Long-term clinical results of autologous bone marrow CD 133+ cell transplantation in patients with ST-elevation myocardial infarction

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Long-Term Clinical Results of Autologous Bone Marrow Cd 133+ Cell Transplantation in Patients with St-Elevation Myocardial Infarction

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Abstract. The aim of the study was investigate the long-term results of autologous bone marrow CD 133+ cell transplantation in patients with primary ST-Elevation Myocardial Infarction (STEMI). Methods and results: From 2006 to 2007, 26 patients with primary STEMI were included in an open randomized study. Patients were randomized to two groups: 1st - included patients underwent PCI and transplantation of autologous bone marrow CD 133+ cell (n = 10); 2nd - patients with only PCI (n = 16). Follow-up study was performed 7.70±0.42 years after STEMI and consisted in physical examination, 6-min walking test, Echo exam. Total and cardiovascular mortality in group 1 was lower (20% (n = 2) vs. 44% (n = 7), p = 0.1 and 22% (n = 2) vs. 25% (n = 4), (p=0.53), respectively). Analysis of cardiac volumetric parameters shows significant differences between groups: EDV of 100.7 ± 50.2 mL vs. 144.40±42.7 mL, ESV of 56.3 ± 37.8 mL vs. 89.7 ± 38.7 mL in 1st and 2nd groups, respectively. Data of the study showed positive effects of autologous bone marrow CD 133+ cell transplantation on the long-term survival of patients and structural status of the heart.

INTRODUCTION

In the last decade, significant progress has been achieved in the treatment of acute myocardial infarction (AMI) with the timely implementation of thrombolytic therapy and early percutaneous coronary intervention (PCI) leading to reduction in mortality from AMI and improved long-term prognosis in these patients. Primary tasks of modern cardiology consist in recanalization of the infarct-related coronary artery (IRCA) and restoration of the myocardial perfusion in patients with AMI. However, novel reperfusion strategies have exhausted their ability to limit the area of myocardial necrosis. Death of cardiomyocytes, arterioles, and capillaries in the myocardial infarction area is irreversible and subsequently results in the formation of scar tissue [1]. Following remodeling of the left ventricle leads to progressive dilatation and disruption of its geometry, which represents morphological substrate of chronic heart failure.

Some time ago, a series of clinical randomized trials were conducted to determine the efficacy and safety of intracoronary infusion of autologous bone marrow cell with various phenotypes including CD133+ bone marrow cell in AMI [2-5].

We conducted a study on the clinical efficacy of autologous bone marrow CD 133+ cell transplantation, short-term results have been published previously [6].

The aim of the of this study was investigate the long-term results of autologous bone marrow CD 133+ cell transplantation in patients with primary ST-Elevation Myocardial Infarction (STEMI).

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MATERIALS AND METHODS

Study Design and Population

A total of 26 patients (pts) with primary STEMI, admitted to the Coronary Care Unit of Research Institute for Cardiology from 2006 to 2007, were included in the open randomized study registered under the title ESTABOMA at http://www.clinicaltrials.gov (trial number NCTO1748383). Inclusion criteria were age 18 to 75 years, primary STEMI, reperfusion time of the IRCA more than 4 h, and admission at the Coronary Care Unit during the 24 hours after onset of STEMI. Randomization procedure conducted by the envelope method after obtaining the written confirmation of patient's consent to participate in the study. Pts were randomized to two groups: group 1 - pts who underwent PCI and autologous bone marrow CD 133+ cells transplantation at day 16±6 after of STEMI (n = 10); group 2 comprised pts with only PCI at day 11±10 of after STEMI (n = 16). Both groups were comparable of baseline characteristics (Tab. 1).

