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Morphological Predictors and Molecular Markers of Progressing Postoperative Remodeling of Left Ventricle in Patients with Ischemic Cardiomyopathy

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1. Introduction

1.1 Background

Coronary artery disease takes a leading role in the etiology of chronic heart insufficiency in 60% of the cases (Belenkov et al., 2002; Kalon et al., 1993; Oganov & Maslennikova, 2000; Simonsen, 2003). According to the data of different authors patients with coronary artery disease experience development of ischemic cariomyopathy preconditioned by diffuse, significantly pronounced atherosclerosis of coronary arteries manifesting as cardiomegaly termed as "heart remodeling" and symptoms of congestive heart failure in 10-35% of the cases (Belenkov et al., 2002; Mareev, 2002).

Postinfarction left ventricular remodeling is one of the most urgent challenges of modern cardiology and cardiac surgery. The heart remodeling process is a combination of changes in cavities' form and volume and in mass of postinfarction heart myocardium in response to significant inadequate hemodynamic conditions of its functioning not connected with sarcomeres elongation caused by their prior overstretching (Jackson, 2002; Maisch, 1996; Rosenberg & Nepomnyashchih, 2003). Among the patients with different cardiomyopathies these are the ones with ischemic cardiomyopathy who have the most unfavorable prognosis, which makes the problem of ischemic heart failure much more significant (Bellenger, 2000; Buckberg, 2005; Frazier, 2000).

Quite frequently the surgical intervention becomes the only treatment method for the patients with chronic heart insufficiency basing on deep changes of functional myocardium morphology. Different approaches to surgical ventricular reconstruction aimed at mechanical changes of the heart cavities sizes in combination with coronary artery bypass grafting (CABG) take the leading place in the complex treatment of this pathology. Nevertheless their outcomes show that in the late postoperative period repeated heart remodeling and CHF progressing i.e. return to the initial preoperative values of the heart cavities sizes and functional capacity of the heart takes place in a part of the operated patients (Batista, 1996; Dickstein, 1997; Dor, 1985; Gradinac, 1998; Menicanti & Di Donato,

2002; Menicanti & Di Donato, 2004; Moreira et al., 2001; Popovic et al., 1998; Popovic et al., 2001; Ratcliffe, 1998; Shah, 2003; Soo, 2005; Stolf, 1998).

The efforts to find clinical and instrumental prognostic criteria of unfavorable late outcomes of surgical treatment in patients with ICMP have not resulted in anything. According to publications, the following preoperative values have been associated with higher postoperative mortality and morbidity of the patients with ICMP: size of left ventricle (LV) (Yamaguchi, 2005), LV end-systolic volume index (LVESVI) (especially > 80 ml/m2 (Athanasuleas, 2004) and > 100 ml/m^2 (Yamaguchi, 2000)), LV ejection fraction (EF) (< 20% (Di Donato, 2001; Yamaguchi, 2005) or < 30%(Yamaguchi, 2000; Athanasuleas, 2004)), mitral regurgitation (Sartipy et al., 2006; Schroder, 2005), number of segments affected by dyssynergia (Di Donato, 1997), pulmonary hypertension >33mmHg (Di Donato, 1997), QRS>130ms (Yamaguchi, 2005), preoperative renal failure (Yamaguchi, 2005), time after previous myocardial infarction (Yamaguchi, 2005), age older than 75 years old (Athanasuleas, 2004). Nevertheless, there have not been any definite preoperative clinical predictors of postoperative LV remodeling offered.

Myocardium is a unique tissue consisting of highly differentiated cells – cardiomyocites which possess a number of morphological features in norm responding by a set of nonspecific structural changes to pathomorphism of cardiovascular diseases. In our opinion, the degree of revensability/irreversibility of advanced pathological processes in myocardium plays a key role in the success of reconstructive cardiac surgical interventions.

It has been 10-12 years since researchers started their first search for morphological predictors of postoperative heart remodeling in patients with cardiomyopathies, carrying out the analysis of the postoperative period course and evaluating morphofunctional condition of LV myocardium by the data of intraoperative biopsies (Gradinac, 1998; Moreira et al., 2001; Popovic et al., 2001; Stolf, 1998). However, the results of these solitary studies have been quite controversial (Moreira et al., 2001; Popovic et al., 2001). We have not found data about any attempts to search for morphological predictors of progressive postoperative LV remodeling in patients with ICMP. In the available Russian publications there are separate articles devoted to studies of morphofunctional condition of LV myocardium and myocardium of RA auricle in patients with coronary artery disease of different functional classes (Kuznetsov, 2003; Salikova et al., 2002). These works can hardly boast wide analysis of morphological parameters (Kuznetsov, 2003) and some authors only provide descriptive morphology without deep investigation of the mechanisms of possible pathogenesis of heart remodeling (Salikova et al., 2002).

At the same time, identification of morphological predictors of postoperative LV remodeling will not solve all the problems which cardiac surgeons face when choose the tactics for surgical intervention and think of the prognosis for each individual patient since pre- and postoperative morphological diagnostics of the pathological processes reversibility degree in ischemic myocardium in reality is limited very much by a definite degree of a risk associated with harvesting biopsies from heart walls which very often becomes a reason to refuse from this diagnostics. In the light of this, one of the perspective directions of scientific research is the finding of molecular predictors of postoperative heart remodeling in peripheral blood of patients together with tissue and cellular aspects of this phenomenon for the blood is always available for laboratory testing and monitoring of its content.

Until today, there have not been performed any complex fundamental scientific works devoted to the identification of tissue, cellular and molecular predictors of postoperative LV remodeling basing directly on real patients cases. The contemporary concept of CHF surgical treatment must be based on peculiarities of functional morphology of each individual patient's myocardium. Thorough patient selection basing on a complex clinical-morphological and biochemical analysis of the prognosis for surgical treatment outcome may improve the efficacy of a standard SVR procedure making a surgeon refuse from it in predeterminedly prospectless patients in favor of alternative methods of surgical treatment such as primary heart transplantation, implant of the devices preventing heart chambers dilatation, cardioresynchronizing therapy, etc.

The latter area has better perspective for the problem of organ transplantation is a very topical issue in modern medicine since the number of recipients exceeds significantly the number of donors. Cardiac support device (CSD) is a special device for suppression of heart chambers dilatation. Multi-centers randomized clinical trial for this device is being carried out at present and according to preliminary data its application is quite efficient for ischemic and dilated cardiomyopathies (Acorn cardiovascular, inc.™ Selected abstracts, 2000; Patel, 1997; Sabbah, 2001).

Objective of the study: The development of a diagnostic algorithm and justification of a modern concept of CHF surgical treatment basing on identification of morphological and blood markers of progressive postoperative LV remodeling in patients with ICMP.

2. Materials and methods

2.1 Design of the study. Object

One hundred and ninety five patients with ICMP and with previous myocardial infarctions have become the object of the study. All the patients were admitted to the cardiovascular surgery department at Tomsk Institute of Cardiology during the period from 2002 to 2009. Preoperative diagnostics included transthoracic EchoCG, Halter ECG monitoring, coronaroventriculography with manometry, SPECT imaging with 99mTc-technetril, MRI imaging of the heart with dye.

The clinical inclusion criteria for the patients enrolled into the study were the following parameters: LV end-diastolic volume index (LVEDVI) > 90 ml/m2, LVESVI > 70 ml/m2 , LV end-diastolic pressure (LVEDP) > 30 mmHg, EF LV < 40%, akinetic and dyskinetic areas of LV, angina II-IV CCS FC, heart failure (HF) II-IV NYHA, coronary artery disease from 1 - 10 years, lesions of coronary arteries – stenosis of more than 75% of LAD or of the trunk, or not less than 75% stenosis in at least two coronary arteries. The age of the patients included into the study was between 37 and 68 years (53.6±8.3), mean number of affected coronary arteries was 2.7±0.4. Lack of organic lesions of heart valves apart from ischemic mitral valve regurgitation was also a clinical criterion for patients' selection.

The reason for the development of ICMP in all the patients was an extensive transmural myocardial infarction. In 135 patients (69.2%) heart insufficiency appeared after their first myocardial infarction. All the patients in conditions of bypass and cardioplegia underwent surgical reconstruction of LV by V.Dor and L.Menicanti methods in different combinations with myocardial revascularization, reduction and reconstruction of LV volume and shape due to exclusion of its scarred septal, anterior and basal parts by endoventriculoplasty and by restoration of the mitral valve (MV) function.

Clinical characteristics		Value
Number of the patients		195 (100 %)
Men		177 (90.8 %)
Women		18 (9.2 %)
Mean age (years)		53.6±8.3
Number of previous MI	1	135 (69.2 %)
	2	39 (20.0 %)
	3	15 (7.7 %)
	4	6 (3.1 %)
Angina functional class (CSS)	Without angina	9 (4.6 %)
	I	15 (7.7 %)
	II	36 (18.5 %)
	III	132 (67.7 %)
	IV	3 (1.5 %)
NYHA functional class		2.86±0.40
Number of the coronary arteries with atherosclerotic lesions	1	72 (36.9 %)
	2	54 (27.7 %)
	3	69 (35.4 %)
LVEDVI (ml/m^2)		114.9±28.4
LVESVI (ml/m^2)		76.7±23.0
LV EF (%)		32.1±5.5
Degree of mitral regurgitation	0	36 (18.5 %)
	I	51 (26.2 %)
	II	63 (32.3 %)
	III	36 (18.4 %)
	IV	9 (4.6 %)
Hypertensive disease		126 (64.6 %)
Diabetes mellitus		30 (15.4 %)
Obesity		39 (20.0 %)
Peripheral atherosclerosis		33 (16.9 %)

Table 1. Initial clinical characteristics of the patients enrolled into the study

Intraoperative control of the remaining LV cavity was performed with the help of special devices (sizers) and satisfied a physiological norm for each patient (55-60 ml/m2). Reduction of LV cavity had to be combined with giving it elliptical shape, which was performed with the use of endovascular patch and retraction of papillary muscles. Interventions on mitral valve were performed in 51 patients (26.2 %). MV repair was made in 36 cases (18.5 %); MV prosthesis was placed in 12 cases (6.2 %). The spectrum of surgical interventions is shown in table 2.

Spectrum of surgical interventions	Absolute value (n = 195)	Relative value (%)
CABG+SVR	132	67.6
CABG+SVR+MV repair	39	20.0
CABG+SVR+MV prosthesis	12	6.2
SVR+TMLR	6	3.1
SVR+CABG+TMLR	6	3.1

Note: CABG – coronary artery bypass grafting; SVR – surgical ventricular reconstruction; MV repair – mitral valve repair; MV prosthesis – mitral valve prosthesis; TMLR – transmyocardial laser revascularization.

Table 2. The spectrum of surgical interventions carried out in the patients with ICMP enrolled into the study

Biopsy samples of RA auricle and LV were taken from the border area of endocardial scar and from the area of the myocardium without visual changes from all the patients (n=195, 100%) with ICMP. RA auricle biopsy was performed during the period of RA cannulation. LV biopsy samples were taken during surgical ventricular reconstruction from the transient zone on the border between scarred tissue and unchanged myocardium.

