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Functional recovery of patients with ischemic cardiomyopathy treated with coronary artery bypass surgery and concomitant intramyocardial bone marrow mononuclear cell implantation – A long-term follow-up study

Funkcionalni oporavak bolesnika sa ishemijskom kardiomiopatijom lečenih implantacijom mononuklearnih ćelija koštane srži tokom aortokoronarne bajpas hirurgije

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Abstract

Background/Aim. Intramyocardial bone marrow mononuclear cells (BMMNC) implantation concomitant to coronary artery bypass grafting (CABG) surgery as an option for regenerative therapy in chronic ischemic heart failure was tested in a very few number of studies, with not consistent conclusions regarding improvement in left ventricular function, and with a follow-up period between 6 months and 1 year. This study was focused on testing of the hypothesis that intramyocardial BMMNC implantation, concomitant to CABG surgery in ischemic cardiomyopathy patients, leads to better postoperative long-term results regarding the primary endpoint of conditional status-functional capacity and the secondary endpoint of mortality than CABG surgery alone in a median follow-up period of 5 years. **Methods**. A total of 30 patients with ischemic cardiomyopathy and the median left ventricular ejection fraction (LVEF) of 35.9 ± 4.7% were prospectively and randomly enrolled in a single center interventional, open labeled clinical trial as two groups: group I of 15 patients designated as the study group to receive CABG surgery and intramyocardial implantation of BMMNC and group II of 15 patients as the control group to receive only the CABG procedure. All the patients in both groups received the average of 3.4 ± 0.7 implanted coronary grafts, and all of them received the left internal mammary artery (LIMA) to the left anterior descending (LAD) and autovenous to other coronaries. **Results.** The group with BMMNC and CABG had the average of 17.5 ± 3.8 injections of BMMNC suspension with the average number of injected bone marrow mononuclear cells of 70.7 ± 32.4 × 10^6 in the total average volume of 5.7 ± 1.5 mL. In this volume the average count of CD34+ and CD133+ cells was 3.96 ± 2.77 × 10^6 and 2.65 ± 1.71 × 10^6, respectively. All the patients were followed up in 2.5 to 7.5 years (median, 5 years). At the end of the follow-up period, siginificantly more patients from the group that received BMMNC were in the functional class I compared to the CABG only group (14/15 *vs* 5/15; $p = 0.002$). After 6 months the results on 6-minute walk test (6-MWT) were significantly different between the groups (435 m in the BMMNC and CABG group and 315 m in the CABG only group; $p = 0.001$), and continued to be preserved and improved on the final follow-up (520 m in the BMMNC and CABG group *vs* 343 m in the CABG only group; $p < 0.001$). Cardiovascular mortality was also significantly reduced in the BMMNC and CABG group ($p = 0.049$). **Conclusion**. Implanatation of BMMNC concomitant to CABG is a safe and feasible procedure that demonstates not only the improved functional capacity but also a reduced cardiac mortality in a 5-year follow-up in patients with ischemic cardiomyopathy scheduled for CABG surgery.

Key words:
coronary artery bypass; bone marrow transplantation; myocardium; intraoperative period; physical endurance; mortality.