TABLE 1. Baseline patient characteristics

Characteristics	**group 1** **M±SD, n, %**	**group 2** **M±SD, n, %**	**p**
Number of pts	10	16	
Mean age	60.31 ± 12.24	58.42±10.41	0.96
Male/female	6 (60)/4 (40)	13 (81)/3 (19)	0.37
Anterior myocardial infarction	10 (100)	16 (100)	-
Reperfusion time of the IRCA, h	5.25 ±0.70	4.90±0.61	0.20
Risk factors for heart disease			
Hypertension	8 (80)	11 (69)	0.66
Smoking	5 (50)	4 (25)	0.35
Family history	3 (30)	10 (62)	0.65
Obesity	3 (30)	10 (62)	0.51
Diabetes	2(20)	2 (12)	0.68
Previous events			
Experience stable angina No 1 year 3 year more than a 3 year	 6(60) 2(20) 0(20) 2(20)	 10(62) 3(19) 3(19) -	 0.41
Preinfarction angina	4(40)	5 (31)	0.11
PCI			
Primary PCI	3(33)	7(44)	0.10
Deferred PCI	6(60)	9(56)	0.45
IRCA LAD	10(100)	16(100)	-
Coronary artery lesion 1-/2-/3 - vascular disease	3(30)/5 (50)/1(10)	-/6(37)/4(25)	0.09
Complications of AMI			
Acute heart failure, functional class I /II /III /IV	9 (90)/1 (10)/0/0	7 (44)/9(56)/0/0	0.06
Postinfarction angina	1 (10)	1 (6)	0.63
Repeated myocardial infarction	-	1 (6)	0.62
Dressler syndrome	-	2 (12)	0.50
New arrhythmias	6 (60)	7 (44)	0.53
Ventricular fibrillation	-	1 (6)	0.60
Thrombosis ofthe left ventricle	3 (30)	3 (19)	0.45
Left ventricularaneurysm	6 (60)	10 (62)	0.61
Pericarditis	-	4 (25)	0.14

IRCA - infarct-related coronary artery, PCI - percutaneous coronary intervention LDA – left descending artery.

Isolation of Cells

Puncture of the anterior superior iliac spine under local anesthesia conducted in patients of groups 1 4–5 hours before the transplantation. Bone marrow aspirate (100 mL) were acquired into two 60-mL syringes containing 10 mL of sterile saline and 25000 IU of heparin. After that, autologous CD133+ bone marrow cells were isolated by the method of gradient centrifugation (density gradient Histopaque-1077) and separated from erythrocytes, thrombocytes, and granulocytes. We allocated 5–10 • 10^6 cells. Cellular phenotyping was performed by the method of flow cytofluorometry (FACSCalibur, Biosciences, USA).

Magnetic labeling with magnetic microgranules CD133 MicroBead (Miltenyi Biotec GmbH, Germany) were carried out to isolate CD133+ BMC. CD133+ progenitor cells labeled using a hapten-conjugated monoclonal primary antibody and anti-hapten antibody bound to MACS MicroBead microgranules. Positive magnetic separation were conducted in the separation column in a magnetic field with the device MidiMACS. The purity of cell populations and their viability will be assessed by flow cytofluorometry after immunofluorescent staining with specific CD133/2 (AC141)-PE dye and vital dye 7-AAD by using BD FACSCalibur device (USA). Cell viability rate was 98-99%. Suspension of 5–10 • 10^6 autologous CD133+ BMC in 1-mL of heparinized solution (20 U of heparin in 1 mL) were prepared for transplantation. Intracoronary infusion of autologous CD133+ BMC were performed by the method of passive passage to the IRA at a rate of 4–8 mL/min.

Follow-up and Outcomes

A follow-up study was performed 7.70±0.42 years after STEMI and consisted in of a physical examination, exercise tolerance assessment by 6-min walk test (6MWT), Echocardiography exam (VIVID 7, GE).

Death, repeated myocardial infarction (RMI), unstable angina, chronic heart failure (CHF) ≥class II NYHA, and stroke were endpoints.

Safety endpoints of autologous bone marrow CD 133+ cell transplantation: clinically significant cardiac arrhythmias, including life-threatening arrhythmias, new oncology diseases.

Ethics

The protocol of the study was approved by the local Ethics Committee (protocol № 27 of November 22, 2003 meeting of the Committee on Biomedical Ethics of Federal State Budgetary Scientific Institution “Research Institute for Cardiology”, Tomsk, Russian Federation). The study complied with the Declaration of Helsinki Guidelines. Written informed consent was obtained from all patients.

Statistical Analyses

Data are presented as n (%), mean ± SD. Mann-Whitney U-test was used as a non-parametric test. Qualitative parameters were analyzed by use of the Pearson's chi-squared test and Fisher's exact test. Survival curves were calculated by Kaplan-Meier analysis and compared using the Cox's F-Test. Two-sided P-values were considered significant when if <0.05. Calculations were performed using the Statistica for Windows version 10.0 (Stat Soft, Inc., USA).

RESULTS

All pts underwent a 7-year follow-up according to the study protocol. Information about vital status and clinical course of cardiovascular disease was obtained from medical documentation (clinical records and patient cards), telephone interviews, and questionnaires. In case of death, information was collected from death certificates and postmortem study protocols. Vital status information of 23 pts (88%) including 8 pts of group 1 and 15 pts of group 2 was obtained. During a median follow-up period of 7.70 years, 9 pts (22%) died. Total incidence of deaths in group 1 was lower compared with that in group 2 (2 pts (20%) vs. 7 pts (44%), p = 0.06, respectively), (Fig. 1).

