result of these structural myocardial tissue shifts, similar patterns of transformation are observed in other cardiac ventricular parameters representing ventricular geometric reconfiguration such as ventricular endocardial surface area. When this parameter is calculated for the free wall of left cardiac ventricle and compared between selected groups of men, endocardial surface area of free wall of left cardiac ventricle in IHD with HF of I-II functional NYHA class increases by 19.46% compared to IHD without HF group and even by 60.76% compared to control group (p<0.001). The endocardial surface area of left ventricular surface of interventricular septum in IHD group with HF is 30.07 cm², and is significantly increased by 13% compared to IHD without HF group and by 57.35% compared to control group (p<0.001).
Reconfigurations of cardiac ventricular geometry is a key feature of the statement that not only the structure of cardiac muscle, but also the form and shape of the ventricles change, as a result of exposure to injuring factors such as IHD. Due to any regional injury of myocardial tissue as a result of IHD, contractility function is affected. The result of this injury is failing myocardium which is unable to eject an adequate blood during a systolic stage, and this inadequate performance of systolic function leads to dilatation of cardiac chamber and stretching of myocardium [21].

Although these processes lead to initially increased contractility function and restoration of cardiac function, eventually this reaction starts to fail, cardiac output decreases, and dilatation of ventricle occurs [21]. Dilatation of the left ventricle may be associated with increasing filling pressure, diastolic wall stress myocyte stretch and up-regulation of stretch response proteins [19]. Furthermore, these shifts of cardiac ventricular geometry are already initiated at significant lengths before the symptoms of HF, and continue on at a less active process, when the heart muscle dysfunction transforms into a clinically symptomatic form.

Considering the facts that there are regular transformations of ventricular tissue structure and shape of ventricular chamber itself, a step towards checking if obtained morphometric data calculations represent regular changes compared between left ventricular tissue weight shifts and reconfigurations of endocardial surface area of interventricular septum is taken. A ratio of left ventricular weight and endocardial surface area (relative weight of left ventricle) is calculated with a purpose to determine if these parameters share a regularly changing pattern in different IHD groups of selected men.

No statistically significant differences were detected between these parameters in different groups of men (p>0.05), meaning that both left ventricular weight and endocardial surface area increase at similar proportions in different groups of IHD men (r>0.7, p<0.05). This correlation detected between left ventricular weight and endocardial surface area can be described by applying a regressive method and expressing this correlation by an equation y=0.3966x – 1.0918 (x – weight of left cardiac ventricle, y – endocardial surface area of left cardiac ventricle). These calculated patterns confirm that not only intracellular regeneration of cardiomyocytes is initiated by injuring factor, but also the geometry itself shifts as a part of complex remodeling process.

The degree of cardiac ventricular remodeling process intensity depends on the extent of injury and exposure time. A moderate correlation between atherosclerotic stenosis index and parameters reflecting left ventricle’s organometric changes is detected (r=0.28, p=0.003 for left ventricular weight and r=0.402, p=0.02 for left ventricular endocardial surface area). These determined correlations between atherosclerotic stenosis index and morphometric parameters of left cardiac ventricle changes suggest that injuring factor initiates the cellular reconstruction of affected myocardial tissue that
manifests on macroscopic level before even HF becomes symptomatic, and has a cumulative effect on failing cardiac muscle when the diagnosis of HF is established.

Persistent myocardial ischemia process (a major factor of chronic IHD) usually manifests when the amount of blood supplying oxygen through coronary arteries is not sufficient for cardiac muscle energy requirements. One of the major shifts in ischemic myocardial tissue is a significant decrease in its contractility function. Therefore, an outflow of blood from left cardiac ventricle decreases significantly inducing a systolic dysfunction with adequate clinical symptoms. Reconfiguration of myocardial tissue by increasing contractility strength of cardiac myocytes relieves pressure to myocardium and improves cardiac function until these mechanisms are exhausted [3, 22].

In conclusion, cardiac ventricular remodeling is a complex of molecular and cellular reactions generated as a response to injuring factors that myocardial tissue is exposed to, like IHD. These organometric remodeling transformations are required to ensure a sufficient and adequate cardiac function by sustaining appropriate configuration. Still, the capacity of transformation of any complex organic structure is limited, therefore a constant transformation process increases metabolic cellular demands and shifts of gene expression that determine cellular differentiation and function. To some level, these processes and mechanisms are exhausted, pathological processes are induced and a vicious circle of myocardial tissue regeneration starts which eventually leads to cardiac failure, where the heart acquires a typical morphometric configuration and cardiac dysfunction progresses further on.
CONCLUSIONS

1. Left ventricular hypertrophy is induced before HF as a result of exposure to injuring factor (IHD), and continues to progress, when HF condition becomes symptomatic. The mean weight of left cardiac ventricle in IHD group with HF of I-II functional NYHA class (149.83 ± 29.58 g) is increased by 15.91% compared to IHD group without HF and by 43.6% compared to control group (p<0.001).

2. Left ventricular dilation already starts before the symptoms of HF at significant lengths, and continues as a less active process, when failing heart muscle condition transforms into a clinically symptomatic form manifesting as HF. Overall endocardial surface area of left cardiac ventricle (ventricular geometry parameter) in IHD with HF of I-II functional NYHA class (90.35 ± 22.91 cm²) increases by 36.12% compared to IHD without HF group and by 59.61% compared to control group (p<0.001).

3. Shifts of left ventricular hypertrophy and shape occurs parallelly. Both left ventricular weight and endocardial surface area increase at similar proportions in different groups of IHD selected men (r>0.7, p<0.05).

4. Myocardial ischemia initiates the cellular reconstruction of affected myocardial tissue that manifests on macroscopic level even before HF becomes symptomatic, and has a cumulative effect on failing cardiac muscle when the diagnosis of HF is established. A statistically significant positive correlation between atherosclerotic stenosis index and parameters reflecting left ventricle’s organometric changes is detected (r=0.28, p=0.003 for left ventricular weight and r=0.402, p=0.02 for left ventricular endocardial surface area).
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