All the patients signed an informed consent form for the participation in the study; the study was approved by the local ethical committee of Tomsk Institute of Cardiology.

In order to find noninvasive molecular markers of postoperative LV remodeling blood samples were taken from 53 patients with ICMP (27.2%) to identify the content of natriuretic peptides and matrix metalloproteinases in blood plasma and serum correspondingly. In 37 patients antibodies titre to myocardial structures was identified in blood serum.

In the early postoperative period (1 month) control transthoracic EcoCG was made. In 12 months after the surgical treatment the patients were hospitalized again for clinical examination and control EchoCG.

In the late follow-up period repeated LV remodeling and HF progressing took place in a part of the patients which resulted in assignment of the patients into two groups: with positive (regressive remodeling – group I) and negative (progressing postoperative remodeling – group II) dynamics.

To achieve the objectives of identification tissue, cellular and molecular markers of postoperative LV remodeling we have used histological, electron-microscopic, morphometrical, biochemical and statistical methods of study.

To compare morphometrical parameters, as a control group we took autopsy samples of the identical sites of LV myocardium and myocardium of RA auricle from 25 cadavers of both sexes and of a comparable age died from an acute trauma with no signs of cardio-vascular pathology.

Seventeen healthy male and female volunteers of comparable age comprised a control group for the evaluation of the content of natriuretic peptide and matrix metalloproteinases in blood plasma and serum, respectively, as well as for identification of the antibodies titre to myocardial structures.

2.2 Histological study methods

Histological methods include preliminary treatment of the studied material necessary for its further microscopic evaluation. Preparation of histological samples was performed as follows (Krivolapov, 2006): the samples of myocardium were being fixed in 10% solution of

neutral formalin during 24 hours, and then they were washed in running water and dehydrated in the solution for histological treatment (dehydration and clearing) based on absolute isopropyl alcohol IsoPrep (BioVitrum, Saint Petersburg, Russia). After dehydration the myocardial samples were placed into homogenized paraffin media HISTOMIX® (BioVitrum, Saint Petersburg, Russia). Paraffin section of about 5-7mcm thick obtained with the use of a sliding microtome MC-2 were stained by hematoxylin and eosine and by Mallori method (stains and staining kits by BioOptica, Italy). The stained samples were place into synthetic monitoring media BioMount (BioOptica, Italy).
Histological samples were studied with a routine light and polarization microscopy on Axioskop 40 microscope (Carl Zeiss, Germany). Microimages of histological samples were taken with the Canon G10 camera (Japan).

2.3 Methods of electron-microscopic study

We took LV myocardium and RA auricle myocardium samples from 58 ICMP patients (50 men, 8 women) for electron-microscopy study. Myocardial samples of not more than $2mm^3$ were fixed in 2.5% glutaric aldehyde solution on 0.2M cacodylate buffer with pH=7.2 with the temperature of +4 °C and postfixed in 1% OsO_4 solution in cold during 4 hours. The bioptates were then dehydrated in ethanol of rising concentration and placed into the mixture of epone and araldite. Semifine and ultrafine sections were prepared on ultratome LKB III (Sweden). The semifine sections were stained by 1% azure II solution and evaluated visually through a light microscope. The ultrafine sections were contrasted by lead citrate and uranyl acetate and studied in an electron microscope JEM-100 CX (Japan).

2.4 Morphological methods of study

For quantitative characteristic of the changes morphometrical methods were applied such as measurement of specific volume (SV) of edema, vessels, parenchyma and myocardial stroma by the point counting methods (Avtandilov, 1990; Glagolev & Chepulin, 1968). Measurement of parenchyma, stroma, vessels and edema SV was performed in 5-7 random microscopic fields of each section with the use of the software for graphic images procession (AxioVision by Carl Zeiss, ImageJ, Germany). One mm^3 of the tissue was considered as a unit volume for the study on the light-optical level (Avtandilov, 1990). Ocular micrometer МОВ-1-16^x («ЛОМО», Saint Petersburg, Russia) was used to measure diameter of cardiomyocytes on longitudinal sections on the level of myocardial cells nuclei. For the quantitative characteristics of the interrelation among myocardial parenchyma, stroma of the organ and exchange link of microcirculatory bed the following morphometrical parameters were evaluated to reveal risk factors of postoperative heart remodeling: parenchyma-stromal ratio (PSR), trophic index (TI) and pericapillar diffusion zone (PcDZ); and for the quantitative characteristics of microvasculature and their capacity Kernogan index (KI) was calculated. PSR is a ratio between myocardial parenchyma SV and stromal SV; TI (the best index reflecting the condition of myocardial trophy) – is the ratio between capillary SV and parenchyma SV; PcDZ (the area of tissue supplied with blood by one capillar) – the ratio between the capillary diameter and their SV; KI (the index of carrying capacity of microcirculatory bed) – is the ratio between arterioles vascular wall and the radius of their lumens (Avtandilov, 1990).
Morphometry of the ultrastructures was performed on digitized negative photoplates with initial magnitude 4800–10000. We calculated SV of myofibrils, mitochondrias and granules

of atrial cardiomyocytes. One mcm^3 of tissue was taken as a unit volume for the study on a light-microscopic level (Avtandilov, 1990). Mitochondrial-myofibrillar ratio was evaluated as the ratio of mitochondrial SV to the SV of miofibrills.
Electron-microscopic study of myocardial microcirculatory bed allowed for evaluation of the ratio between open (functioning) and closed (not functioning) capillaries. For the open capillaries we performed quantitative evaluation of their lumen and active transport through endothelium with the help of micropinocytic vesicles. For that, we identified the number of pinocytic vesicles associated with a length unit of a luminal contour on an area unit of capillary lumen, as well as density of free pinocytic vesicles per a volume unit of endothelial cells cytoplasm.

2.5 Biochemical methods of study of blood plasma and serum of the ICMP patients

The content of natriuretic peptides (pro-ANP и NT-proBNP), matrix metalloproteinases (pro-MMP-1, MMP-3, MMP-9) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in blood plasma and serum of the ICMP patients was identified by immunoenzyme method with standard kits by: pro-ANP and NT-proBNP – Biomedica (Austria); MMP-1 and MMP-9 – Quantikine® (R&D Systems, USA); MMP-3 and TIMP-1 – Biosource (Belgium). Evaluation of circulating antibodies to myocardium in blood serum had been performed in 37 patients with ICMP by the method based on the indirect immunofluorescence reaction («IMMCO Diagnostics» set, USA): we registered the presence of antifibrillar, antisarcolemmic and antinuclear antibodies by localization of fluorochrome on histological preparations by the method of fluorescent microscopy using the Axio Scope A1 microscope (Carl Zeiss, Germany). The concentration of the antibodies was expressed in titre.

2.6 Clinical and instrumental methods of study

Every patient underwent twelve-lead ECG study (Schiller AT-6 machine, Switzerland) before and after the surgery. The degree of cardiomegalia and the condition of pulmonary circulation was evaluated by radiographic methods of study. Each patient underwent EchoCG examination with color dopplerography on Acuson 128 XP/10 (Japan). To study global systolic function of LV the following parameters were evaluated: end-diastolic size of LV, end-systolic size of LV, LV stroke volume and their indexes, LV EF, thickness of ventricular septum and that of posterior LV wall. Contractility of 16 segments was evaluated by a 4-score system and the index of local contractility disturbance was calculated to study disturbances of local LV myocardial contractility. To study LV diastolic function we evaluated transmitral blood flow by dopplerography. We also assessed the sizes of left atrium, degree of mitral regurgitation, estimate pressure in pulmonary artery. All the patients underwent EchoCG study before the surgery and in the postoperative period. Using the obtained data of echocardiography we calculated an estimate indicator – the index of specific thickness of LV wall by the following formula: thickness of ventricular septum + thickness of LV posterior wall/end-diastolic LV size. To assess this indicator we used the following grading: with remodeling index <0.30 – maladaptive remodeling; with the index from 0.30 to 0.45 – adaptive remodeling; >0.45 – asymptomatic remodeling.
Coronary angiography and left ventriculargraphy were performed on Philips maximus C1250, Philips polydiagnost C20 angiographic units. Selective coronary angiography was performed by Judkins method (1967) with freezing monitoring images.
To calculate changes of ESVI expressed as a percentage we used the formula:

$$\Delta ESVI = \left(\text{preoperative ESVI / ESVI in a year after the surgery x } 100\right) - 100.$$

To study the values of central hemodynamics the patients were subjected to catheterization of heart chambers with measurements of LVEDP and pressure in the pulmonary artery.

2.7 Statistical analysis of the results

The results were statistically analyzed with the software package SSPS 11.5 for Windows. Normality of a distribution law of quantitative values was assessed by Shapiro-Wilk test. Parameters which obey the normal distribution law were described with the help of the mean value (M) and standard deviation (m); those which do not obey the normal distribution law - with the use of median (Me) and interquintile interval (Q_{25}–Q_{75}). Qualitative data were described by the frequency of occurrence or its percentage. If the distribution law was normal, Student t-test was used for the assessment of reliability of quantitative values differences in the compared groups; Mann-Whitney test – in the case of not normal distribution law. To evaluate reliability of quantitative data χ^2 criterion was used (or Fisher exact test in cases when χ^2 test was not possible). To find statistical dependences of linear character, to identify their strength and direction Pearson correlation coefficient (r) (among quantitative values obeying the law of normal distribution) and Spearsman correlation coefficient (for quantitative values not obeying the law of normal distribution and for qualitative values in the ordinal scale) were calculated. All statistical values were considered significant with $p<0.05$.

3. Results and discussion

3.1 Morphological predictors of postoperative LV remodeling in ICMP patients

During the study of morphofunctional condition of LV myocardium and myocardium of RA auricle in the patients with ICMP there was found that the density of vessels distribution was significantly decreased in comparison with that in the control group. Irrespectively of the blood vessels diameter the signs of hemodynamic disturbances were noticed everywhere: perivascular edema, venous plethora, desolation and spasm of arterioles and small arteries (fig. 1). Nuclei of endothelial cells in spasm arterioles were visually "extruded" into the vessels lumen. Microcirculatory link of vascular bed was plethoric; phenomena of erythrocyte stasis in capillaries, pericapillaries and arterioles were observed quite often. In separate capillaries we noticed rounding of endothelial cells manifesting as extrusion of endothelial cells nuclei into the capillaries lumen which, without any doubt, lowered their carrying capacity and the level of myocardial trophy.

In LV myocardium and in that of RA auricle of the ICMP patients stroma had enlarged volume and was edematous; its collagen fibers were curved and sometimes swollen. Mixed (lymphocytic-macrophage) infiltrate (> 14 per mm2 of tissue by Marburg classification (World Heart Federation Consensus Conferences Definition of Inflammatory Cardiomyopathy (Myocarditis), 1997) was found in some patients' LV myocardial stroma and RA auricle which was considered as myocarditis (fig. 2).

