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# Dynamics of Biochemical Markers in Patients Suffering from Heart Failure under the Influence of Cell Therapy

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**Abstract**

This article provides information about the nature of changes in biochemical markers on the background of stem cell therapy in patients suffering from coronary artery disease (CAD) and chronic heart failure (CHF).

Methods: The study included 60 patients (54 men and 6 women) suffering from chronic heart failure and coronary artery disease. The age of patients ranged from 42 to 75 years. All patients have received medical therapy according to current clinical protocols. Patients from the 1st group, in addition to drug therapy, have received intravenous cell therapy (culture of autologous bone marrow stromal stem cells with cellularity 50 million). The changes in hemodynamic parameters have been studied according to echocardiography and dynamics of indicators of biochemical markers of heart failure, brain natriuretic peptide (BNP) and serum lactate. Evaluating of performance has been carried out initially and at 12 and 24 weeks of observation.

Results: Performance evaluation of hemodynamic parameters have shown an increase in ejection fraction to 38.6 ± 2.3% by the end of 12 weeks of observation, by the end of 24 weeks of observation also there have been registered

an increase in this indicator to 38.2 ± 2.4%, compared with the initial state (35.2 ± 2.5%). Performance analysis of biochemical markers have identified, by the end of 12 weeks of observation, statistically significant reduction of the level of BNP to the value 311±26 pg / mL compared on the one hand with baseline 407±35 pg / ml and on the other - with the control group. By the end of 24 weeks of observation, this trend has been driving. When assessing the performance of serum lactate at the end of 12 weeks of observation, there have been revealed a statistically significant decrease in its value to 4.37 ± 0.28 mmol / L when compared with the initial state (5.08 ± 0.26 mmol/ L).

As can be seen from the above, the cell therapy in combination with standard medical therapy has a positive effect on the course of CHF, as confirmed by the improvement of basic hemodynamic parameters and decreasing of the level of biochemical markers of CHF due to CAD.

**Keywords**: brain natriuretic peptide, heart failure, cell therapy

## Introduction

Despite a significant advances in the study of etiology, pathogenesis, recent advances in the diagnosis and treatment of this disease, chronic heart failure (CHF) remains a burning medical and social problem. The most common cause of CHF is cardiovascular disease including coronary heart disease (CAD) [8]. Although the success of drug therapy and revascularization procedure of CAD), proven effectiveness in numerous studies, CHF is still characterized by high levels of mortality. This requires the development and application of new methods of treatment, one of which is a regenerative cell therapy [8]. Conducted in the last decade studies have shown that the use of cellular technologies can improve the functional state of the heart muscle [4, 6, 9]. A brain natriuretic peptide has been found as the most important indicator of myocardial dysfunction of the left ventricle in patients with CHF. Determination its plasma concentration is recommended as a diagnostic laboratory test for assessing the risk of cardio - vascular events in patients with heart failure, as well as monitoring the effectiveness of the therapy [3, 7, 10]. Also of interest the study of lactate levels in patients with CHF on the background of conducted therapy [1, 5, 11].

## Materials and methods

The study prospectively included 60 patients (54 men and 6 women) suffering from chronic heart failure and coronary heart disease. The age of patients ranged from 42 to 75 years. All patients signed a voluntary agreement to participate in the study. The diagnosis of stable CAD has been verified on the basis of the availability of stable angina, confirmed by the daily monitoring of ECG method using "Cardio 04" ("Inkart" St-Petersburg, Russia), and the positive results of the test on the treadmill. Treadmill test is performed on KardioLab "KhAI Medica" (Kharkiv, Ukraine) using Bruce’ protocol. The protocol was used

in connection with high functional class of angina and the severity of CHF in the study participants. Hemodynamically significant atherosclerotic lesions (more than 50% stenosis of at least one coronary artery disease), coronary artery were angiography confirmed. Research was carried out in accordance with M. Judkins method [5] by transfemoral S. Seldinger access using nonionic contrast "Visipaque-270" installation «Integris-3000" Phillips Company (Netherlands) according to the method M. Judkins [5] S. Seldinger transfemoral access by using nonionic contrast "Visipaque-270" at the installation «Integris-3000" Phillips Company (Netherlands).

Quality of life was assessed with the help of questionnaire (MLHFQ), when the patient was included in the research and at 12 and 24 week follow-up [14].