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Apstrakt

Uvod/Cilj. Intramiokardna implantacija mononuklearnih ćelija koštane srži (MNĆKS) tokom hirurške revaskularizacije miokarda aortokoronarnim premošćivanjem (HRMAKP), kao pokušaj regenerativne terapije u lečenju hronične ishemijske srčane slabosti, testirana je u manjem broju kliničkih studija sa nekonzistentnim zaključcima po pitanju popravljanja funkcije leve komore i sa periodom praćenja najčešće od šest meseci do godinu dana. Primarni cilj studije bio je da pokaže bolji i dugotrajniji funkcionalni kapacitet za vežbanje bolesnika sa ishemijskom kardiomiopatijom (IK) operisanih kombinacijom implantacije MNĆKS i HRMAKP u odnosu na bolesnike operisane samo HRMAKP. Sekundarni cilj bio je da pokaže njihovo duže preživljavanje i kvalitetniji život u nižoj NYHA klasi u dugoročnom praćenju, prosečno pet godina posle operacije. **Metode**. Trideset bolesnika sa IK i srednjom ejekcionom frakcijom leve komore od 35,9 ± 4,7% bili su prospektivno i nasumično uključeni u otvorenu interventnu kliničku studiju jednog centra i podeljeni u dve grupe: prva grupa od 15 bolesnika označena kao studijska grupa i predviđena da dobije uz HRMAKP i implantaciju MNĆKS i druga grupa od 15 bolesnika, označena kao kontrolna grupa, kojoj je rađena samo HRMAKP. **Rezultati**. Svi bolesnici obe grupe dobili su prosečno 3,4 ± 0,7 aortokoronarnih graftova, i to svi po graft leve unutrašnje torakalne arterije na prednju silaznu međukomorsku granu leve koronarne arterije (LIMA-LAD) i po potrebi autovenske graftove na druge koronarne arterije. Grupa kojoj je rađena i implantacija MNĆKS imala je u proseku 17,5 ± 3,8 injekcija rastvora MNĆKS sa prosečnim brojem ubrizganih mononuklearnih ćelija od 70,7 ± 32,4 × 10^6 u totalnom prosečno ubrizganom volumenu od 5,7 ± 1,5 mL. Izračunato je da je prosečno ugrađeno 3,96 ± 2,77 × 10^6 CD34+ ćelija i 2,65 ± 1,71 × 10^6 CD133+ ćelija. Svi bolesnici su postoperativno praćeni od 2,5 do 7,5 godina (prosečno 5 godina). U vreme poslednje postoperativne kontrole statistički značajno veći broj bolesnika iz grupe HRMAKP uz intramiokardnu implantaciju MNĆKS nalazio se u NYHA I funkcionalnoj klasi u poređenju sa bolesnicima iz grupe kojoj je rađena samo hirurška revaskularizacija (14/15 *vs* 5/15; $p = 0.002$). Pređeno rastojanje izmereno šestominutnim testom hoda pokazalo je statistički značajnu razliku već na postoperativnoj kontroli posle šest meseci (435 m u grupi MNĆKS i HRMAKP u odnosu na 315 m u grupi HRMAKP; $p = 0{,}001$) i održavala se sve vreme praćenja i neznatno povećavala do poslednje kontrole (520 m u odnosu na 343 m; $p < 0{,}001$). Kardiovaskularni mortalitet bio je takođe statistički značajno manji u grupi lečenoj implantacijom MNĆKS uz HRMAKP nego u grupi tretiranoj HRMAKP ($p = 0{,}049$). **Zaključak**. Implantacija mononuklearnih ćelija koštane srži uz hirurške revaskularizacije miokarda aortokoronarnim premošćivanjem bezbedna je i izvodljiva procedura kojom se postiže bolji funkcionalni oporavak bolesnika i smanjuje kardiovaskularni mortalitet.

Ključne reči:
aortokoronarno premošćavanje; transplantacija kostne srži; miokard; intraoperativni period; sposobnost, fizička; mortalitet.

Introduction

Despite long time improvements in treating ischemic cardiomyopathy, disability and mortality rates remain high and 50% of the diagnosed, will die within 5 years [1]. Alongside of improved medical therapy and surgical and interventional revascularization, regenerative therapy emerged as one of promising additional options over the last decade [2–8]. One of the first clinically utilized options for regenerative attempts in myocardium was bone marrow aspirate mononuclear cells (BMMNC) intracoronary injections in the early postinfarctional recovery period for improvement in left ventricular function [9, 10]. Few smaller scale randomized studies have proved that intramyocardial application of BMMNC in ischemic cardiomyopathy is safe and feasible, however, the results in a short-term follow-up period of 6–12 months were different regarding improvement in ventricular function and patients condition [11–15].

The aim of this study was to test hypothesis that intramyocardial BMMNC implantation concomitant to coronary artery bypass grafting (CABG) surgery in ischemic cardiomyopathy patients leads to better postoperative results than CABG surgery alone, regarding the primary endpoint of patients functional capacity and secondary endpoint of cardiovascular mortality in the median follow-up period of 5 years.