In the ICMP patients LV myocardial cardiomyocytes were, as a rule, hypertrophic and located either singly or in small foci surrounded by the areas of scarred tissue which had been formed at the sites of previous infarctions. It is worth mentioning a pronounced

polymorphism of myocardial cells nuclei: their enlargement, changing shape and tinctoral properties. In the most cases the shape of cardiomyocytes was oval with fuzzy contours. Quite often the nuclei had the shapes of "eights", "bow", "spring", etc. Chromatin in such the nuclei was condensed and located mostly along the nuclei periphery. Oxyphilous inclusions looking like apoptotic bodies were noticed either close to some nuclei or inside them. Such cardiomyocytes had eosinophilic cytoplasm and irregular contours of the cells.

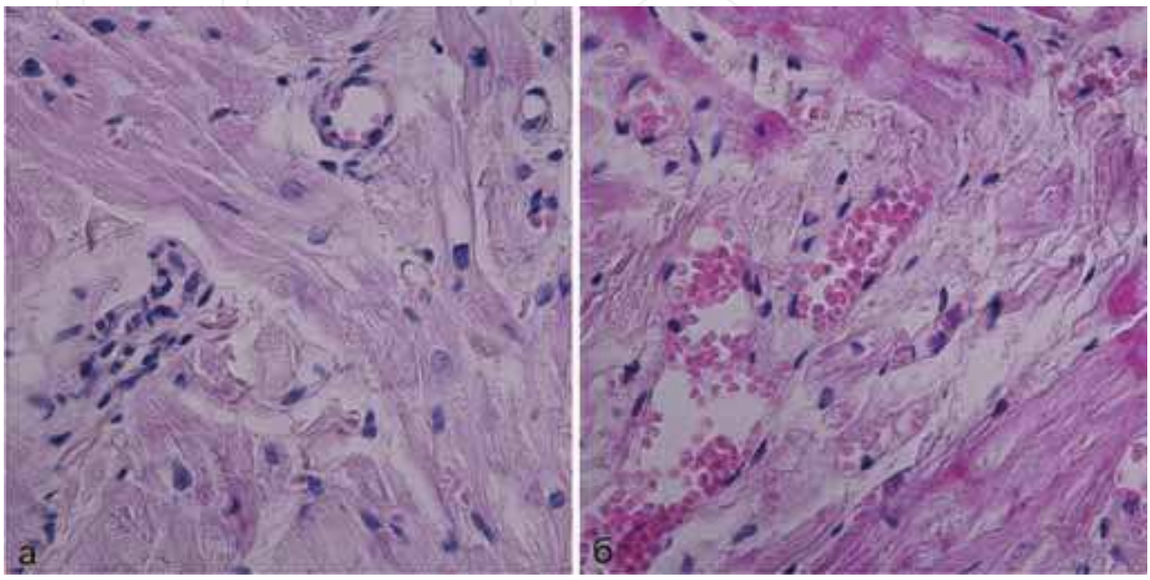

Fig. 1. LV myocardium of ICMP patients: a – perivascular edema; b –venous plethora. Stained with hematoxylin and eosin. X 450 (a) and 400 (b)

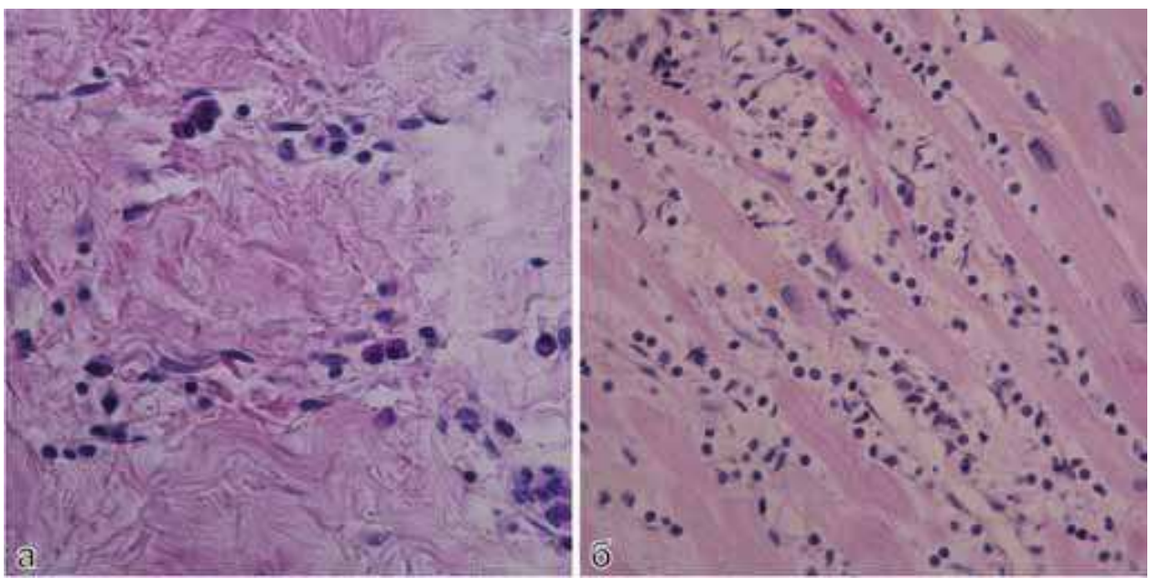

Fig. 2. LV myocardium of ICMP patients: a – mixed (lymphocytic-macrophage) focal infiltration; b – diffuse lymphocytic infiltration of myocardial stroma. Stained with hematoxylin and eosin. X 500 (a) and 300 (b)

Intracellular edema in cardiomyocytes of LV was noticed everywhere and its degree varied very much from cell to cell. In a polarized light on histological preparations stained with hematoxyline and eosine alongside with unchanged areas of cardiomyocytes' cytoplasm we observed damaged areas of sarcoplasm with predominantly subsegmental contractures, contracture lesions of the Ist, IInd and less often IIId degree which differed by the enhancement of luminescence of disdiaclasts with different degrees of isotropic discs shortening; we also noticed isolated areas of intracellular myocytolysis and primary clump disintegration of myofibrillas and cytolysis of cardiomyocytes. The described changes of mosaic nature were also noticed in RA auricle myocardium.

Study of morphofunctional condition of LV myocardium in patients with ICMP revealed very interesting peculiarities: cardiomyocytes organization disturbance in muscular fibers and disturbance of fibers orientation relative to each other, disintegration of myocardial cells on intercalated disks, elimination of cardiomyocytes along muscular fibers (fig. 3). Myocardium in these cases had not fibrous but cellular structure which resulted in its poor contractility (Anderson, 2005). Sometimes cardiac myocytes were star-shaped. Besides, on the longitudinal sections of LV myocardium of ICMP patients one could observe wave-shape deformation of cardiomyocytes along myocardial fibers.

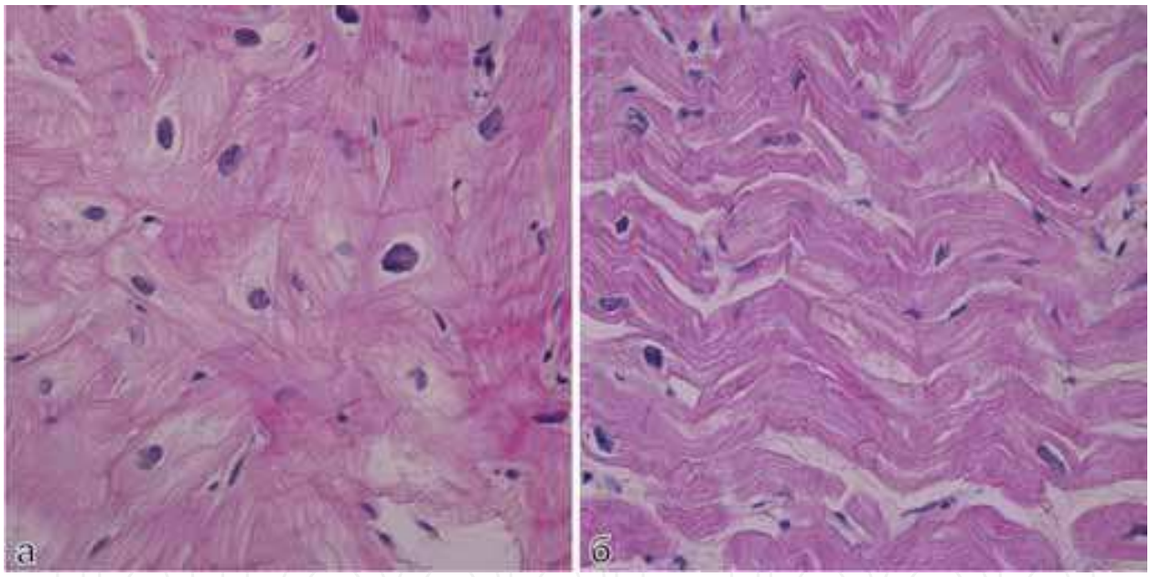

Fig. 3. LV myocardium of ICMP patients: a- disturbance of muscular fiber orientation, star-shaped cardiomyocytes; b- wave-shaped deformation of muscular fibers. Stained with hematoxylin and eosin. X 370 (a) and 330 (b)

During electron-microscopic study of myocardial cardiomyocytes of LV and RA auricle our attention was drawn to the polymorphism of ultrastructures of myocardial cells. Nuclei often having irregular scalloped shape with multiple intussusceptums and outgrowths of nuclear membrane were located in the center of the cells, but in some cardiomyocytes they were displaced into subsarcolemmic zone. Genetic material was observed mostly as euchromatin which took a central position. In some cardiomyocytes' nuclei, vice versa, heterochromatin prevailed and was situated mostly in juxtamembrane zone. Chromatin aggregation (compaction) was noticed quite often. Nuclear envelope was continuous throughout and had pores.

Perinuclear space was dilated; it was not filled with mitochondrias, granular reticulum and elements of Golgi organ but consisted mostly of rare matrix containing glycogen of β-shape and having round or elongated shape (monogranular glycogen) (fig. 4). Rosettes of α-glycogen were present in a very insignificant number in intermiofibrillar spaces.

