DOI: 10.15825/1995-1191-2019-4-54-66

EVALUATION OF THE EFFECTIVENESS OF COMBINED TREATMENT OF CORONARY HEART DISEASE – CORONARY ARTERY BYPASS GRAFTING, TRANSPLANTATION OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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Introduction. Despite resounding success in treatment of patients with coronary heart disease (CHD), researchers are yet unable to significantly reduce mortality in this disease. With this in mind, there are ongoing studies everywhere, which are aimed at investigating new techniques in order to boost the efficiency of existing standards. One of such promising techniques is cell/regenerative therapy with autologous bone marrow mononuclear cells (ABMMCs). However, even though ABMMCs have been studied for more than 10 years, there are no unambiguous data yet on several issues. **Objective:** to evaluate the outcome of ABMMC transplantation during coronary artery bypass grafting (CABG) surgery in combined treatment of CHD. **Materials and methods.** The data of 408 patients admitted to the clinic from 2013 to 2016 for planned surgical treatment of CHD were analyzed. The work included 117 people based on the design of the study. Patients were randomized in 3 groups: Group 0 (control group) – CABG surgery and intramyocardial injection of 0.9% NaCl solution, Group 1 – CABG surgery and intramyocardial injection of ABMMCs, Group 2 – CABG surgery, intramyocardial and intragraft injection of ABMMCs. The dynamics was assessed 12 months later – functional class of angina pectoris and heart failure, echocardiography, speckle tracking (assessment of the degree of myocardial deformation), treadmill test, 6-minute walk test, daily ECG monitoring, quality of life questionnaires, coronary angiography. Qualitative indicators were calculated using the Pearson's chi-squared test and Fisher criteria. Quantitative indicators were calculated using the Kruskal–Wallis and Wilcoxon tests. Factor analysis was used to identify certain severity factors and to study data homogeneity. Discriminant analysis was performed to investigate the leading characteristics that determine differentiation between the groups. For analysis of variance, taking into account various factors, the model of variance analysis for dependent samples – Repeated Measures ANOVA – was used. **Results.** In the observation groups, an improvement in both systolic and diastolic myocardial function was universally noted. A six-minute walk test showed statistically significant increase in Groups 1 and 2 compared with the control Group 0 – 315.06 ± 17.6 (433.54 ± 20.6), Group 1 – 319.8 ± 24.5 (524.4 ± 28.7), Group 2 – 329.9 ± 25.3 (452.7 ± 29.7) meters. A significant decrease in the functional class of exertional angina pectoris in Groups 1 and 2 was noted unlike in the control group. The percentage of functioning coronary shunts after a 12-month follow-up period was 87.6% in Group 0. In Groups 1 and 2, this ratio was 96.2% and 97.3%, respectively. Predictors of overall effectiveness were identified: smoking, initial diastolic myocardial dysfunction, left ventricular ejection fraction. **Conclusion.** In addition to surgical treatment of coronary heart disease, ABMMC transplantation can improve myocardial contractility, boost exercise tolerance, and increase the duration of the functioning of coronary shunts at the follow-up period of 12 months. The study showed the need for stage-by-stage analytical calculations with the aim of possible correction of further work.

Keywords: clinical trial, coronary artery bypass grafting, revascularization, reperfusion injury, coronary heart disease, speckle tracking, diastolic dysfunction, diastolic heart failure, autologous bone marrow mononuclear cells, intramyocardial injection.

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INTRODUCTION

The National Research Center for Preventive Medicine estimates that about 10 million able-bodied people in Russia suffer from CHD, and more than a third of them have stable angina pectoris. Economic burden resulting from such morbidity in 2016 alone exceeded 2.7 trillion roubles – 3.2% of the country's GDP. Among all cardiovascular diseases, CHD provides the highest economic burden – over 1 trillion roubles [1].

The World Health Organization (WHO) predicts a further increase in cardiovascular morbidity and mortality in both developed and developing countries, due to the aging population and lifestyles.

Despite the undeniable successes achieved in the treatment of patients with ischemic heart disease as a result of modern breakthroughs in rational pharmacotherapy, as well as development and implementation of various techniques of restoring coronary blood flow – bypass surgery, angioplasty and coronary artery stents, it has not yet been possible to significantly reduce mortality in this disease.

Mortality from cardiovascular disease (CVD) is projected to rise to 23.3 million by 2030. This indicator was 16.7 million in 2002 [2].

According to the American Heart Association, over 2150 Americans die of CVD each day (approximately 1 death every 40 seconds). It is noteworthy that about 155,000 people who died from this heart disease in 2011 were less than 65 years old [3].

In the Russian Federation, CVDs are the leading cause of death among the population (57% of total mortality rate). This figure is one of the highest in the world. CVD is the most common cause of hospitalization and disability in the Russian Federation.

Given these data, in order to combat age-related pathologies at the end of the 20th century, innovative programs were launched in economically developed countries to develop priority research and practical innovations on regenerative medicine as one of the promising methods for improving the effectiveness of existing standards.