Methods

Between June 2006 and April 2011, 30 consecutive patients with ischemic cardiomyopathy (at least one previous myocardial infarction) were scheduled for CABG surgery and planned for prospective interventional, single center, open labeled, randomized clinical trial. The patients were randomized into two groups: group I of 15 patients designated as the study group to receive CABG surgery and intramyocardial implantation of BMMNC and group II of 15 patients as the control group to receive only CABG. In the both groups CABG was performed predominantly in "on pump" fashion, with the heart arrested by cold crystalloid cardioplegia or in "off pump" method on beating heart, and with left interval mammary artery (LIMA) graft on the left arterior descending artery (LAD) and venous aortocoronary bypasses to other coronary arteries if needed. All the participating patients provided informed consent and signed a form approved by the local Ethics Committee. Inclusion criteria for patient enrolment in the study were as follows: scheduled for CABG surgery due to LAD occlusion or multi-vessel coronary disease, age between 35 and 72 years, previous myocardial infarction older than 30 days, established diagnosis of ischemic cardiomyopathy with left ventricular ejection fraction (LVEF) < 40% and in the New York Heart Association (NYHA) III–IV functional class; full medical treatment for heart failure [β-blockers, angiotensin converting enzyme (ACE) inhibitors, diuretics].

Exclusion criteria were any of the following: non-ischemic dilatative cardiomyopathy, aneurism of the left ventricle, chronic obstructive pulmonary disease, valves insufficiency or stenosis indicated for surgical correction, serious and chronic cardiac rhythm disturbances, hepatic or renal dysfunction, malignancy, cerebrovascular insult in the previous 3 months, hematological diseases, unable to understand explanation of the procedure.

The heart team consisting of the interventional cardiologist/radiologist, heart surgeon and clinical cardiologist evaluated coronary angiography and all clinical and imaging data and made decisions on coronary revascularization and stem cell implantation.

Echocardiography was performed on Vivid 7 GE Ultrasound Systems. LVEF was calculated using the biplane method of discs (modified Simpson's rule) in the apical 4- and 2-chamber views, as recommended by the American Society of Echocardiography [16].

Single-Photon Emission Computed Tomography (SPECT) study was performed 60 min from intravenous injection of 740MBq Technetium labeled contrast media Tc99m MIBI (methoxyisobutylisonitrile) et rest. Perfusion defect extent was determined by a semiquantitative method using Auto Quant software, Cedars-Sinai (QPS/QGS component of Auto Quant) on 20 segmented heart model and was expressed in percentage.

The level of brain natriuretic peptide (BNP) was evaluated preoperatively and in every postoperative control.

The severity of symptoms was assessed using the New York Heart Association functional classification [17]. NYHA functional class was estimated for all the patients preoperatively and on postoperative controls.

Six a minute walk test (6-MWT) was performed as a simple and useful test to evaluate functional capacity in patients with heart failure [18–20]. 6-MWT was not performed preoperatively due to poor condition, dyspnea and/or angina at rest. The results were assessed postoperatively after 6 months, 1 year and on final follow-up for all the patients.

Technical elements of the procedure

After induction of general endotracheal anesthesia, before proceeding with CABG, bone marrow was obtained by multiple aspirations from the posterior iliac crest in the amount of 150 mL, mixed with 25 mL of heparinized saline, and transferred into a sterile bag to the Institute for Transfusiology and Hemobiology, Military Medical Academy in Belgrade. After filtering, 10 mL of heparinized saline and 25 mL of citrate phosphate dextrose (CPD) anticoagulant solution were added and centrifuged on 370 g force spin for 10 min in a Hettich–Roto Silenta RP (Hettich, Germany). After supernatant removal, 15 mL of hydroxyetil starch (HES) solution, 5mL of CPD and 10 mL of IWM-phosphate buffered saline were added in the sediment and incubated for 60 min at the temperature of 22 ± 2°C and then underwent to ''heavy-spin" centrifugation (g-force = 3890) for 10 min. After new removal of supernatant, in the sediment ("buffy coat" + erythrocytes) 5 mL of HES solution was added and left for 40 min to gravity sedimentation at the temperature of 22 ± 2°C. Supernatant was removed and whitish clouded sediment in the middle was aspirated, approximately 15 mL of "buffy coat" rich in BMMNC. This was the final 15 mL BMMNC product that was brought back in the operative room and injected intramyocardially. All the procedures from harvesting to cell injection were performed in a closed-circuit system using asterile conection equipment (Sterile Tubing Welder TSCD-Terumo, Japan) with a sterile plastic bag system designated for cell transplantation in peroperative conditions.