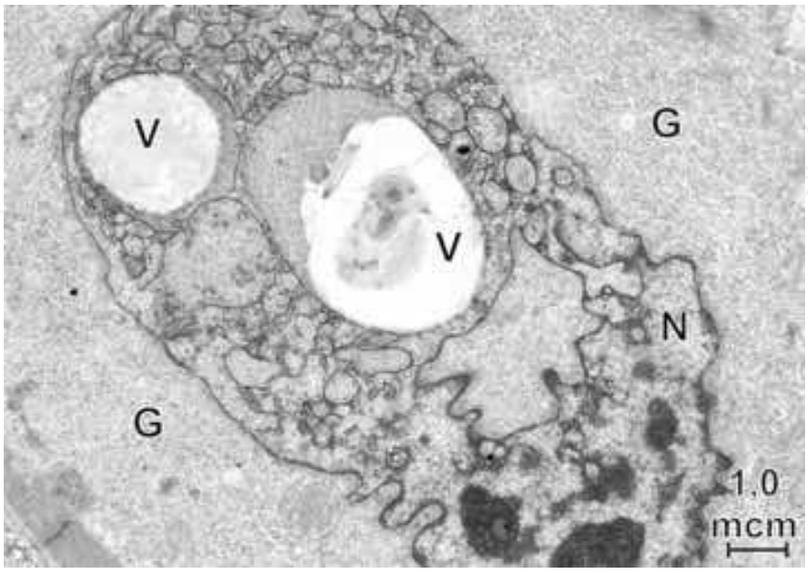

Fig. 4. A fragment of a left ventricular cardiomyocyte of an ICMP patient: vacuolization (V) of a nucleus (N) with its further desolation; dilatation of perinuclear space filled with β- glycogen (G)

Dispersed nucleoli, segregation of fibrillar and granular components of nucleolonema, ring-shaped nucleoli were noticed which evidenced suppression of rRNA biosynthesis (fig. 5).

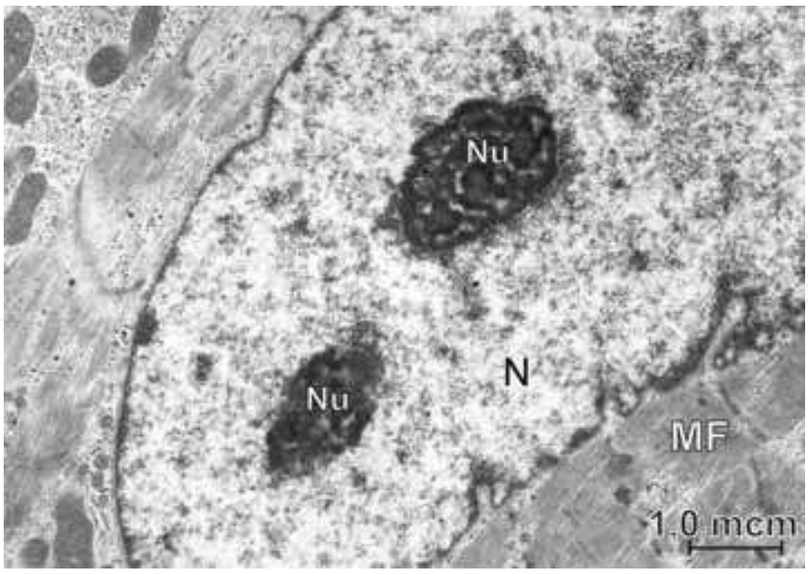

Fig. 5. A fragment of a left ventricular cardiomyocyte of an ICMP patient. Dispersion of nucleoles (Nu), segregation of granular and fibrillar components. N- a nucleus of a cardiomyocyte; MF – myofibrils

We also found wide variety of myofibril lesion forms in LV and RA auricle myocardial cardiomyocytes in ICMP patients such as: contracture lesions of myofibrils of the Ist, less often of the IInd and III^{d} degrees, isolated areas of primary clump of fibrillar disaggregation (fig. 6). The presence of contracture lesions of the III^{d} degree in a cell was associated with formation of sarcolemma festoons, contraction and deformation of a nucleus and mitochondrias due to overcontraction of myofibrils and with displacement of mitochondrias into the space between myofibrils and formation of compact assemblies.

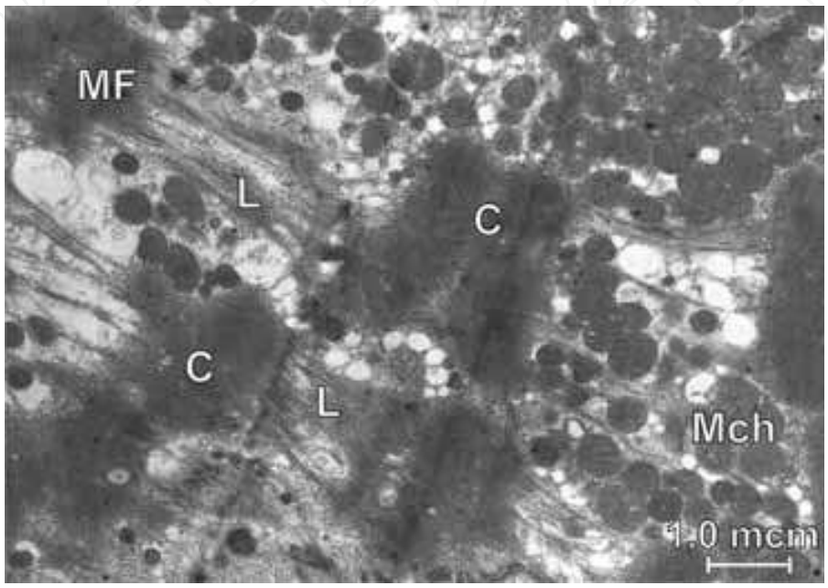

Fig. 6. Primary clump disintegration of LV myocardial cardiomyocite's myofibrils of an ICMP patient: alteration of the areas of mosaic lysis (L) and contractures (C) in isolated groups of myofibrillar (MF) sarcomeres; MCh- mitochondrias

In the most cardiomyocytes small-focal and diffuse lysis of myofibrillar bundles, myofibril "melting" were registered. I-disks with thin (actin) filaments in them were lysed to a greater extent. Myofibrils were becoming less dense, cavities appeared in some sarcomeres, total lysis of myofilaments within a sarcomere was noticed. Sarcomeres in the area of intercalated disks and in perinuclear zone were significantly destructed.

It was very seldom when foci of intracellular regeneration of ultrastructures were found in LV and RA auricle myocardial cardiomyocytes. The foci were evaluated by the accumulation of free ribosomes on the stumps of survived myofibrils contributing to the synthesis and neoformation of contractile proteins. As the newly formed myofilaments synthesized on polyribosomes and got matured they gathered into the bundles of myofibrils. But in the process of their neoformation their normal orientation is disturbed, they elongate excessively and it results in growing distance between Z-bands.

Apart from disturbance of normal orientation of the newly formed myofibrils we observed chaotic orientation of "mature" contractile proteins. Myofibrilar bundles and even individual myofilaments were oriented at different angles in relation to each other. Besides, wave-shaped deformation of a contractile apparatus of LV and RA auricle myocardial cardiomyocytes took place in the patients with ICMP. All these circumstances, with no doubt, made their contribution into desynchronization of contractile processes, thus preconditioning systolic dysfunction of myocardium (Anderson, 2005).

Polymorphism of mitochondrias attracted our attention. We observed large mitochondrias reaching a length of 2-3 sarcomeres in the space among myofibrils. On the contrary, more often we were observing small round mitochondrias located randomly or in clusters. Sometimes we saw mitochondrias with destructive and degenerative changes, with cleared matrix, destroyed and reduced cristas, few mitochondrias with electron-dense matrix in condition of vacuolization.

Cisterns and vacuoles of cytoplasmic reticulum and Golgi apparatus in LV and RA auricle myocardium cardiomyocytes were deduced and sometimes dilated in the samples of ICMP patients. Dilation of the cisterns was found mostly in perinuclear zone and less often in the space between fibrils (in the area of Z-bands) and mitochondrias.

Atrial cardiomyocytes contained electron-dense granules of atrial natriuretic peptide of various sizes.

Thus, electron-microscopic study revealed mixed, alterative and regenerative-plastic insufficiency of cardiomyocytes in both LV and RA auricle.

Electron-microscopic study of peculiarities of microvasculature functional morphology was performed for intraoperative samples of LV and RA auricle from 47 ICMP patients (44 men, 3 women). Hemocapillaries having a sufficient lumen for passage of blood cells or containing these cells in their lumen were considered open (functioning). Hemocapillaries with a minimal lumen between plasmalemmas of endothelial cells insufficient for blood cells circulation were considered closed (non-functioning).

In endothelial cells of myocardial capillaries a large number of micropinocytic vesicles of different diameters (from 30 to 120nm) was found; these vesicles were present in both closed and open capillaries. The content of small vesicles was electron-optically low dense. On the contrary, large vesicles were electron-optically transparent. The number of micropinocytic vesicles in endotheliocytes in different capillaries was variable and their number in closed capillaries was very insignificant.

In cytoplasm of endothelial cells unexpanded canaliculi of granular and granular endoplasmic reticulum were observed as well as multiple crests and dints on a luminal surface of the cells.

Electron-microscopic study of microvasculatory link of LV and RA auricle myocardial vascular bed one could notice a bulging endotheliocytes into the capillaries lumen, reduction of their lumen and their decreased capacity. Rounding of endotheliocytes, probably, has adaptive meaning and is directed toward slowing blood flow in capillaries and more efficient use of blood mass in transcapillar exchange (Kawamura et al., 1974).

Matrix of endothelial cells cytoplasm in one and the same capillary is vividly cleared in one cells evidencing swelling of cytoplasm and in other cells it becomes electron-optically dense. If an endothelial cell matrix was cleared, we could visualize mostly singular small size micropinocytic vesicles situated predominantly on free and basal edges of cytoplasm. In cytoplasm of dark endotheliocytes larger pinocytic vesicles were present quite often (fig. 7).

Often one could see mitochondrias in capillary endotheliocytes of LV and RA auricle myocardium. Usually no changes in functional morphology of cellular energy apparatus were noticed, but sometimes we observed mitochondrias with cleared matrix, dilated intracristal spaces and destroyed crysts. Myelin-like structures were found in some mitochondrias.

In clear endotheliocytes were swallow and chromatin was loose. In osmium dark cells nuclei remained unchanged. Chromatin aggregation and pyknotic nuclei were noticed eventually. In nuclei of singular endotheliocytes we noticed the lost connection between heterochromatin and nuclear membrain. Heterochromatin was displaced deeper into the nuclei of endothelial cells and was present there as ring-shaped structures.

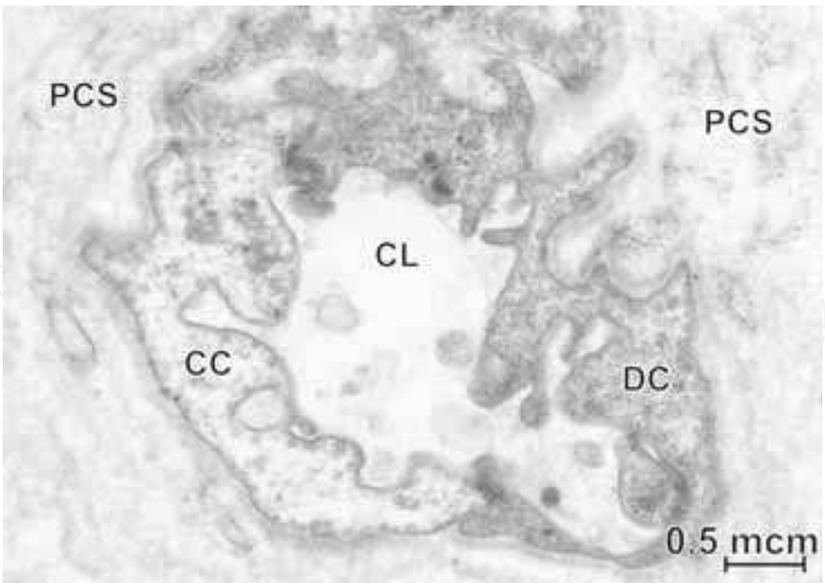

Fig. 7. A capillary from LV myocardium of an ICMP patient: Capillary lumen (CL) is limited by an endothelial cell with osmium dark matrix (a dark cell - DC) and by an endothelial cell with cleared matrix (a clear cell - CC). PCS – pericapillar space

Sometimes large lipid inclusions were noticed in cytoplasm of endothelial cells of capillaries in both LV and RV auricle myocardium; large vacuoles – only in singular cardiomyocytes. Glycogen granules were eventually found in insignificant amount.