Ethical declaration

The research protocol was reviewed and approved at the meeting of the Commission on Bioethics GI "Institute of Urgent and Recovery Surgery named after V.K. Gusak of NAS of Ukraine" (protocol № 1 of 02.06.2008).

There had been formed 2 groups of patients: the first (primary) group consisted of 30 patients who received conventional medical therapy of CHF, including ACE inhibitors, blockers of ß-adrenergic receptors, diuretics, statins, cardiac glycosides, aldosterone antagonists, in addition - cell therapy using autologous mesenchymal stem cells (MSCs).

Autologous bone marrow MSCs were obtained by the standard technique in the laboratory of cell and tissue culturing IURS. Aspiration of the bone marrow (BM) was carried out under aseptic conditions from an iliac crest bone in quantities of 50 ml the addition of 0.5 ml of heparin (5000ED / 5 ml) in 15 ml of physiological saline ("Dartitsa", Ukraine). Syringes used for the explantation of the BM, previously had been washed with working solution of heparin. Aspirate of BM was mixed with with Hanks solution ("BioloT", Russia) in a ratio of 1: 1 and centrifuged during 30 min at 447 g.

The resulting BM cell suspension from supernatant had been seeded into the culture flasks with an area of 75 cm² («Cjrning-Costar», USA) in 15 ml. A cultivation of MBM was carried out in a mixture of culture medium DMEM / F 12 1: 1 ("Sigma", USA) supplemented with 20% fetal calf serum ("BioloT", Russia), 0.75 g / L glutamine ("BioloT", Russia) $4 \cdot 10^{-6}$ g/l basic fibroblast growth factor («Sigma», USA), $5 \cdot 10^{-2}$ g/l L-ascorbic acid («Sigma», USA) and primary culture - 100 u/ml penicillin and $10^{-4}$ g/l streptomycin ("Dartintsa", Ukraine), a $CO_2$-incubator ("Jouan", France) at 37 ° C and 5% CO2 atmosphere. The medium was changed every 3-4 days of cultivation. Cultures reached an initial monolayer at 8-14 days of culture. Passage or subculturing was performed by using a mixture solution of 0.25% trypsin / EDTA ("BioloT", Russia) in a ratio of 1: 3 or 1: 5. The cells were cultured in the $CO_2$-incubator under the same conditions. As a result of

these manipulation an uncommitted culture of MSCs had been obtained [15]. Highlighted cells at marking by antibodies ("BD Biosciences Pharmingen", US) had the phenotype of CD105 +, CD73 +, CD90 + (> 95% positive) and CD45-, CD34 (<2% positive) [5].
The transplantation of obtained culture with cellularity of 50 million was performed by intravenous infusion to patients from the 1st group. The second (control) group was consisted of 30 patients who received conventional medical therapy heart failure [8].

## Statistical analysis

The results for quantitative traits during analyzing are presented as $\overline{X} \pm m$ (the average value ± standard error). For the analysis 95% confidence interval (95% CI) had been calculated for the average value. Student's test (normal distribution) and W-Wilcoxon test (non - normal distribution) were used during comparison of the groups in the corresponding periods of measurement. ANOVA was used for repeated measurements analyzing the dynamics of change in the indicator, while making comparisons for the 3 groups, the Bonferroni correction was taken into account [12]. Differences were considered as statistically significant at a value of $p < 0.05$
Assessment of the patients' state was performed at baseline and later at 12 and 24 weeks of observation. Data was analyzed using the statistical package «MedCalc 15.6» (MedCalc Software bvba, 1993-2015).

## Results

The main patient characteristics from the research are presented in Table 1. Both groups were comparable between them by clinical and anthropometric indicators. Initial evaluation of the patient's functional state of the cardiovascular system showed that there is no statistically significant differences in the CHF functional class, as evidenced by test results of physical activity (6-minute test walking, treadmill test). Initial evaluation of quality of patients' life defined according to the Minnesota questionnaire "Living with heart failure", which is specific for this group of patients. There weren't any statistically significant differences between the two groups of patients.