Such strong focus on regenerative therapy is because stem cells promote neoangiogenesis. Such procedures can improve myocardial perfusion, extent of local and global contractility, and prevent myocardial remodeling and cell apoptosis [4–10].

The same results were obtained for ABMMC fraction [11–14].

However, even though ABMMCs have been studied for more than 10 years, there are no unambiguous data yet on several issues: type of cellular material, transplantation methods, volume of introduced cellular material, general safety and effectiveness.

These questions inspire researchers towards new studies [15–16].

In randomized, double-blind, placebo-controlled trial TAMIS (Autologous Bone Marrow Mononuclear Cells in the Combined Treatment of Coronary Heart Disease), ClinicalTrial.gov Identifier: NCT02059512, comprehensive assessment of the safety, efficacy and predicted outcome in combined treatment of ischemic heart disease (IHD) in combination with coronary artery bypass grafting (CABG) and ABMMC transplantation in patients with coronary artery disease and heart failure (left ventricular ejection fraction in reference values) was carried out.

The trial was conducted after approval by the ethics committee of Pavlov First St. Petersburg State Medical University (minutes No. 147 of February 26, 2013). The work was done as part of state assignment to the university, under a grant on the topic: "*Improving the methods of surgical treatment of coronary heart disease using cell technology*". Platform "Cardiology and Angiology" (115091630053).

MATERIALS AND METHODS

For the period of the trial from 2013 to 2016, the data of 408 patients who were admitted to the clinic of the Research Institute of Surgery and Emergency Medicine, Pavlov First St. Petersburg State Medical University for elective CABG surgery with cardiopulmonary bypass (CPB). According to angiographic study, these patients had 3 or more branches of stenosed coronary arteries. The study included 117 people who met the inclusion criteria: men and women from 18 to 80 years old, class III–IV angina pectoris, and informed voluntary consent. Exclusion criteria: intolerance to heparin and hydroxyethyl starch (HES), thyroid pathology (hypothyroidism, hyperthyroidism), concomitant pathology with predicted life expectancy of up to 3 years, infectious diseases, simultaneous participation in another study, patient's refusal to participate in the trial. Randomization was performed, according to the table of random numbers, in the following groups: group 0 (46 people) – control group – CABG surgery and intramyocardial injection of 0.9% NaCl solution, group 1 (34 people) – CABG and intramyocardial injection of ABMMCs, group 2 (37 people) – CABG, intramyocardial and intragraft injection of ABMMCs (Fig. 1).

Final quantity of the material obtained was calculated on a sample of patients – 37, 25 and 23 in groups 0, 1 and 2, respectively. The remaining patients included in the study felt satisfactory at the time of control examination, but for various reasons, there were no objective data on their clinical condition. They were excluded from final statistical analysis.

To evaluate the safety, efficacy, and prediction of outcomes of ABMMC transplantation in combination with CABG with CPB, several parameters were analyzed (Table 1). Analysis of the dynamics of these variables

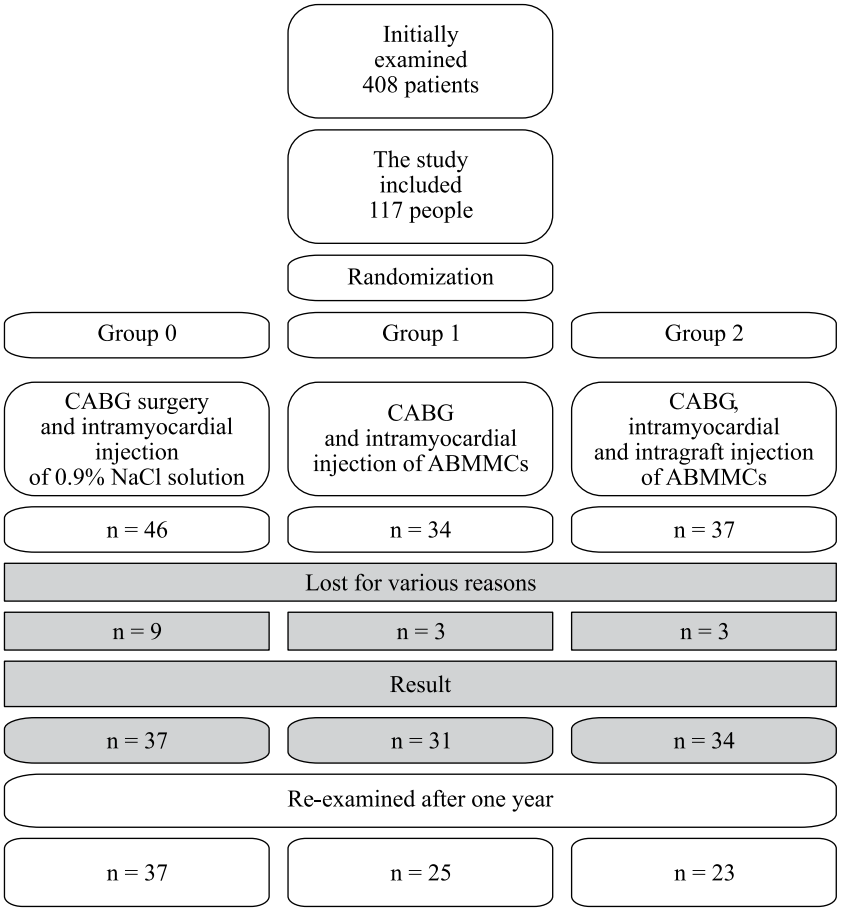


Fig. 1. Study design of TAMIS (Autologous Bone Marrow Mononuclear Cells in the Combined Treatment of Coronary Heart Disease)