After finishing revascularization with LIMA to LAD and sufficient number of autovenous aortocoronary bypass grafts to achive total targeted revascularization (either "on pump" or "off pump", and if "on pump" when heart resumed its function from cardiopulmonary bypass), intramyocardial implantation was performed with a 1 mL insulin syringe through a 27G needle. BMMNC injection was targeted in to the hypocontractile periinfarcted viable myocardium that was visually identified and performed transepicardialy in 30–45 degree manner, intramyocardially by multiple, average 17.5 ± 3.8 injections, with a single injecting volume of 0.2–0.5 mL to the final injected volume of the average 5.7 ± 1.5 mL as shown in Figure 1.

From the rest of BMMNC suspension, the cell viability, precisely cell "membrane integrity" of mononuclear cells (MNC) was determined by trypan blue exclusion test, and was above 95% in each patient. The number of CD34+ and CD133+ cells was counted in a sample diluted with phosphate buffered saline and fixed with paraformaldehyde.

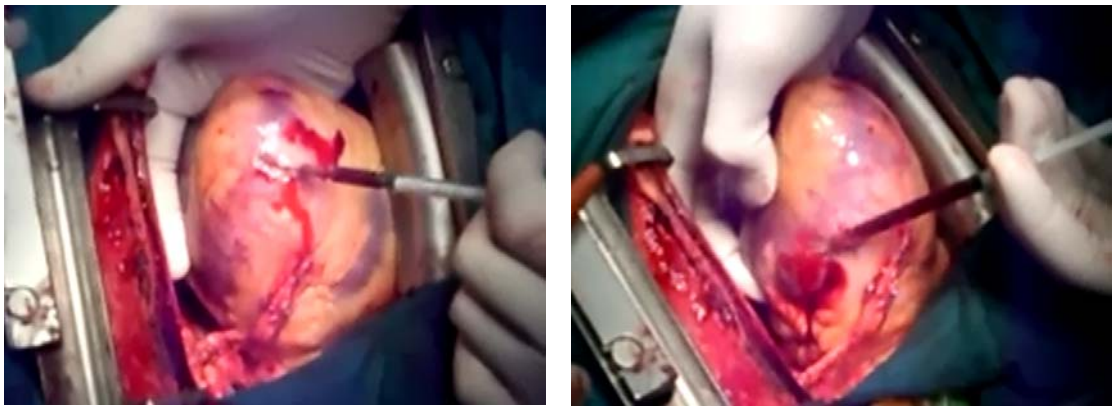

Fig. 1 – Sequences of bone marrow aspirate mononuclear cells implantation procedure in the anteroapical and anterolateral wall of the left ventricle during coronary artery bypass grafting.

Fixed cells were incubated with monoclonal antibodies specific for CD34+ and CD133+ surface antigens conjugated with FITC (fluorescein isothiocyanate) or PE (phycoerythrin) and then were investigated by the flow cytometry method with the EPICS XL–MCL device (Coulter, Germany).

Follow-up of the patients

All the procedures elapsed uneventfully. The patients had good postoperative course with hospital discharge on the 10th postoperative day. Clinical follow-up was performed 2 months, 6 months, one year, and every year after, during the follow-up period. Echocardiography study was performed preoperatively and during each follow-up visit. Gated SPECT was performed pre-operatively and 6 months postoperatively and if needed later at 2-year intervals. MSCT was performed to evaluate grafts after 1 year and if needed later at 2-year intervals. 6-MWT was performed after 6 months to test functional capacity of the patients and then after 1 year and every year after.

The median follow-up was 5 years (range 2.5–7.5 years).

The primary aim of the study was to determine patients postoperative functional capacity and also to find out the cardiac related mortality in the median follow-up period of 5 years.