Table 1: Characteristics of study groups (n = 60)

| **Variables** | Mean value, $\overline{X} \pm m$ | | P value |
|---|---|---|---|
| | 1st group (primary) (n = 30) | 2nd group (control) (n = 30) | |
| Age (years) | 58.0±1.5 | 60.1±1.7 | 0.21 |
| Weight, kg | 82.3±1.5 | 84.9±1.3 | 0.24 |
| Height, sm | 176.7±1.2 | 176.1±1.0 | 0.67 |
| BMI, kg / m2 | 26.4±0,5 | 27.4±0.4 | 0.16 |
| HR, beats. min. | 79.3±1,9 | 77.3±1.9 | 0.45 |
| BP sist, mmHg | 127.8±3.1 | 130.3±2.2 | 0.17 |
| BP diast, mmHg | 79.8±1,4 | 79.7±1.2 | 0.97 |
| SMWT, m | 315±21 | 316±20 | 0.98 |
| Tredmil-test, m | 262±26 | 335±26 | 0.05 |
| MLHFQ, point | 54.9±2.4 | 56.1±2.1 | 0.71 |

The evaluation of dynamics of the LVEF under the influence of traditional drug therapy and combination of traditional drug therapy with cell transplantation has been made.

Table 2: Dynamics of index LVEF in the research group

| The study average | Mean value LVEF, % $\overline{X} \pm m$ | | P value |
|---|---|---|---|
| | 1st group (primary) (n = 30) | 2nd group (control) (n = 30) | |
| baseline | 35.2±2.5 | 37.6±1,2 | 0.38 |
| 3 months later | 38,6±2,3* | 36,6±1,2 | 0.46 |
| 6 months later | 38,2±2,4* | 35,0±1,2* | 0.23 |

Notes: Student's test (normal distribution) and W-Wilcoxon test (non - normal distribution) were used during comparison of the groups in the corresponding periods of measurement. ANOVA was used for repeated measurements analyzing the dynamics of change in the indicator.

* - It was noted that there was Statistically significant difference from the value of the index values in the initial state, ($p < 0,05$, taking into account the Bonferroni correction).

The analysis found no statistically significant differences in the average values of the LVEF between the groups in any of the measurement points ($p > 0.05$ in all cases).

In the first group there have been found the presence ($p = 0.004$) of a rising trend line indicator LVEF. Moreover there have been an increase ($p = 0.001$) of index LVEF at the end of 12th-week up to 38.6 ± 2.3% and also an increase ($p = 0.01$) of index LVEF indicator at the end of 24th weeks of observation to 38.2 ± 2 4% compared with the initial state (35.2 ± 2.5%).

The presence of a trend of the LVEF index was identified ($p < 0.001$) and in the second group, but in this case the trend was downward (see. Figure 1). Thus decline of LVEF index was observed at the end of 24 th weeks of observation to 35.0 ± 1.2% in comparison with its value at end of 12 th weeks of observation to 36,6 ± 1.2% ($p = 0.01$) and with the baseline state, the value of 37.6 ± 1.2% ($p < 0.001$).

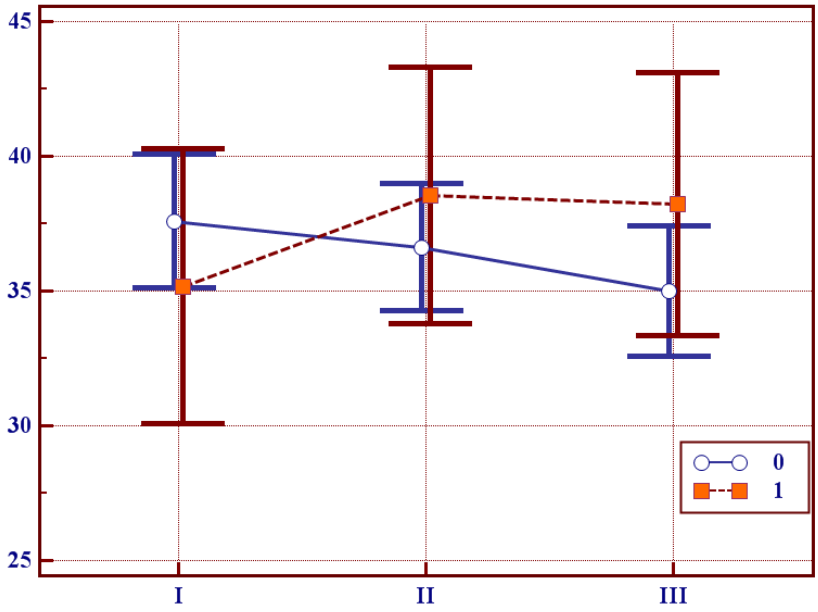


Figure 1. The dynamics of variation of the LVEF in the control group (0) and major groups (1): I - initial state, II - value of the index 3 months later, III - index value of 24th weeks after starting treatment. The average value was showed (points) and 95% of confidence interval (upper and lower limits of the confidence interval - a mustache)

The dynamics of biochemical marker of brain natriuretic peptide (BNP) in patients with chronic heart failure under the influence of both treatment options was estimated.