was performed within the time frame established by the study design.

Initially, patients in the observation groups were comparable in terms of key parameters (Table 2).

Patient's baseline examination for inclusion in the study corresponded to the standard volume (laboratory, instrumental) before CABG surgery with CPB.

The risk of an adverse outcome for upcoming surgery was rated using the EuroScore II scale.

All patients received adequate medication according to international guidelines depending on the individual clinical situation. Correction was carried out as necessary during the hospitalization period.

Echocardiography was performed on a GE Vivid 7 ultrasound machine using standard technique.

Structural indicators obtained from the study were calculated according to recommendations by ASE and EAE 2015 [17].

Left ventricular linear and volumetric indicators were calculated taking into account the body mass index (BMI), which means that these parameters were taken into account.

The parameters for assessing the left ventricular diastolic function are: transmitral flow velocity (peak E/peak A) and their ratio, deceleration time of peak E (DT), and left ventricular isovolumetric relaxation time (IVRT).

Additional assessment of the left ventricular regional systolic function was done using an automatic functional imaging (AFI) application based on the 2D Strain function, which calculates myocardial tissue deformation by tracking the deformation by two-dimensional images. After software processing, the result was obtained in the form of 6 sectors (17-segment scheme), which shows maximum systolic longitudinal strain in arbitrary colors and numerical values, global strain data for each projection, and global strain averages for the entire left ventricle. The dynamics of these indicators were: initially, when the patient was included in the study, on the 7–14th day of postoperative period, and 12 months after the study.

Treadmill test was aimed at determining exercise tolerance, and at identifying clinical and electrocardiographic signs of myocardial ischemia. The patient was given a physical activity (treadmill), consisting of several stages of progressively greater workloads in accordance with the R. Bruce protocol.

At the same time, cardiac performance (pressure, pulse, electrocardiogram) and their changes depending on the load were recorded.

Table 1

Clinical study sections

I. Safety assessment for additional ABMMC transplantation	II. Efficacy evaluation	III. Prediction of treatment outcomes (influence of several parameters)
1. Assessment of the risk of adverse outcome of upcoming surgery according to the EuroScore II scale. 2. Assessment of intraoperative indicators of ACT; Hb, Ht, K+ at the end of CPB and at the end of surgery. 3. Heart rate recovery at the end of main stage of surgery (defibrillation/self-recovery). 4. Cardiopulmonary bypass time. 5. Duration of anoxia. 6. Volume of discharge through tube in day 1 and day 2 of postoperative period. 7. Level of CPK-MB, myoglobin, troponin I on the 1st and 3rd day of postoperative period. 8. Data obtained from echocardiography performed on the 7–14th day of postoperative period (assessment of myocardial contractility – presence of additional hypo- or akinetic zones compared with baseline). 9. Frequency of postoperative complications (hydrothorax, hydropericardium, rhythm disorders). 10. Length of ICU stay. 11. Length of hospital stay (bed-day).	1. Assessment of systolic and diastolic myocardial function (echocardiography). 2. Assessment of stress test data: treadmill test, daily ECG monitoring. 3. Assessment of data from 6-min walk test (6MWD). 4. Assessment of NYHA angina functional class and heart failure. 5. Quality of life assessment (Minnesota Questionnaire, Seattle Questionnaire, SF-36 Questionnaire). 6. Bypass patency 12 months after treatment (angiographic examination). 7. Assessment of myocardial condition (before and after treatment) – AFI 2D Strain, speckle tracking. 8. Dependence and duration of positive clinical effect on the size of introduced cellular material.	1. Age 2. Sex 3. Body mass index 4. Diabetes 5. Smoking 6. Family history of cardiovascular disease 7. Length of CHD history 8. Level of total cholesterol and its fractions 9. Leukocytosis and CRP levels (initial and dynamics of decline in postoperative period). 10. Creatinine level 11. Presence/absence of extracardiac arteriopathy 12. ABMMC injection intramyocardially or intracoronally 13. Bone marrow examination: number of nucleated cells, CD34+, CD133+.

Note. ACT – activated clotting time; Hb – hemoglobin; Ht – hematocrit; CPK-MB – MB fraction of creatine phosphokinase; CRP – C-reactive protein.