Statistical analysis

The continuous variables were calculated as mean value ± standard deviation (± SD) and median values, whereas the absolute and relative frequencies were measured for categorical variables. Continuous variables distributions were determined by the Kolmogorov-Smirnov test. The differences between the groups in continuous data were examined by the Student *t*-test or the Mann-Whitney test as appropriate. In case of categorical variables, the group differences were examined by the Fisher exact test, mixed-design ANOVA for repeated measures or Friedman's test as appropriate. The correlations were assessed by the Pearsons correlation analysis. All the statistical tests were two-tailed. A p value < 0.05 was considered significant. Statistical analysis was performed with commercially available software (SPSS Statistics 17.0.).

Results

The study included 30 patients (28 males and 2 females) mean age 56.9 ± 9.0 years. The two groups were well matched as shown in Table 1, with no statistically significant differences between the patients in the group 1 (BMMNC and CABG) and the group 2 (CABG only; $p > 0.05$), except in a higher number of patients with hypercholesterolemia in the group 1 ($p = 0.035$). All the patients had at least one previous anteroseptal myocardial infarct, whereas 5 patients in the BMMNC and CABG, and 1 patient in the CABG only group had two previous myocardial infarctions at different locations of the myocard.

Table 1

Preoperative clinical and demographic characteristics of the patients

Patients characteristics	Study group (n = 15) CABG&BMMNC	Control group (n = 15) CABG alone	*p*
Gender – male, n (%)	14 (93.3)	14 (93.3)	1.000
Age (years), $\bar{x}$ ± SD	53.8 ± 10.1	60 ± 6.8	0.059
Number of previous MI, n (%)			
1	10 (66.7)	14 (93.3)	0.169
2	5 (33.3)	1 (6.7)	
Time from the first MI, $\bar{x}$ (range)	3.20 (6–12 months)	3.07 (6–12 months)	0.692
Localisation of the first MI, n (%)			
anteroseptal	9 (60.0)	11 (73.3)	0.857
anteroseptal and lateral	1 (6.7)	0 (0.0)	
inferoposterolateral	3 (20.0)	3 (20.0)	
inferior	2 (13.3)	1 (6.7)	
Hypertension, n (%)	7 (46.7)	10 (66.7)	0.462
Smoking status, n (%)			
active smoker	1 (6.7)	1 (6.7)	
previous smoker	11 (73.3)	11 (73.3)	1.000
non smoker	3 (20.0)	3 (20.0)	
Hypercholesterolemy, n (%)	14 (93.3)	8 (53.3)	0.035*
Diabetes mellitus, n (%)			
insulin dependent	3 (20.0)	2 (13.3)	0.762
oral hypoglycemic therapy	3 (20.0)	2 (13.3)	
NYHA class, n (%)			
III	11 (73.3)	13 (86.7)	0.651
IV	4 (26.7)	2 (13.3)	
BMI [kg/m^2], $\bar{x}$ ± SD	27.90 ± 3.35	27.40 ± 3.75	0.703
LVEF (%), $\bar{x}$ ± SD	35.3 ± 3.9	36.5 ± 5.3	0.490
SPECT defect extent (%), $\bar{x}$ ± SD	26 ± 9	28 ± 9	0.597
BNP (pg/mL), $\bar{x}$ ± SD	471.86 ± 375.79	632.85 ± 444.55	0.293

***Statistically significant difference; MI – myocardial infarction; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cells; NYHA – the New York Heart Association; BMI – body mass index; LVEF – left ventricular ejection fraction; SPECT – Single Photon Emission Computed Tomography; BNP – brain natriuretic peptide.**

All the patients in both groups received the average of 3.4 ± 1.0 implanted coronary grafts, all of them LIMA to LAD and autovenous to the other coronaries. There were no significant differences in the number of grafts between the groups (3.3 ± 1.1 in the BMMNC and CABG group *vs* 3.5 ± 0.9 in the CABG only group; $p = 0.476$).

The group BMMNC and CABG had the average of 17.5 ± 3.8 injections of BMMNC suspension with the average number of injected bone marrow mononuclear cells 70.7 ± 32.4 × 10^6 in the total average volume of 5.7 ± 1.5 mL. In this volume the average count of CD34+ and CD133+ cells were 3.96 ± 2.77 × 10^6 and 2.65 ± 1.71 × 10^6, respectively, as shown in Table 2.