Table 3: Dynamics of the BNP indicator in groups under research

| The study average | Mean value LVEF, % $\overline{X}$ ±m | | P value |
|---|---|---|---|
| | 1st group (primary) (n = 30) | 2nd group (control) (n = 30) | |
| baseline | 407±35 | 408±30 | 0,95 |
| 3 months later | 311±26* | 453±34* | 0,002 |
| 6 months later | 322±44* | 486±35* | <0,001 |

Notes:

Student's test (normal distribution) and W-Wilcoxon test (non - normal distribution) were used during comparison of the groups in the corresponding periods of measurement. ANOVA was used for repeated measurements analyzing the dynamics of change in the indicator.

* - It was noted that there was Statistically significant difference from the value of the index values in the initial state, (p <0,05, taking into account the Bonferroni correction).

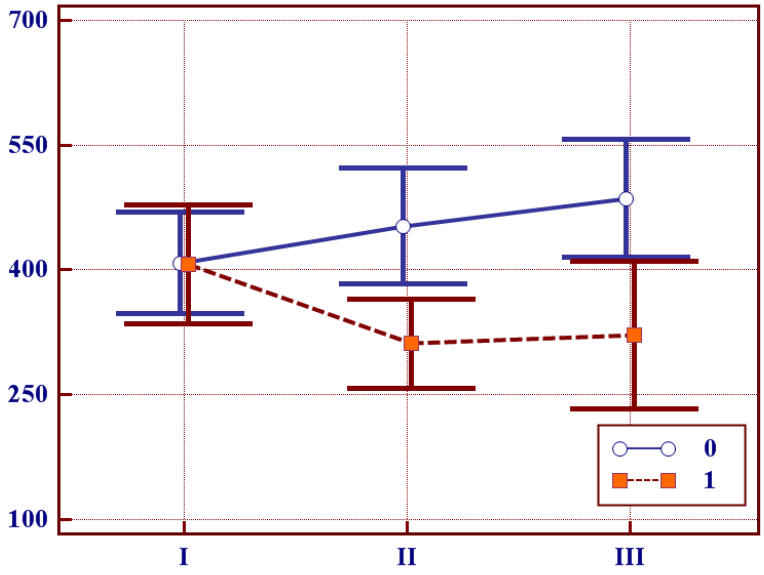


Figure 2. Dynamics of the NUP indicator in the control group (0) and primary group (1) shows the average value (points) and 95% confidence interval (upper and lower limits of the confidence interval - a mustache). Where: I - initial state, II - 12 weeks from starting treatment, III - after 24 weeks of treatment.

There was no statistically significant difference (p = 0.95) of average value of the BNP between the groups before treatment in the analysis (407±35 pg / ml in the main group and 408±30 pg/ ml in the control group). By the end of 12$^{th}$ weeks of observation the average value of the BNP in the study group was statistically significant (p = 0.002), lower than in the control group (311±26 pg / ml and 453±34 pg / ml, respectively). Similarly, at end of the 24 weeks of observation the average value of BNP in the study group was statistically significant (p <0,001) lower than in the control group (322±44 pg/ ml in the main group and 486±35 pg / ml in the control group). Moreover in the control group there were found the rising and in the main group – downward trend indicators of BNP change during time of observation (p <0,001).

There were no statistically significant differences (see. Figure 3) in the average values of the serum lactate between the groups in any of the measurement points (p> 0.05 in all cases).

Table 4: The changes of serum lactate in groups study

| The study average | Mean value LVEF, % $\overline{X} \pm m$ | | P value |
|---|---|---|---|
| | 1st group (primary) (n = 30) | 2nd group (control) (n = 30) | |
| baseline | 5.08±0.26 | 4.52±0.25 | 0.12 |
| 3 months later | 4.37±0.28* | 4.8±0.25* | 0.18 |
| 6 months later | 4.86±0.25 | 5.03±0.25* | 0.63 |

Note:

Student's test (normal distribution) and W-Wilcoxon test (non - normal distribution) were used during comparison of the groups in the corresponding periods of measurement. ANOVA was used for repeated measurements analyzing the dynamics of change in the indicator;

* - It was noted that there was Statistically significant difference from the value of the index values in the initial state, (p <0,05, taking into account the Bonferroni correction).