Table 2

Basic characteristics of patients in observation groups

	Group 0 (n = 37)		Group 1 (n = 25)		Group 2 (n = 23)		p
Age (years)	61 ± 8	45–79	61.7 ± 6.8	47–73	59.5 ± 5.4	51–70	0.44
Sex (m/f)	6/31		4/21		3/20		
BMI (kg/m^2)	28.6 ± 3.59	21.1–34.8	28.05 ± 4.46	22.9–44.8	28.09 ± 3.57	22.5–35.4	0.46
Diabetes	6	16.22%	5	20%	4	17.39%	0.93
High blood pressure (grade)							
2	1	2.70%	0	0	0	0	0.52
3	36	97.30%	25	100%	23	100%	
Arterial hypertension (grade)							
0	2	5.41%	3	12%	2	8.70%	
1	6	16.22%	5	20%	5	21.74%	0.82
2	14	37.84%	11	44%	7	30.43%	
3	15	40.54%	6	24%	9	39.13%	
Peripheral artery disease	11	29.73%	4	16%	6	26.09%	0.46
AMI before							
1	14	37.84%	17	68%	9	39.13%	0.13
2	4	10.81%	3	12%	6	26.09%	
3	2	5.41%	1	4%	1	4.35%	
Smoking	7	18.92%	2	8%	4	17.39%	0.78
Family history of CVD	19	51%	11	44%	10	43.48%	0.78
Debut angina pectoris (months)	71.9 ± 70.9	6–300	62.9 ± 79.2	5–240	76.9 ± 59.4	6–240	0.11
up to 5 years	23	62.2%	16	64%	12	52.2%	
6–10 years	8	21.6%	4	16%	8	34.8%	
>10 years	6	16.2%	5	20%	3	13.04%	

End of table 2

	Group 0 (n = 37)		Group 1 (n = 25)		Group 2 (n = 23)		p
Functional class of angina pectoris							0.85
3	32	86.49%	23	92%	21	91.30%	
4	3	8.11%	2	8%	1	4.35%	
painless	1	2.70%	0	0	1	4.35%	
NYHA heart failure functional class							0.24
2	29	78.38%	24	96%	21	91.30%	
3	3	8.11%	0	0	0	0	
TC (mmol/L)	4.49 ± 1.08	2.6–6.46	4.5 ± 1.24	3.05–7.69	4.6 ± 1.49	2.3–8.3	0.99
VLDL (mmol/L)	0.96 ± 0.35	0.41–1.65	0.94 ± 0.5	0.44–1.92	0.77 ± 0.3	0.26–1.34	0.30
HDL (mmol/L)	2.7 ± 0.89	1.34–4.44	2.58 ± 1.05	1.35–4.4	2.7 ± 1.26	0.76–5.63	0.90
LDL (mmol/L)	0.98 ± 0.31	0.62–1.73	1.16 ± 0.37	0.65–1.63	1.13 ± 0.29	0.56–1.58	0.20
TG (mmol/L)	2.04 ± 0.68	0.84 ± 3.59	1.69 ± 0.72	0.82–3.35	1.63 ± 0.62	0.57–2.92	0.07
AC	4.07 ± 1.32	2.1–6	3.27 ± 1.9	1.4–7.1	4.5 ± 5.2	1.3–24.4	0.3
Creatinine (mmol/L)	0.09 ± 0.02	0.06–0.1	0.09 ± 0.02	0.06–0.15	0.08 ± 0.02	0.06–0.1	0.05
CRP (mg/L)	5.9 ± 11.07	1–59.3	2.56 ± 1.96	0.7–7.7	5.3 ± 5.38	1–24.4	0.06
GFR (mL/min) CKD-EPI	79.89 ± 15.8	49–116	77.6 ± 16.03	42–111	84.2 ± 17.26	49–127	0.05
EuroScoreII	1.3 ± 0.68	0.7–3.6	1.07 ± 0.47	0.55–2.54	1.02 ± 0.4	0.5–1.85	0.24
Nitrates (took)	21	56.76%	11	44%	10	43.48%	0.49
Diuretics	5	13.51%	10	40%	3	13.04%	0.07
ACE inhibitors	18	48.65%	9	36%	16	69.57%	0.06
ARBs	5	13.51%	3	12%	4	17.39%	0.86
bETA-2 blockers	35	94.59%	20	80%	23	100.00%	0.05
CCB	5	13.51%	6	24%	4	17.39%	0.57
Antiplatelet drugs	18	48.65%	12	48%	9	39.13%	0.75
Statins	27	72.97%	16	64%	15	65.22%	0.7
Anticoagulants	6	16.22	0	0	2	8.7%	0.1

Note. BMI – body mass index; AMI – acute myocardial infarction; CVD – cardiovascular diseases;; TC – Total cholesterol; VLDL – very low-density lipoprotein; HDL – high-density lipoproteins; LDL – low density lipoproteins; TG – Triglycerides; AC – atherogenic coefficient; CRP – C-reactive protein; GFR – glomerular filtration rate; ACE inhibitors – angiotensin-converting-enzyme inhibitors; ARBs – angiotensin II receptor blockers; CCB – calcium channel blockers.