Table 2
Operative/procedural characteristics in the group BMMNC and CABG

Variable	x̄ ± SD
Injection, n	17.5 ± 3.8
Volume, mL	5.7 ± 1.5
MNC, n	70.7 ± 32.4
CD34+ (× 10^6), n	3.96 ± 2.77
CD133+ (× 10^6), n	2.65 ± 1.71

BMMNC – bone marrow aspirate mononuclear cells; CABG – coronary artery bypass grafting; MNC – mononuclear cells.

The early postoperative course was unevetful in both groups with no significant differences between them in regard to adverse side effects during hospital stay. There were no significant differences in cardiac specific enzymes activities after the operation or the number of atrial fibrilation episodes or appearance of pericardial effusion between the groups.

In a follow-up period, 6 and 12 months postoperatively, there were no statistically significant difference between the patients in the group 1 (BMMNC and CABG) and the group 2 (CABG only) in regard to the NYHA functional class ($p = 0.224$; $p = 0.169$). At the time of the last recorded control, that was between 2.5 and 7.5 (median 5) years after the cardiac surgery, statistically significantly more patients from the BMMNC and CABG group were in the NYHA functional class I *vs* the CABG only group (14 *vs* 5; $p = 0.002$) as presented in Table 3.

On the other hand, the distance that was measured by 6-MWT showed a significant difference between the groups even after 6 months postoperatively (435 ± 90 m in the BMMNC and CABG group and 315 ± 80 m in the CABG only group; $p = 0.001$), and continued to be preserved and improved after 12 months (499 ± 85 m in the BMMNC and CABG group *vs* 338 ± 103 m in the CABG only group; $p < 0.001$) and on final follow-up (520 ± 79 m in the BMMNC and CABG group *vs* 343 ± 114 m in the CABG only group; $p < 0.001$). The cardiac related mortality was also significantly reduced in the BMMNC and CABG group (0/15 *vs* 4/15, respectively; $p = 0.049$) as shown in Table 4.

There were no significant difference in mean LVEF preoperatively between the groups (35.3 ± 3.9% *vs* 36.5 ±

Table 3
Changes in the New York Heart Association (NYHA) functional class in a follow-up period of median 5 years

Variable NYHA class	Study group (n = 15) CABG&BMMNC	Control group (n = 15) CABG alone	*p*
after 6 months			
I	15 (100.0)	12 (80.0)	
II	0 (0.0)	2 (13.3)	0.224
III	0 (0.0)	1 (6.7)	
after 1 year			
I	14 (93.3)	10 (66.7)	0.169
II	1 (6.7)	5 (33.3)	
final control			
I	14 (93.3)	5 (33.3)	
II	1 (6.7)	8 (53.3)	
III	0 (0.0)	2 (13.3)	0.002*

Results are given as number (%) of patients.

***Statistically significant difference; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cells.**

Table 4
Functional capacity measured by walking distance test (6-MWT) the and the mortality in a 5-year follow-up period

Variable	Study group (n = 15) CABG&BMMNC	Control group (n = 15) CABG alone	*p*
6MWT (m), x̄ ± SD			
after 6 months	435 ± 90	315 ± 80	0.001*
after 1 year	499 ± 85	338 ± 103	< 0.001*
final control	520 ± 79	343 ± 114	< 0.000*
Vital status at final follow-up, n (%)			
live	13 (86.7)	11 (73.3)	
death from cardiac event	0 (0.0)	4 (26.7)	0.049*
death from non cardiac event**	2 (13.3)	0 (0.0)	

***Statistically significant difference; **cerebrovascular hemorrhage 6 years after operation in 41-year old patient, and pulmonary malignancy 6 years after the operation in 78 year old patient; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cells.**

5.3%, respectively; $p = 0.490$) but on final postoperative follow-up the mean LVEF in the BMMNC and CABG group was significantly higher than in the CABG only group (45.3 ± 4.9% *vs* 33.9% ± 8.8%, respectively; $p < 0.001$) as shown in Figure 2.