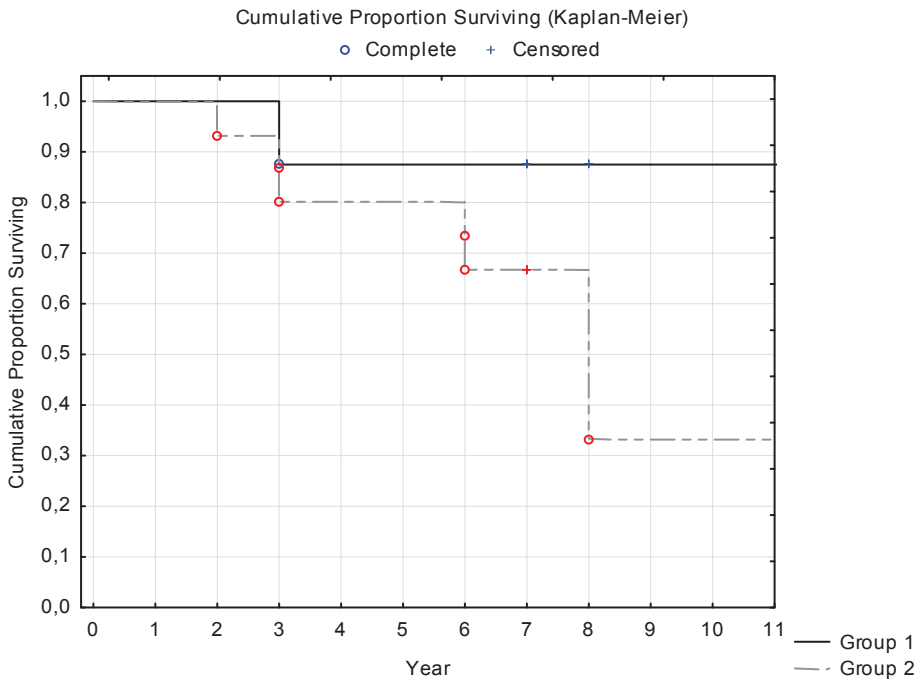

FIGURE 1. Kaplan-Meier survival estimates for cause of death (Cox's F-Test, p = 0.11)

Cardiovascular deaths occurred in 2 cases (20%) in group 1 vs. 4 cases (25%) in group 2. Non-cardiovascular deaths occurred in 2 cases in group 2 (12%). Cause of death of 1 pts (6%) from in group 2 was unknown (Table 2).

Fourteen pts (54%) underwent a follow-up exam 7.70±0.42 years after STEMI. The results are shown in Table 2. At a 7-year follow-up the mean age of pts in group 1was 60.31 ± 12.10 years, the 2nd group– 60.92 ± 9.61 years. Both groups were comparable of age (p = 0.86).

TABLE 2. Outcomes and safety for 7 years, n (%)

Outcome events	**Group 1 (n=10), n, %**	**Group 2 (n=16), n, %**	**p**
Cardiovascular death	2 (20)	4 (25)	0.53
Non-cardiovascular death	-	2 (12)	-
The cause is of death is unknown	-	1 (6)	-
Total incidence of deaths	2 (20)	7 (44)	0.09
Repeated myocardial infarction	-	8 (50)	-
Unstable angina	2 (20)	11 (69)	0.049
Stroke	-	-	-
Chronic heart failure ≥class II	2 (20)	8(50)	0.06
Stable angina ≥class II	1 (10)	2 (12)	0.54
Repeated PCI	2 (20)	3 (19)	0.65
Aneurysmectomy	-	-	-
Pacemaker implantation	1 (10)	-	-
Correction of heart valve disease	-	1 (6)	-
CABG	-	1(6)	0.62
Safety endpoints			
New arrhythmias	2 (20)	1 (6)	0.38
Oncology diseases	-	1(6)	-

Analysis of stroke incidence rates, stable angina ≥class II, the frequency of heart surgery (repeated PCI, aneurysmectomy, pacemaker implantation, correction of heart valve disease, CABG) did not show significant differences between groups.

CHF ≥class II was found more often in group 2: 2 cases (20%) vs. 8 cases (50%) in group 2, (p = 0.06). However, no differences in exercise tolerance (6MWT: 468.3±83.3 m in group 1 vs. 437.5±113.9 m in group 2, p=0.24) between groups were found.