The 6-minute walk test (6MWT) was performed according to standard method.

An angiographic examination (coronarography) was performed under local (S. Lidicaini / S. Novocaini 0.5% – 30.0 mL) and intravenous anesthesia (S. Propofoli 1% – 200 mL) according to the Seldinger technique. Condition of coronary arteries was assessed in standard positions.

The SYNTAX Score was used to assess coronary artery disease severity. Risk stratification was evaluated based on summation of points obtained from this scale: low-risk group – 0–22 points, intermediate risk group – 23–32 points, high-risk group – >32 points.

Quality of life parameters were assessed using specialized questionnaires: SF-36, Minnesota Questionnaire, Seattle Questionnaire.

CABG surgery was performed based on the standard procedure in accordance with the Heart Team decision.

Bone marrow exfusion was performed in the operating room, in the position of the patient lying on the back, under general anesthesia to the skin incision from the sternum; bone marrow 140 mL in Teruflex 450/400 blood preservation bags (Terumo). Heparin 5000 U/100 mL of 0.9% NaCl solution was used as stabilizer. Thus, in one syringe, 7 mL of bone marrow had 3 mL of 0.9% NaCl solution and 150 units of heparin (15 units/mL).

Mononuclear fraction was isolated by hydroxyethyl starch precipitation in a density gradient.

Sequential removal of fatty inclusions, plasma, and red blood cells was performed using plasma extractor.

Before transplantation, additional mononuclear fraction filtration was carried out using a standard blood transfusion system and blood substitutes with a nylon liquid microfilter with minimum possible cell diameter of 200 μm.

Cell composition of mononuclear fraction was estimated using cytofluorimetry in a specialized laboratory.

Intragraft injection of ABMMCs was performed after application of distal anastomoses of 5 mL per bypass graft, 15-minute exposure.

Intramyocardial transplantation was carried out transepicardially at 0.2 mL per 1 cm^2 (1 mL divided into 5 points) – a total of 10 points in the blood supply pool of the left coronary artery from proximal to the distal sites.

A BD Miro-Fine Plus insulin syringe – 0.30 mm (30G) × 8 mm – was used for intramyocardial administration of both ABMMCs and 0.9% NaCl solution.

Data obtained were processed using the STATISTICA software for Windows program (version 7.0). Calculations were made taking into account exclusion of omissions, which were not considered when drawing up conclusions.

The normal/non-normal distribution was initially estimated to determine the method for statistical processing of obtained data. Nonparametric statistics methods were used to analyze data having a non-normal distribution.

Quality indicators were calculated using the Pearson (chi-squared) test and Fisher's exact test.

Quantitative indicators were analyzed using the Kruskal–Wallis and Wilcoxon tests.

Factor analysis was used to identify certain severity factors and study data homogeneity.

Discriminant analysis was performed to investigate the leading characteristics defining differentiation between the groups.

The model for analysis of variance of dependent samples – Repeated Measures ANOVA – was used for variance analysis, taking into account various factors.

A p-value less than 0.05 was used to assess the statistical significance of findings.

RESULTS

I. Safety assessment for additional ABMMC transplantation

ABMMC transplantation in combination with CABG surgery with CPB in patients with coronary and heart failure is a safe technique [18].

II. Efficacy evaluation

Systolic and diastolic myocardial function parameters, the state of heart valve based on echocardiography that was carried out 12 months after treatment were comparable and did not have statistically significant differences.

Similar data were obtained in analysis of left ventricular diastolic function indicators.

Dynamics of the treadmill test data tended to improve in the observation groups without statistically significant differences among the observation groups ($p = 0.7$). Ischemic changes were detected in 2 cases of group 0.

6MWD showed significant dynamics of increase in the number of meters covered in groups 1 and 2 compared with group 0 ($p = 0.03$) (Fig. 2).

During control examination, ECG daily monitoring data showed ischemic changes in 2 cases from group 0. No rhythm disorders were observed in the observation groups.

Functional tests after 12 months revealed class II-angina in 8 cases only in group 0 ($p = 0.001$).

Recurrent angina pectoris was noted in 8 group 0 patients. The average recurrence period was 6.5 ± 2.9 (1–11) months.

A decrease in heart failure functional class (diastolic) was observed in the observation groups without significant differences ($p = 0.13$).

There were improvements in quality of life parameters in observation groups, according to SF-36, Seattle and Minnesota questionnaires, without significant differences.

The percentage of functioning bypass grafts after a 12-month follow-up period was 87.6% (initially 113 superimposed bypass grafts, 99 were tracked over time) in group 0. In group 1, this ratio was 96.2% (79 and 76), and in group 2 – 97.3% (75 and 73) ($p = 0.048$).

In the presence of non-functioning bypass grafts, ischemic changes according to stress tests, as well as symptoms of at least class-II angina pectoris, native coronary arteries were stented. In group 0 – in 5 cases (13.5%) and in group 1 – in one case (4%) ($p = 0.38$).