Subendocardial capillary zone as a rule was not changed. Sometimes on longitudinal sections one could see isolated local dilations. Basal membrane was uninterrupted throughout and in most cases had the same thickness with only rare local thickened parts. Noncellular element of a basal layer was thickened locally and loose, occasionally thinned and interrupted.

The space between basal capillary membrane and cardiomyocytes (adventitious layer) was, as a rule, dilated and filled mostly with fibrous component of a loose connective tissue (with the prevalence of collagen fibers) and less often – with electron-transparent amorphous substance of low electron density (with separate fibrous structures like collagen fibers). In the latter case pericapillar space was sharply cleared; areas of a substance with low electron-optical density identical to that of plasma in a capillary lumen were found. The contours of these areas were not clear.

Cytoplasmic crest of pericytes were close to cytoplasm adjusting immediately to it. There were no any pronounced changes of functional morphology in pericytes found.

Pericapillar edema was registered in almost all the patients with ICMP, its degree varied significantly.

Thus, a thorough analysis of functional morphology of microvasculature in ICMP patients revealed specific changes of endothelial capillaries situated in foci of chronic ischemia.

Early postoperative mortality during 30 days after the surgery in the group of ICMP patients was 7.7% (n=15). First year postoperative mortality comprised 9.2% (n=18). Mean period of the control follow-up comprised 369±147 days. Only 153 (78.5 %) patients took part in the further study since 33 patients (16.9%) died in the early and mid-term postoperative period and communication with 9 patients (4.6%) was lost. During control

examination (in a year after the surgery) clinical and echocardiography evaluation of the surgical treatment outcomes was performed.

In January 2009 all the patients who had underwent reexamination (n=153) were interviewed over the telephone. Mean follow-up period was 4.24 years, maximal follow-up period – 6 years. We managed to acquire information about all the patients who had had control examination. By the time of the interview there had been 108 alive patients of all included into the study. Mortality in the group during all the follow-up period comprised 29.4% (n=45), 27.5% (n=42) died from cardiac diseases.

Echocardiography was used as an instrumental method of the evaluation of heart cavities sizes and for the calculation of their indexed values. Despite obvious benefits of this method of study, it has low reproducibility of results, which varies within 20% from obtained values (Geidel, 2005; Gelsomino, 2008).

With an artificially introduced efficacy endpoint (ΔESVI – delta ESVI- postoperatively less than 20% from preoperative values) we managed to divide the initially homogeneous group of the patients into two subgroups, different by the character of clinical course of HF after surgical treatment. To calculate ΔESVI parameter we used the following formula:

$$\Delta ESVI = \left(\text{postoperative ESVI} / \text{preoperative ESVI} \times 100\right) - 100$$

To evaluate changes of LV ESVI 20% interval in comparison with preoperative values was used: changes > 20% into positive or negative side were considered sufficient to take these changes as true. The patients in which LV ESVI was <80% from preoperative values were assigned to group I. In these patients the process of LV remodeling was stopped and reversed by surgical intervention i.e. reverse (regressive) remodeling type. The calculated value of ΔESVI was < minus 20%.

The patients in which ESVI was > 80% from preoperative values were assigned to group II of the study. In these patients the course of HF remained unchanged due to the complex surgical treatment of ICMP: remodeling process was either resistant to the exposure or went on progressing after surgical treatment, i.e. had progressing remodeling type. The calculated value of ΔESVI was > minus 20%.

The distribution of the patients between the groups basing on the ΔESVI value was the following: the 1st group consisted of 97 patients (63.4%) with the reverse remodeling type (i.e. with positive dynamics of the late postoperative period); the 2nd group consisted of 56 patients (36.6%) with progressing remodeling type (i.e. with negative dynamics of the late postoperative period).

Analysis of the control EchoCG data showed that in the early postoperative period in all the patients end-diastolic and end-systolic heart volumes decreased: LV EDVI from (114.9±28.4) ml/m^2 to (98.0±25.3) ml/m^2, LV ESVI from (83.9 ±21.6) ml/m^2 to (64.3±21.0) ml/m^2; and LV EF significantly increased from (32.1±5.5) % to (36.7±8.9) %.The values of echocardiography study performed during control follow-up period are shown in Table 3.

Performed comparison of EchoCG data in the group of the patients with reverse LV remodeling showed that LV EF was significantly higher and the values of LVESVI and LVEDVI – significantly lower.

It should be noted that the great majority of the patients continued medical treatment recommended at the time of their dismissal from the hospital after the surgery. The groups with reverse and progressing types of remodeling were comparable in respect to the frequency of taking of different drug groups and their mean dosages.

Value	Before the surgery (n=153)	In a year after surgical intervention		
		All the pts (n=153)	Group I (n=97)	Group II (n=56)
FC NYHA	2.6±0.5*	2.3±0.3*	2.1±0.2**	3.0±0.2**
LV EDVI (ml/m²)	114.9±28.4*	98.0±25.3*	84.4±13.7**	102.6±14.6**
LV ESVI (ml/m²)	83.9±21.6*	64.3±21.0*	50.4±11.6**	71.9±17.3**
LV EF (%)	32.1±5.5*	36.7±8.9*	43.0±3.7**	33.0±5.1**

Note * – significance of the differences between the groups of the patients before surgical intervention and in a year after the surgery; ** – significance of the differences between the groups of the patients with reverse and progressive LV remodeling ($p < 0.05$).

Table 3. The values of HF functional class and the data of EchoCG study in the patients with ICMP in the control follow-up period

Among 153 patients included into the study and examined in a year after surgical treatment mean value of ΔESVI was minus 24.0: maximum value – 36.8, minimum – minus 64.7.
It is obvious that SVR results in higher values of ΔESVI in comparison with isolated CABG providing more significant decrease of LVESVI in a year after the surgery. To avoid statistical and methodological mistakes in the analysis of correlation relationships, the patients with maximum and minimum values of ΔESVI and the patients with hypercorrection associated with diastolic LV dysfunction with postoperative EDVI less than 60ml/m² were excluded from the further study. One hundred and thirty eight patients out from 153 were included into the further analysis: 90 patients with reverse remodeling (group I) and 48 patients with progressive remodeling (group II).
Screening analysis of correlation relationships between clinical data and ΔESVI with the use of Spearman test showed moderate reverse correlation relationship with the age of a patient (Table 4). This correlation relationship was also proved during detailed analysis; an absolute p value was 0.0001. Thus, taking into account that the smallest (negative) values of ΔESVI are optimal for a favorable clinical course, older patients with ICMP are prone to the development of progressive remodeling in postoperative period.
Initial clinical data characterizing the degree of coronary artery disease and heart failure did not demonstrate any correlation with the values of ΔESVI after surgical treatment.

Parameters	Value			R value	p<0.05
	ME	max	min		
Age	54.3	43	68	0.600	S
Number of MI	1	1	4	–0.097	NS
Hypertonic disease	3	0	3	–0.060	NS
Angina FC	III	I	IV	–0.244	NS
NYHA FC	3	2	4	0.127	NS
Men				–0.166	NS
Women				–0.166	NS
Diabetes mellitus				–0.088	NS
Obesity				–0.147	NS

Note: NS – statistically insignificant difference; S – statistically significant difference.

Table 4. Correlation relationships of clinical signs with the degree of LVESVI changes after surgical treatment of the patients with ICMP.

Analysis of preoperative EchoCG data and the values calculated on their basis revealed correlation relationship with ΔESVI shown in Table 5.

Mixed lymphocytic-macrophage infiltrate in LV myocardium of the IInd group patients was found in 42 out of 48 ICMP patients (87.5%) and in only 24 out of 90 patients (26.7 %) with reverse remodeling ($p<0.01$). In LV myocardium, as a rule, fibrosis was moderate (degree II according to Marburg classification) in the Ist group of patients; in the IInd group of patients with ICM in the most cases it was severe (unfavorable, or the III^{d} degree fibrosis) and in rare cases – moderate.

In 8 out of 24 patients from group I (33.3%) and in 33 out of 42 patients from group II (78.6) infiltration had diffuse nature ($p<0.01$), in the rest of the cases – focal and even less often – confluent.

Besides, in 18 patients (37.5%) with repeated LV dilatation and in 13 patients (14.4%) with favorable late outcomes of surgical treatment the infiltrate of a similar nature was found in myocardium of RA auricle ($p<0.01$). As a rule, fibrosis in RA auricle myocardium was I-II degrees lower by Marburg classification than that in LV myocardium.

Parameters	Values			R value	p<0.05
	ME	max	min		
Mitral regurgitation (degree)	2	4	0	–0.149	NS
Tricuspid regurgitation (degree)	0	3	0	–0.120	NS
Mean pressure in RV(mm Hg)	40	60	30	–0.025	NS
LV ESV (ml)	142	266	65	0.294	S
LV EDV(ml)	245.5	395.0	104.0	0.199	NS
LV ESVI (ml/m^2)	75.5	133.6	45.6	0.843	S
LV EDVI (ml/m^2)	111.1	190.0	71.2	–0.215	NS
LV EF M-mode (%)	34	40	20	–0.131	NS
LV EF B-mode (%)	32	38	19	–0.213	NS
Cardiac index (l/min/m^2)	2.2	5.0	1.6	–0.099	NS
Thickness of ventricular septum (mm)	10.5	15.0	6.0	–0.055	NS
Index of relative LV wall thickness	0.307	0.222	0.410	–0.026	NS

Note: NS – statistically insignificant difference; S – statistically significant difference.

Table 5. Correlation relationships of EchoCG parameters with the degree of LV dilatation in the patients with ICMP after combined surgical treatment.

It should be mentioned that there was no a case of cellular inflammatory infiltrate found in myocardial stroma in the autopsy material of the similar sites of LV myocardium and RA auricle myocardium taken from 25 cadavers which were the relative control group.