In group 2, 8 cases (50%) of recurrent myocardial infarction were recorded, and 4 were fatal. While repeated MI was not registered in patients in group 1. During the entire period of the follow-up study, unstable angina were more in the group 2 (11 (69%) in the group 2 to 2 (20%) in the group 1, p = 0.049).

In addition, we assessed mean change from baseline in left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic index (LVEDI), left ventricular end-systolic index (LVESI), left ventricular ejection fraction (LVEF) and wall motion score index (WMSI). At a 7-year the following Echo parameters was significantly lower in 1st group as compared group 2: LVEDI 51.41±21.22 mL/m² vs. 75.43±18.14 mL/m², p=0.03, LVEDV 100.74±50.21 ml vs. 144.40±42.72 ml, LVESV 56.31±37.81 vs. 89.73±38.76, p=0.049. Analysis of other Echo parameters did not show significant differences between the groups: LVESI was 31.16±16.78 mL/m² vs. 46.31±16.85mL/m², p=0.19, LVEF 46.83±9.00 % vs. 39.15±9.81 %, p=0.17, WMSI 1.46±0.43 vs. 1.72±0.41, p=0.40 in group 1 and group 2, respectively. Note that mean values of LVESI, WMSI were lower in the group. While the LVEF was higher in group 1. However, due to the small number of patients we could not find statistically significant differences between groups.

TABLE 3. Echo parameters for 7 years, M±SD

Echo parameters	**Group 1 (n=10), M±SD**	**Group 2 (n=16), M±SD**	**p**
Left ventricular end-diastolic volume, ml			
Baseline	111.00±25.51	125.91±48.92	0.67
After 7years	100.74±50.21	144.40±42.72	0.049
% Change LVEDV after 7 years	-2.93±31.87	38.51±45.72	0.11
Left ventricular end-systolic volume, ml			
Baseline	54.22±23.93	68.31±32.11	0.24
After 7years	56.31±37.81	89.73±38.76	0.049
% Change LVESV after 7 years	16.50±32.22	74.31±90.65	0.27
Left ventricular end-diastolic index, ml/m^2			
Baseline	51.9±15.2	58.5±11.6	0.37
After 7years	51.41±21.22	75.43±18.14	0.03
% Change LVEDI after 7 years	1.2±32.9	49.8±27.2	0.11
Left ventricular end-systolic index, ml/m^2			
Baseline	31.71±5.57	35.00±11.22	0.63
After 7years	31.16±16.78	46.31±16.85	0.19
% Change LVESI after 7 years	-11.3±51.7	74.4±55.3	0.22
Left ventricular ejection fraction, %			
Baseline	52.51±13.13	47.55±9.43	0.29
After 7years	46.83±9.00	39.15±9.81	0.17
% Change LVEF after 7 years	-11.60±18.71	-18.81±15.73	0.67
Wall motion score index			
Baseline	1.61±0.43	1.84±0.31	0.21
After 7years	1.46±0.43	1.72±0.41	0.40
% Change WMSI after 7 years	15.23±50.51	28.55±40.28	0.85

The safety parameters determined by study groups did not different. During the reporting period, the frequencies of clinically significant cardiac arrhythmias was not significantly different in both groups (20% (2) to 6% (1), p =0.38). Life-threatening arrhythmias and new oncology diseases were not registered in group 1.

CONCLUSION

The first short- and mid-term results most of previous trials have shown safety and efficacy of CD133+ cell transplantation in patients with AMI [4-9]. However, the unit of previous experimental studies have demonstrated that transplanted progenitor cells may provide the substrate for electrical instability, leading to fatal arrhythmia [10-12]. In addition, cases of myocardial calcification after intramyocardial injection in acute myocardial infarction were identified of experimental works [13]. Our study indicated that CD133+ cell transplantation was safe. We have not found calcification infarction, life-threatening arrhythmias and new oncology diseases patients exposed autologous bone marrow CD 133+ cell transplantation. We installed the tendency to statistically significant differences in overall mortality and the incidence of CHF ≥class II in favor of a group 1. In addition, patients of group 1 had significantly less unstable angina. For the parameters of the left ventricle, we observed a positive effect of transplantation on the left ventricular end-diastolic and end-systolic volumes during the follow-up period. Thus, results of the study showed positive effects of autologous bone marrow CD 133+ cell transplantation on the long-term survival of patients, clinical course of coronary heart disease and structural status of the heart. We believe that clinical trials with more patients in this direction are promising.

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