Speckle tracking did not have significant differences in the observation groups at the control points of examination.

III. Prediction of treatment outcomes (influence of several parameters)

Analysis demonstrated that smoking has significant effect on 6MWD results – smaller distance was covered in the observation groups ($p = 0.048$).

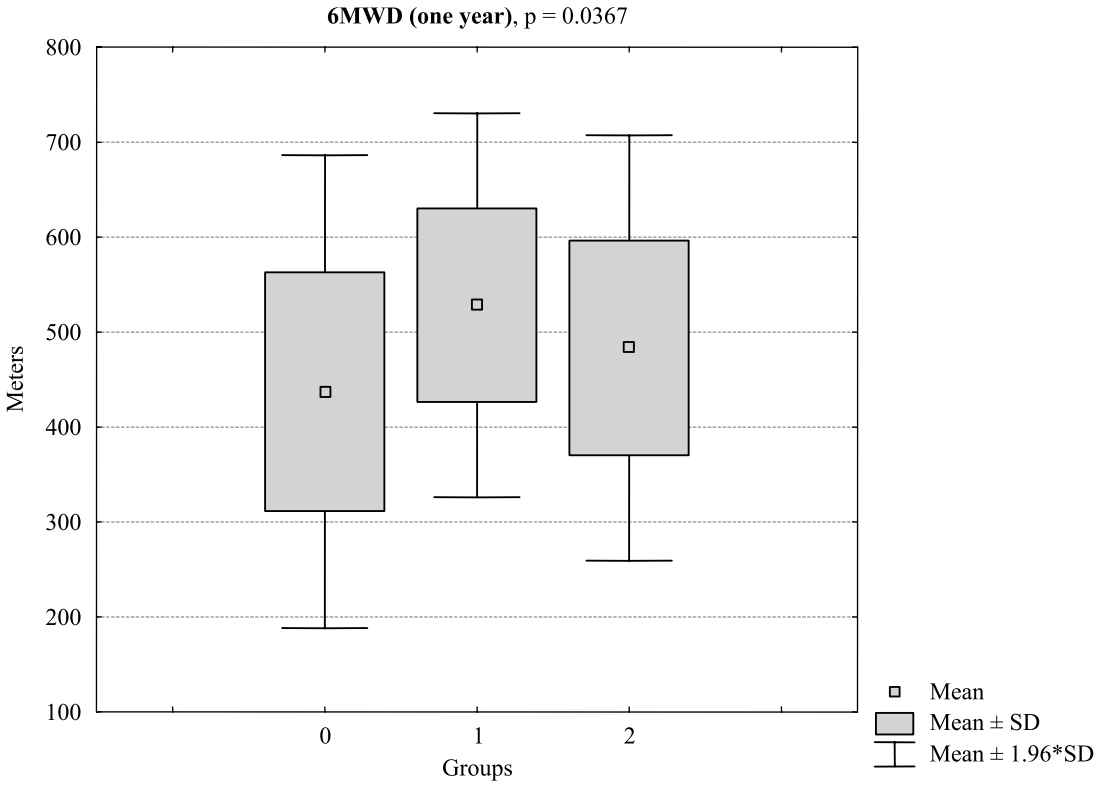
The effect of the initial left ventricular ejection fraction (LVEF) on dynamics of 6MWD parameters and the combined effect of initial LVEF and pulmonary artery pressure on the dynamics of 6MWD indicators were also noted.

The cell composition of obtained mononuclear fraction did not have statistically significant differences in the observation groups (Table 3).

When comparing ABMMC intramyocardial and/or intracoronal transplantation techniques, no statistically significant differences between the groups were found.

DISCUSSION

TAMIS (Autologous Bone Marrow Mononuclear Cells in the Combined Treatment of Coronary Heart Disease) is a single-center, randomized, double-, placebo-controlled trial. The aim of the study was to evaluate the safety, efficacy, and the influence of various factors on the overall effect of transplanted ABMMCs in the surgical treatment of coronary heart disease. This work was carried out after many years of research in the field of cell/regenerative medicine. Previously performed studies in groups of patients in the treatment of chronic IHD, both in isolation and in combination with surgical treatment (angioplasty and stenting, CABG), as well as in



	Initially		1 year later	
Group 0	315.06 ± 17.6	279.9–350.2	433.54 ± 20.6	392.2–474.9
Group 1	319.8 ± 24.5	270.8–368.8	524.4 ± 28.7	466.8–581.9
Group 2	329.9 ± 25.3	279.4–380.5	452.7 ± 29.7	393.3–512.1

Fig. 2. Dynamics of the number of meters covered during the 6-min walk test (6 MWT) in the observation groups

* – Avg. value ± St. Dev./interval

Table 3

Cellular composition of the mononuclear fraction obtained via flow cytometry

	Group 1 n = 25		Group 2 n = 23	
Total cytosis (10^8/L)*	20.3 ± 8.9	1–84.5	6.8 ± 3.2	2–12
(%)*	21.04 ± 2.9	16.09–28	20.0 ± 2.7	15–25
CD34+ (%)*	1.16 ± 0.22	0.9–1.57	1.18 ± 0.34	0.8–1.7
CD133+ (%)*	0.4 ± 0.08	0.3–0.5	0.38 ± 1.14	0.2–0.7

* – Avg. value ± St. Dev./interval.

the treatment of acute myocardial infarction (AMI) served as the basis for development of the design [19–23].