Statistical analysis of the obtained morphometrical data did not reveal any significant differences of the values of specific volume of edema and vessels among the patients with progressive and regressive remodeling. The specific volume of parenchyma was significantly higher and stroma specific volume – lower in both LV myocardium and in myocardium of RA auricle of the Ist group patients. Morphometrical parameters of mean value of capillary diameter and diameter of LV and RA auricle myocardial cardiomyocytes did not differ significantly among the patients with different late outcomes of surgical treatment. Specific volume of capillaries in the aforementioned heart parts was significantly higher in the patients with reverse remodeling (group I). All the morphometrical parameters mentioned above differed statistically significantly from those of the control study group.

Parenchymo-stromal ratio reflecting quantitative relationships between parenchyma and stroma of cardiac muscular tissue was significantly lower in LV myocardium and in myocardium of RA auricle in 48 ICMP patients with unfavorable late outcomes of surgical treatment (Table 6).

Patients	Myocardium	PSR	TI	PcDZ (mcm)	KI
Group I (n=90)	RA	2.17±0.31*	0.042±0.016*	245.6±27.9*	1.61±0.37*
	LV	2.18±0.27**	0.027±0.011**	385.5±51.3**	1.55±0.32**
Group II (n=48)	RA	1.85±0.38*	0.024±0.008*	481.2±61.7*	1.84±0.30*
	LV	1.65±0.31**	0.008±0.003**	1315.7±88.2**	1.85±0.21**
Control group(n=25)	RA	5.96±0.14	0.086±0.006	81.0±1.7	1.13±0.10
	LV	9.48±0.20	0.087±0.003	66.1±2.6	1.15±0.08

Note: *, ** – significance of morphometrical values differences between the patient groups with positive and negative dynamics of late postoperative follow-up period for RA auricle and LV myocardium respectively ($p<0.05$).

Table 6. Morphometrical parameters of LV and RA auricle myocardium in ICMP patients with different late outcomes of surgical treatment, Van-der-Waerden test (M±m)

Trophic index of these parts of myocardium which better reflects the condition of cardiac muscular tissue and is assessed as a ratio between capillary specific volume and specific volume of cardiomyocytes, was statistically lower in patients with progressive remodeling; according to the data of intraoperative biopsies of LV myocardium the condition of myocardial trophy in these patients was 8-12 times lower in comparison with that of the control study group.

Pericapillar diffusion zone (the value reflecting load on capillary bed) and Kernogan index (the value showing carrying capacity of microvasculature – the bigger index is the less is carrying capacity of arterioles and the worse are the values of tissue trophy) of LV and RA auricle myocardium were statistically higher in the patients of group II. Values of PcDZ in ICMP patients with unfavorable late outcomes of surgical treatment assessed as a ratio of capillary diameter to their specific volume, 15-20 times exceeded those of the IInd group patients.

There was no significant difference between the groups concerning cardiomyocytes diameter in both LV and RA auricle myocardium found.

As a result of the complex clinical-morphological study we were granted the patent "The prognostic method for postoperative heart remodeling in patients with ischemic cardiomyopathy" #2310372 of 20.11.2007 by the Federal Intellectual Property, Patent and Trademark Service. The method was based on the evaluation of morphofunctional condition of LV myocardial samples on histological preparations stained with hematoxylin-eosin and by Mallory with the help of light microscopy. With simultaneous presence of pathomorphological picture of myocarditis and values of parenchyma-stromal ratio < 1.7, trophic index < 0.010, pericapillar diffusion zone > 1000 mcm and Kernogan index > 1.6 postoperative heart remodeling and progressive chronic heart failure can be predicted.

The offered prediction method of postoperative heart remodeling based on complex evaluation of myocardial morphology in preoperative period or during surgical intervention allows to avoid unfavorable late outcomes of surgical treatment. Clinical cases are the best proof of reliability of the patented prognostic method:

Clinical case 1. Patient N., 56 years old

Diagnosis: CAD. *Obliterating atherosclerotic stenosis of coronary arteries. Postinfarction cardiac sclerosis. LV aneurism. HF of III functional class by NYHA.*

CAD history – 5 years.

Preoperative examination

EchoCG: LV EDVI – 102.4 ml/m²; LV ESVI – 68.2 ml/m²; LV EF – 34 %. Mild mitral regurgitation.

Surgery: SVR by Menicanti, CABG. Intraoperative biopsy of LV myocardium taken for histological study.

During histological study of intraoperative samples of LV myocardium pathomorphological signs of myocarditis were found: presence of diffuse-macrophage inflammatory infiltration of myocardial stroma, the 3d degree fibrosis (severe or unfavorable by Marburg classification of myocarditis).

Picture 8 shows a microimage of a histological preparation of LV from the patient N of 56 years old.

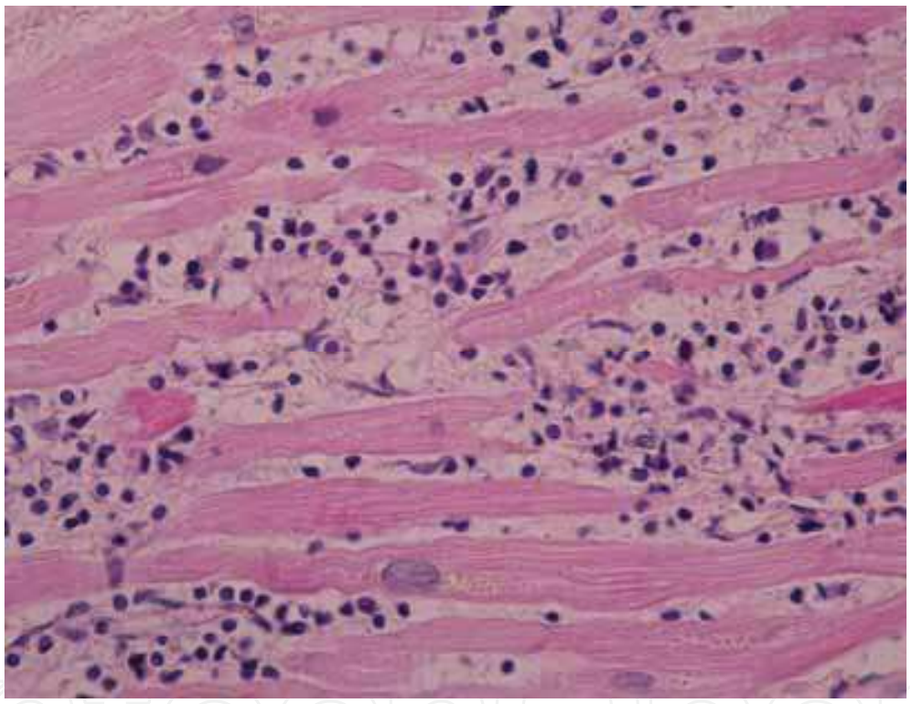

Fig. 8. Intraoperative biopsy sample of LV myocardium. Patient N, 56 year old. Diffuse-macrophage inflammatory infiltration of myocardial stroma. Severe (unfavorable) fibrosis. Stained with hematoxylin and eosin. X 300.

Morphometrical values of LV myocardium: parenchyma-stromal ratio – 1.04; trophic index – 0.0097; pericapillar diffusion zone – 1375.0 mcm; Kernogan index – 1.74; diameter of cardiomyocytes – 20.6 mcm. Thus, the morphological study revealed prognostic criteria associated with unfavorable late outcomes of surgical treatment.

Hospitalization in a year after the surgery.

CHF II FC. LV EDVI – 96.6 ml/m²; LV ESVI – 62.2 ml/m², LV EF – 36%. Mild mitral regurgitation. Pre- and postoperative EchoCG images are shown in Figure 9.

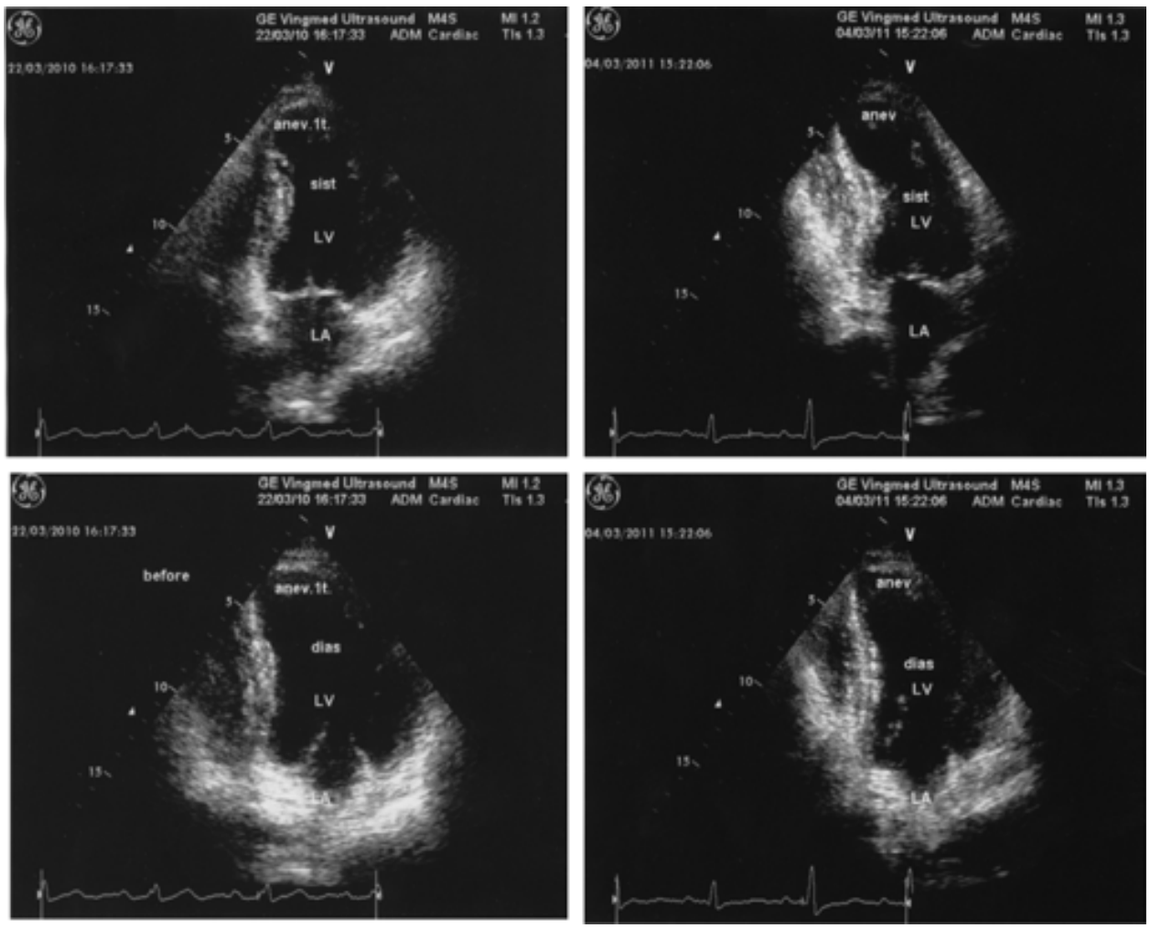

Fig. 9. Pre- and postoperative EchoCG images of the 56 year old patient N. Notice repeated postoperative LV remodeling and progression of chronic HF.