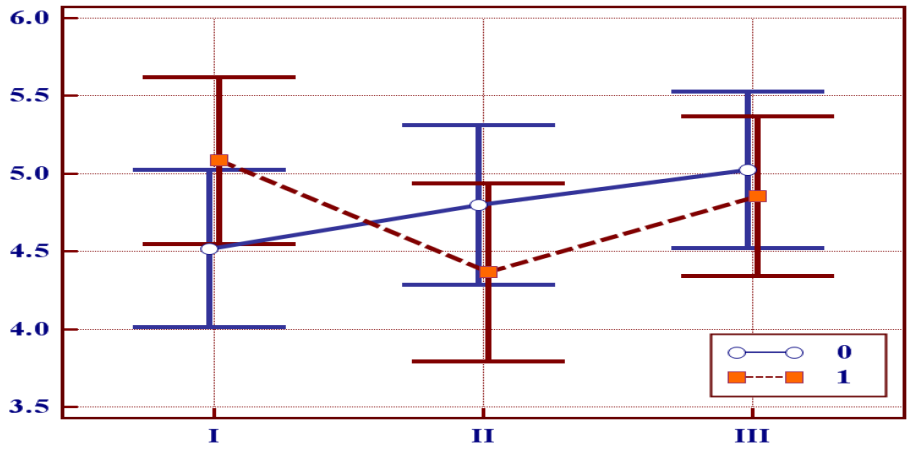


Figure 3. Dynamics of changes in the rate of lactate (mmol / L) in the control group (0) and primary group (1) - the average value (points) and 95% confidence interval (upper and lower limits of the confidence interval - a mustache). Where: I - initial state, II - 12 weeks from the beginning of treatment, III - after 24 weeks of treatment

For the control group revealed the presence ($p < 0,001$) of the rising trend line of variation of the serum lactate with time. During it the increase of the indicator was revealed ($p = 0.02$) at the end of 12 weeks of observation (4,80 ± 0.25 mmol / l) and ($p < 0,001$) by the end of 24 weeks (5.03 ± 0.25 mmol / l) compared to baseline (4.52 ± 0.25 mmol / l). In the basic group there was revealed the ame presence ($p < 0,001$) of downward linear trend of changes in the rate of lactate serum over time. At the same time was revealed reduction ($p < 0.02$) of this index by the end of 12 weeks of observation (4.37 ± 0.28 mmol / l) to a value comparison with the initial state (5.08 ± 0.26 mmol / l).

## Discussion

Modern views on the pathogenesis of CHF determine the inclusion in pharmacotherapy of the disease state of medical such preparations as ACE inhibitors, beta-adrenergic blockers, diuretics, cardiac glycosides, aldosterone antagonists. The surgical methods of correction of diseases leading to the development of heart failure, particularly myocardial revascularization in ischemic heart disease also play a very important role. Despite this, to achieve significant results in the treatment of heart failure in patients who have expressed systolic dysfunction, resulting from the presence of large post-infarction scar areas, it is extremely difficult. Development of the fundamental branches of cell and molecular biology offers new opportunities in the treatment of heart and vascular disease [4]. Our research has shown the efficacy of MSCs in patients with CHF due to CAD, which manifests itself in increasing the LVEF, reducing the level indicators of biochemical markers and improving the quality of life of patients.

According to Patel A.N. et al. on the background of cellular therapy there was a decrease of severity of clinical symptoms of heart failure and of the level of BNP in 3-6 months after the introduction of MSCs [11], which was also observed in the present research paper. As part of the research POSEIDON [4] there have been established a moderate positive effect on LV systolic function and quality of life of patients after application of stem cell therapy, which is coincide with the results presented in the research under consideration. The differences in the results of the research can be caused by the inhomogeneity of characteristics of the patients, administration routes, and the cellularity of MSCs.

## Conclusions

As a result of conducted research has been found that the inclusion in traditional drug therapy of CHF of intravenous transplantation of autologous MSCs can achieve the best results in treatment what is clinically confirmed as well as during the instrumental and laboratory research. During the control testing the improvement of the quality of patients' life is marked. However, the effect of autologous transplantation of MSCs is relatively short-term and requires the repetition of the procedure at intervals of 4-5 months. The positive impact of cell

transplantation of MSCs allows to introduce this type of cellular cardiomyoplasty in clinical practice, especially in cases where revascularization procedure is impossible.

**Competing interests**
The authors declare that they have no competing interests.

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