Positive outcomes were obtained in a separate observation group of patients with severe systolic heart failure (dilated cardiomyopathy) [24].

Preliminary calculations indicated the required capacity of the study.

Analysis of literature data pointed to some parameters that are integral to this kind of work. In particular, incidence of complications during such operations varies: 30 ± 10%. According to the formula:

$$n_1 = kn_2,\ n_2 = \frac{(z_\alpha + z_\beta)^2}{(\varepsilon - \delta)^2}\left[\frac{p_1(1-p_1)}{k} + p_2(1-p_2)\right]$$

Let's denote by n_1 the sample size, n_2 – the sample size in the control group. In the test group, there are k = 2/3 times more observations than in the control. Difference between the test and control frequencies $\varepsilon = p_1 - p_2$ [25].

So, with $p_1 = 0.2$, $p_2 = 0.4$, $\delta = 0.1$, we get $n_1 = 49.5$, $n_2 = 33$. In practice, the size of the control group was 36, the two test groups were 25 + 25 = 50.

The null hypothesis is that the frequency of complications in the test sample does not exceed the frequency in the control sample with equivalence limit δ, that is, Ho: $p_1 < p_2 + \delta$. The value p_1 can be $< p_2 + \delta$ by any value. An alternative hypothesis means that the frequency of complications in the test sample is significantly higher

than the control. Accordingly, these statistical indicators are significant.

In overall, this work has indicated an increase in the effectiveness of combined treatment of IHD by combining ABMMC transplantation and CABG surgery with CPB. In addition, common predictors of adverse outcomes, which were not previously considered as such, were identified.

Statistical analysis showed no significant differences in echocardiography data, both in relation to systolic and diastolic myocardial function. There was general tendency towards improvement of these indicators to a more significant degree in groups 1 and 2 without statistical significance in comparison with the control group.

Between observation group and control group, previous studies have found no significant differences in systolic and diastolic myocardial functions [26–29].

With this in mind, Doppler ultrasonography (speckle tracking) was included in the study design to improve the quality of the data obtained. The method showed significant changes in local myocardial contractility on the 7–14th day (average 10th day) of postoperative period. These changes were accompanied by reduced left ventricular ejection fraction (LVEF) and increased left ventricular linear and volumetric indicators (Fig. 3).

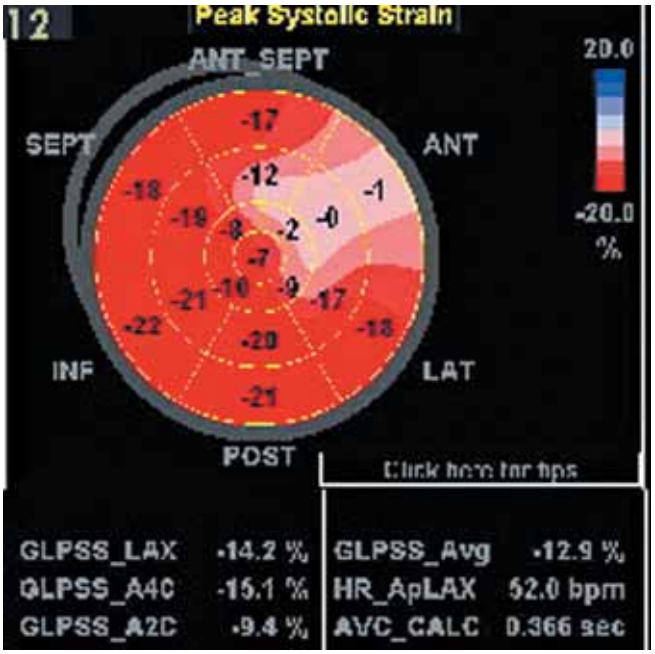
However, discriminant analysis showed that these changes were not significant.

At the same time, there was no significant increase in markers of myocardial injury (troponin I, CPK-MB, myoglobin), and ECG data were nonspecific. After a 12-month follow-up period, speckle tracking echocardiography was able to confirm improvement in revascularization areas. No statistically significant differences in echocardiography parameters were obtained either.

This pattern was directly associated with surgical intervention (with CPB) and reperfusion of previously ischemic myocardial segments (hibernation and stanning zones). Different volume of these segments, peculiarities of the individual anatomical structure of coronary arteries, and differences in surgical revascularization could lead to insignificant echocardiography results.