Clinical case 2. Patient B. 53 year old.

Diagnosis: CAD. *Obliterating atherosclerotic stenosis of coronary arteries. Postinfarction cardiosclerosis. LV aneurism. HF FC III by NYHA.*

Six year CAD history.

Preoperative examination

EchoCG: LV EDVI – 98.7 ml/m^2; LV ESVI – 61.3 ml/m^2; LV EF – 37 %. Mild mitral regurgitation.

Surgery: SVR by Menicanti, CABG. Intraoperative biopsy of myocardium taken for histological study.

Histological study of intraoperative samples of LV and RA auricle myocardium did not reveal any signs of inflammatory infiltration of myocardium. In Figure 10 one can see a microimage of the histological sample of LV myocardium from the 53 year old patient B.

Morphometrical values of LV myocardium: parenchyma-stromal ratio – 2.83; trophic index – 0.0417; pericapillar diffusion zone – 311.2 mcm; Kernogan index – 1.21; diameter of cardiomyocytes – 28.7 mcm. Thus, combination of the prognostic criteria for the progression of heart failure was not found, postoperative heart remodeling in the late follow-up period is not predicted.

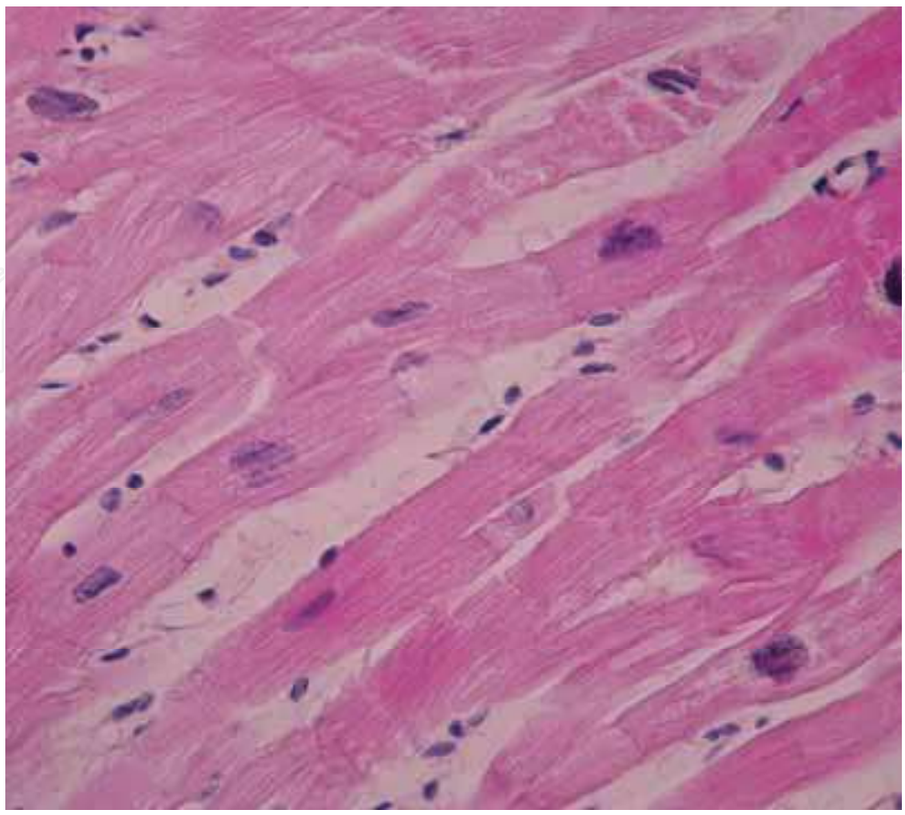

Fig. 10. A microimage of the histological sample of LV myocardium from the 53 year old patient B. No stromal inflammatory infiltration and fibrosis. Stained with hematoxylin and eosin. X 350.

Hospitalization in a year after the surgery.

CHF I FC. LV EDVI – 62.5 ml/m^2; LV ESVI – 33.8 ml/m^2, LV EF – 46%. Mild mitral regurgitation. Pre- and postoperative EchoCG images are shown in Figure 11.

Clinical examples support our conclusions about improper use of a mean value of LV myocardial cells diameter as a predictor of postoperative heart remodeling for ICMP patients: in a patient with the LV cardiomyocytes diameter > 28mcm signs of progressive HF were not found, however with the diameter of cardiac muscular cells <21mcm repeated heart remodeling was registered in the late postoperative follow-up period (as it was described by Moreira et al for the patients with dilated CMP (2001)).

Out from 58 ICMP patients whose myocardium was studied by electron-microscopy, 47 were included into the further analysis (43 men and 4 women): 34 with the reverse remodeling (group Ia) and 13 with the progressive remodeling (group IIa).

Morphometrical analysis of the specific volumes of LV and RA auricle myocardial cardiomyocytes ultrastructures confirmed our hypothesis about reduction of myocardial cells contractile apparatus as HF progressing as a result of exhaustion of compensatory adaptation processes: myofibrillar SV of cardiomyocytes significantly decreases. As myofibrillar SV decreases in cardiomyocytes, specific rate of mitochondrias grows, mitochondrial-myofibrillar ratio increases.

SV of natriuretic peptide of RA auricle cardiomyocytes insignificantly grows as chronic HF progressing. Nuclear SV does not change significantly.

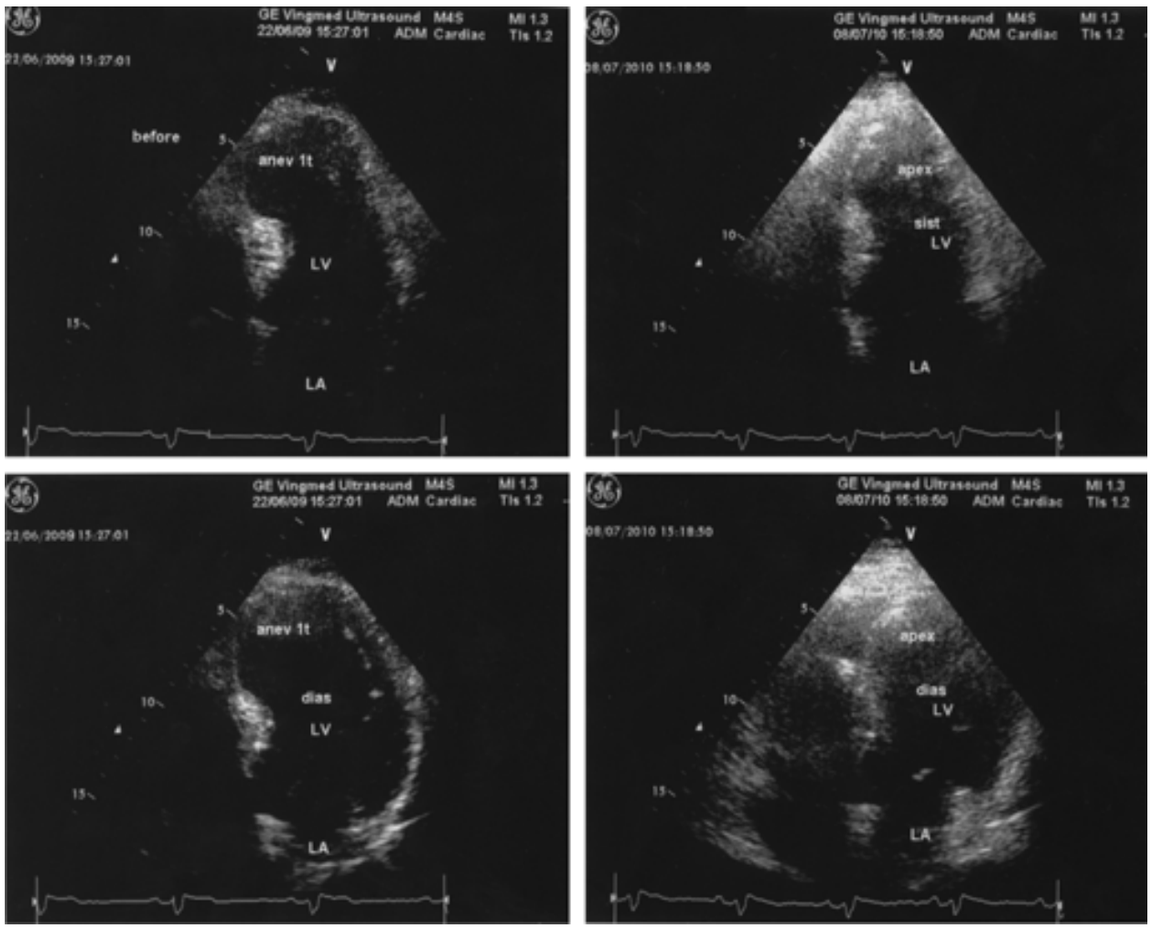

Fig. 11. Pre- and postoperative EchoCG images of the 53 year old patient B. Repeated postoperative LV remodeling and progressing chronic HF were not noticed.

Out from 47 ICMP patients whose myocardium was taken for the investigation of functional morphology of microcirculatory link of the LV and RA auricle vascular bed at an ultrasonic level, 39 patients were included into the further analysis (37 men and 2 women): 33 with the reverse remodeling (group Ib) and 6 with the progressive remodeling (group IIb).

Morphometrical values of functional morphology of microvasculature of the LV and RA auricle myocardium of ICMP patients are presented in Table 7.

The rate of open capillaries in myocardium of LV and RA auricle myocardium in ICMP patients with reverse remodeling type was significantly higher than that in the patients of Group II (73.0% vs 56.4% respectively). A similar value for RA auricle myocardium did not differ significantly among the patients with different late outcomes of surgical treatment. Luminal area of the open capillaries in ICMP patients with the reverse and progressive remodeling types as well as the number of pinocytic vesicles connected with a luminal contour of endotheliocytes per a unit of capillary luminal area were identical for both LV myocardium and myocardium of RA auricle.

Density of free pinocytic vesicles in capillary endotheliocytes of LV myocardium has become another value of functional morphology of microvasculature which differed significantly in ICMP patients with different late outcomes of surgical treatment: 0.0796 mcm^3/mcm^3 in the group of patients with positive postoperative dynamics versus

0.0678 mcm^3/mcm^3 in the group of ICMP patients with progressing chronic HF in the late follow-up period. All in all morphofunctional condition of microvasculature in ICMP patients with the reverse type of heart remodeling looks favorable in comparison with ICMP patients from group II.

Patients	Myocardium	Open capillary Rate (%)	Luminal area of open capillaries (mcm^2)	Density of free pinocytic vesicles in endotheliocytes (mcm^3/mcm^3)	Number of pinocytic vesicles per 1 mcm^2
Group Ib (n=33)	RA	82.0	10.5±1.9	0.0547±0.0079	5.7±0.6
	LV	73.0*	10.5±1.4	0.0796±0.0113*	6.8±0.7
Group IIb (n=6)	RA	82.8	10.4±2.1	0.0539±0.0092	5.5±0.8
	LV	56.4*	10.5±1.8	0.0678±0.0127*	6.3±0.9

Note: * – significance of morphological values differences between the groups of patients with positive and negative dynamics in the late follow-up period ($p<0.05$).