A step-by-step analysis of results obtained noted that left ventricular (LV) diastolic dysfunction can be a factor influencing treatment results, that is, the functional viability of bypass grafts (Table 4).

Initially



14th day of postoperative period

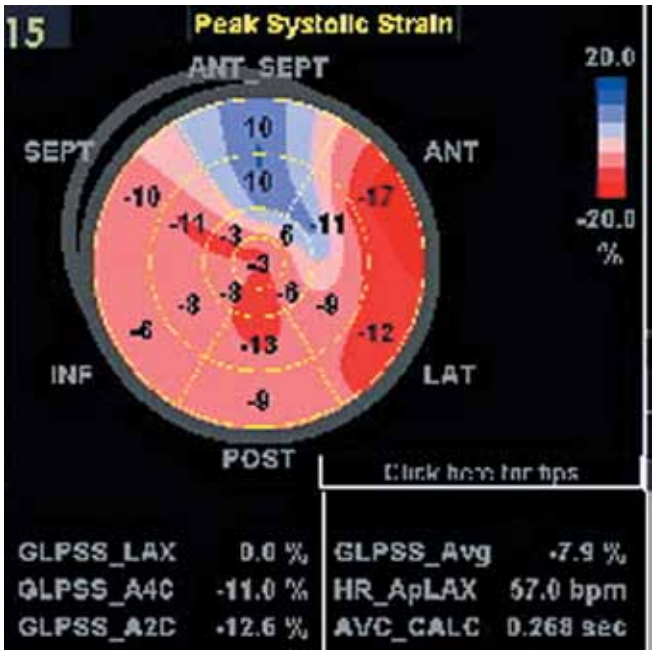


Fig. 3. Dynamics of speckle tracking echocardiography (initially and on the 14th day of postoperative period)

Table 4

Effect of diastolic myocardial dysfunction on the functional viability of bypass grafts after CABG surgery (echocardiography)

	Group 0	Group 1	Group 2
Initial number of patients with LV diastolic dysfunction (n)	19	16	15
Presence of LV diastolic dysfunction after 1 year of follow-up (n)	15 (78.9%)	7 (43.7%)	3 (20%)
Number of patients with non-functioning bypass grafts / with LV diastolic dysfunction after 1 year of observation (n)	10/8	1/0	2/1

During control examination in group 0, LV diastolic dysfunction remained in 78.9% of cases, 43.7 and 20% in groups 1 and 2, respectively. At the same time, LV diastolic dysfunction remained in 9 out of 13 patients with non-functioning bypass grafts, that is, 69.2% ($p = 0.04$).

Aggregate results confirmed the effect of LV diastolic dysfunction on duration of bypass graft functional viability, regardless of the type of surgery performed.

In this case, influence of LV diastolic dysfunction on the integral indicator – functional viability of bypass grafts – manifested through increased elasticity, decreased myocardial stiffness, decreased number of ischemic segments, and increased LV ejection fraction.

Previous studies on the use of ABMMCs have shown that myocardial diastolic function improved [30–31], but none of the studies reported a relationship with the functional viability of coronary bypass grafts.

Quality of life assessment was carried out comprehensively using the SF-36, Minnesota, and Seattle questionnaires. The main purpose of using all these questionnaires was to obtain reliable and comprehensive results. Statistical analysis did not show significant differences in the observation groups. The results are linked to possible error of subservience. This assumption was made, given that all patients were instructed to strictly abide by the prescribed recommendations (lifestyle, drug therapy, frequency of dynamic and follow-up examinations) at all stages of work. In case of deviation from these guidelines, patients were warned of exclusion from further research.

Probably, in order to obtain adequate data regarding quality of life parameters, it was necessary to identify patients who responded to therapy (responders) and did not respond (non-responders).

Cell substrate analysis and ABMMC administration method analysis also showed no statistically significant benefits. These results were associated with insufficient sampling required to calculate the indicated differences. Besides, the observation period of 12 months established by our study design was determined to be insufficient to obtain information on significant difference in the effectiveness of a particular cellular material delivery method.

Absence of statistically significant differences in the data, which previously confirmed the significant influence of ABMMCs on overall effectiveness of treatment, is most likely attributable to the small size of the sample, which makes it impossible to detect small but still significant differences. In addition, since this study was randomized, significant selective sampling was conducted in order to form equally-weighted groups as much as possible. The selection criteria may have been softer in earlier studies. Despite the probably small size of sample, we, nevertheless, obtained new results that were not previously reflected in any study. These findings have more significant implications for subsequent research in this section of scientific medicine.

CONCLUSIONS

ABMMC transplantation along with CABG surgery can boost the overall effectiveness of combination therapy for ischemic heart disease. It was noted that compared with the control group, the observation groups with additional transplantation of indicated type of cellular material achieved significant difference – higher exercise tolerance (6MWD), lower exertional angina functional class, and better bypass graft functional viability. The study showed the need for stage-by-stage analytical calculations with the aim of possible correction of further work.

The authors declare no conflict of interest.

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The article was submitted to the journal on 26.08.2019