Table 7. Morphometrical values of functional morphology of the LV and RA auricle myocardial microvasulature of ICMP patients with different outcomes of surgical treatment, Van-der-Waerden test (M±m)

To make sure of diagnostic reliability of intraoperative myocardial biopsies we studied LV and RA autopsy material of 9 ICMP patients (7 men and 2 women) died at different periods after surgical intervention whose intraoperative myocardial samples we had taken during surgical intervention. We have not found any false-positive or false-negative results of diagnostics of pathomorphological myocarditis signs according to intraoperative biopsy samples (with the control on the autopsies material). Morphometric values of autopsy and biopsy samples were also comparable. Intraoperative biopsy of myocardium should not be considered as an absolute true, but on the whole, without any doubt, it reflects functional morphology of cardiac muscular tissue. First of all it depends on the volume of the material obtained for morphological studies and on the possibility of a targeted harvesting of a necessary LV area.

3.2 Molecular markers of postoperative LV remodeling in patients with ICMP

Samples of peripheral blood from 53 ICMP patients were taken 1-2 days prior to the surgical intervention. The control group consisted of 17 healthy volunteers.

Analysis of the obtained data revealed that in ICMP patients the content of pro-ANP and NT-proBNP in blood plasma was significantly higher than that in the group of healthy volunteers ($p<0.001$). The study of the content of matrix metalloproteinase in blood serum showed that between the groups of ICMP patients and healthy volunteers there were no differences in the content of matrix metalloproteinases of types 1, 3, 9 and tissue inhibitor MMP-1 (TIMP-1). But only MMP-9 content in blood serum obeyed normal distribution law.

During evaluation of the detection rate of antimyocardial antibodies of difference specificity in the healthy volunteers group in 53% of the cases there were no found autoantibodies to the cardiac tissue; in 47% of the cases antibodies to fibrillar structures were detected, but in only 6% of them these antibodies were present in a titre exceeding acceptable values. In the group of patients with ICMP the distribution of antibodies titres to fibrillar structures was the following: in 46 % of the patients the antibodies were detected in titre 20, in 24.3 % – in titre 40, in 21.6 % – in titre 80, in 2.7 % the titre reached 160 and in only 5.4 % of the patients autoantibodies were not detected.

In determining antibodies to sarcolemmic structures it was found that in 53% of the cases these antibodies in healthy donors were absent. Antibodies to sarcolemmic structures were

not found in 10.8 % of ICMP patients, in 24.3 % – they were detected in titre 20, in 43.3 % – in titre 40, in 16.2 % – in titre 80, in 5.4 % – in titre 160.
Maximal detection rate of antibodies to nuclear structures in titre 80 in the control group was 11.8%, in 88.2% of the patients did not have these antibodies. The highest titre in ICMP patients where nuclear antibodies were found was equal to 80 (2.7%); in 5.4% of the cases antibodies to nuclear structures were detected in titre 40, in 32.4% - in titre 20, in 59.5% autoantibodies of this specificity were absent.
In a year after the surgical treatment all the patients included into the study were examined (n=53, 100%). In accordance with the aforementioned algorithm for the evaluation of late postoperative period 40 patients were assigned into group I (with positive dynamics of the late postoperative period), 13 patients – into group II (with negative dynamics of the late postoperative period). The levels of pro-ANP, NT-proBNP and pro-MMP-1, MMP-3, MMP-9, TIMP-1 in blood plasma and serum In the ICMP patients with different dynamics of the late postoperative period are shown in Table 8.

Value	Normality of distribution law		Significance of differences between the groups		Group I			Group II		
	Shapiro–Wilk	p	Mann–Whitney t- TEST		n=40			n=13		
Nonnormal distribution law			U	p	Q_{25}	Me	Q_{75}	Q_{25}	Me	Q_{75}
pro-ANP (nmol/l)	0.917	0.03	57	0.73	3.69	6.34	8.59	3.31	5.30	7.78
NT-proBNP (fmol/l)	0.729	<0.01	59	0.82	12.30	38.45	58.42	19.48	39.93	90.37
MMP-3 (ng/ml)	0.861	0.002	25	0.03*	5.16	5.70	7.05	6.25	7.11	8.49
TIMP-1 (ng/ml)	0.838	0.001	46	0.32	426.0	455.8	502.6	447.4	480.8	512.4
Normal distribution law			t	p	Mean		Std. Dev.	Mean		Std. Dev.
MMP-9 (ng/ml)	0.954	0.26*	–2.255	0.03*	64.51		24.23	90.64		27.97
MMP-1 (ng/ml)	0.948	0.19*	–0.651	0.52	6.10		3.72	7.25		4.28

Note: * – statistically significant data.

Table 8. The content of natriuretic peptides and matrix meatlloproteinases in blood plasma and serum in ICMP patients with different dynamics of the late postoperative treatment

We managed to follow the late postoperative period of 32 (86.5%) out from 37 patients whose blood was tested for antimyocardial antibodies. Postoperative LV remodeling took place in only 5 patients which does not allow to reliably associate the activity of inflammatory response in myocardium with postoperative heart function.
Thus, evaluation of the content of MMP-3 and MMP-9 in blood serum at the preoperative stage let us "foresee" the outcome of possible surgical treatment since their content is significantly higher in the group of patients with postoperative heart remodeling. We made

an attempt to calculate sensitivity and specificity of molecular prognostic criteria of postoperative heart remodeling basing on the obtained material.
Test sensitivity (Se) may be identified as a probability of a positive outcome in the patients: Se=p(P/D). Calculation is performed as follows:

Se=(the number of positive outcomes in the group of patients P / number of the patients D) × 100 %.

Specificity (Sp) – is the probability of negative results of the test in healthy volunteers: Sp=p(N/H). Calculated as follows:

Sp=(the number of negative results among the healthy N / number of the healthy H) × 100 %.

A test with high specificity, as a rule, does not refer healthy people to the category of patients.

For MMP-3

Cut-off value = 7.7 ng/ml.
Se = (11/13)×100 % = 84.6 %.
Sp = (40/40)×100 % = 100.0 %.

For MMP-9

Cut-off value = 102.4 ng/ml.
Se = (7/13)×100 % = 53.8 %.
Sp = (40/40)×100 % = 100.0 %.

As for antibodies to myocardial structures, their titre is much higher in ICMP patients. This fact proves our hypothesis about the presence of inflammatory infiltrate in myocardial stroma as the key factor for unfavorable outcome of surgical treatment. Nowadays it is obvious that taking blood of the patients with massive postinfarction cardiac sclerosis at the preoperative stage for the evaluation of MMPs content and antibodies titre to myocardial structures in order to make prognosis for the late postoperative period is not only perspective but reasonable. Identification of the antimyocardial antibodies titre in blood serum preoperatively as well as in the dynamics of early and mid-term postoperative period will indirectly allow for monitoring of inflammatory process in myocardium and evaluation of the efficacy of a complex medical treatment avoiding repeated biopsies of myocardium.

4. Conclusion

Our clinical observations demonstrate progress of chronic HF in the late postoperative period and inefficiency of SVR in ICMP patients in 35% of the cases. These patients should be refused from the standard procedure of surgical restoration of normal left ventricular geometry in favor of alternative methods of surgical treatment – isolated bypass grafting for the patients with symptomatic CAD, cardiac resynchronization therapy for patients with QRS >120 msec and left bundle branch block, primary heart transplantation and implant of the devices preventing ventricular dilatation. Attempts to find clinical, instrumental or other markers of chronic HF progression have been made many times but the results are very modest. The age older than 55 years and high values of LV ESVI in preoperative period have been considered as risk factors of such interventions.
In our opinion the reason of unfavorable outcomes of surgical treatment lies in peculiarities of myocardial functional morphology of an each and every patient and depends on irreversibility of the far gone pathological changes in cardiac muscular tissue. Assessment of an initial morphofunctional condition of myocardium (at the moment of surgical treatment)

based on pre- and/or intraoperative LV biopsies taken for the detection of irreversible pathological changes in myocardium by a number of qualitative and quantitative histomorphometrical values taking into account the condition of tissue trophy to the full extent, should contribute into prevention of repeated heart remodeling and progression of chronic HF in the late postoperative period.

Cellular-stromal relationships on the background of chronic ischemia of myocardium precondition destructive processes of a heart muscular tissue remodeling which is reflected also in peripheral blood of ICMP patients: the content of MMPs and MMP-3 and MMP-9 in particular, which becomes molecular prognostic markers of the late postoperative period.

Basing on the obtained data we offer the following algorithm of surgical treatment of patients with ICMP: for the candidates for a complex surgical treatment older than 55 years old with preoperative LV ESVI >80 ml/m² it would be reasonable to widen indications for endomyocadial biopsy at the preoperative stage or to perform intraoperative biopsy of LV myocardium in order to identify prognostic criteria of the postoperative progressive heart remodeling and take blood samples for the detection of blood markers of HF progression. If the combination of unfavorable prognostic criteria takes place: the presence of diffuse inflammatory infiltration of myocardial stroma in combination with pronounced fibrosis, low TI (<0.010) and high values of KI (>1.5) and PcDZ (>1000 mcm) of LV myocardium as well as high concentrations of MMP-3 (>7.7 ng/ml) and MMP-9 (>102.4 ng/ml) in blood serum, the patient should be refused from a standard procedure of surgical reconstruction of normal left ventricular geometry in favor of alternative methods of surgical treatment (in the case of taking preoperative biopsy of myocardium) or surgeons should refuse from SVR in favor of a surgical procedure with less risk (in case of taking intraoperative biopsy of myocardium).

5. Study limitations

The success of reconstructive surgeries in patients with ICMP depends not so much on functional morphology of cardiomyocytes and the condition of myocardial trophy as on cellular-stromal interactions in cardiac muscular tissue with the background of chronic ischemia. In our opinion, identification of myocarditis etiology with the purpose of its etiotropic treatment is one of the key factors of prevention of heart failure progression in the late postoperative period in patients with ICMP. By far the correlation of the presence of myocardial inflammatory infiltration with activation of metalloproteinase system has not been fully understood. Probably, quantitative and, what is also important, qualitative content of myocardial stroma (collagen types, fibronectin, laminin, etc) determines "tolerance" of the tissue to progressive dilatation.

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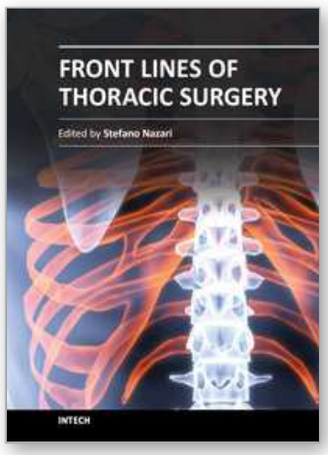

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