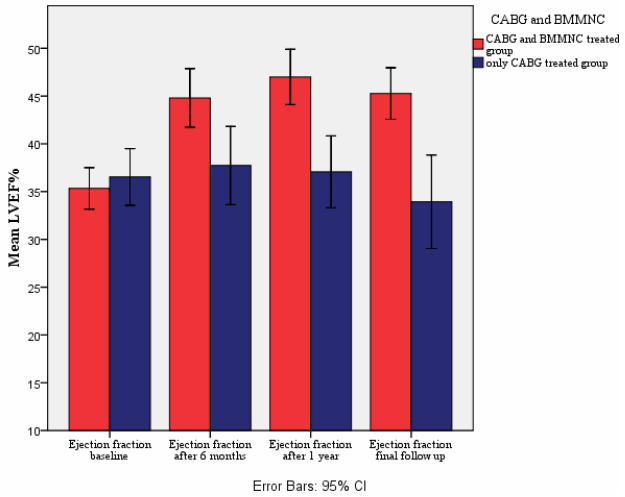

Fig. 2 – Differences in the mean left ventricular ejection fraction (LVEF) between the groups; CABG – coronary artery bypass grafting; BMMNC – bone marrow aspirate mononuclear cell; CI – confidence interval.

Discussion

Since 2001 clinical trials have been initiated to investigate the safety and efficacy of cardiac cell therapy in patients. Most studies were conducted in patients with acute MI and intracoronary application of BMMNC (about 30 randomized trials) and demonstrated conflicting results [21–26]. On the other hand, 12 studies reporting inconsistent results were performed in the patients with chronic ischemic cardiomyopathy, among them several with intramyocardial implantation concomitant to CABG [27, 28]. In a meta-analysis performed and published by Donndorf at al. [29] six studies were included with the sample size of 20–40 and follow-up duration of only 3–6 months. All of the patients had improvement in LVEF and tending to reduced LVEDV sugesting that a decrease of cardiac remodeling was achieved. The authors also pointed out to methodologically heterogenity of the analized studies in few important moments, such as time from MI, preparing BMMNC, points of implantation, evaluation of ventricular function and the range of preoperative LVEF values. To overcome these limitations the authors launched a controlled, prospective, randomized, double blinded multicenter trial (PERFECT) in July 2009 [30]. The present status of this study is that currently recruiting participants (http://clinicaltrials.gov/show/NCT00950274).

In the present study, the approach was to use BMMNC due to the presumption that there is no favorable cell in bone marrow that can be isolated and that can behave better in myocardial recovery than the equilibrium between cells subpopulations and their products present in a mononuclear fraction of bone marrow and their influence on cardioprogenitor cells resided in myocardium. For delivery the method of intramyocardial transepicardial route was chosen. Since the patients were operated due to postinfarctional ischemic cardiomyopathy (median LVEF of 35.9 ± 4.7%) sheduled for CABG surgery, it was very natural to try to identify peri-infarction viable myocardium in the region of fibrous scared as a targeted area, and inject the amount of BMMNC that seemed to be acceptable not to disturb the milieu of myocardium, but to achieve the goal of cell engraftment. During the procedure there were no adverse side effects noted, and in the early postoperative period recovering was uneventfull, as it was in the control group. Markers of myocardial damage were in the same rank in both groups, so as postoperative pericardial effusion which were quite a rare event and did not require additional intervention other than the use of diuretics. According to the results from the patients this procedure seems to be safe, not aggravating the risk for CABG itself. Postoperatively, the procedure contributes to better quality of life and further reduces cardiovascular mortality. Postoperative improvement in the NYHA class is evident for all the patients, which is consistent with other studies and follow-up periods of 6–12 months [14, 31, 32]. Also, functional capacity of patients in the BMMNC group was significantly better according to the results achived by 6-MWT in all time frames during a long-term follow-up. This is in accordance with short-term results of others investigations for concomitant procedures of CABG and BMMNC implantation. Comparing long-term results to that of intracoronary implantation studies, these results might be superior, because in a meta-analysis of the results beyond 12 months good efects of intracoronary procedure vanish, and clinical benefit because marginal and transient, with no durable effect [33]. The possible reason could be cell retention and homing ability in different implantation technics. Despite some leakage from the point of injection, the amount of BMMNC that retained and engrafted the myocardium by intramyocardial method remained to be highest, 30–75% after 24 h, and more than 25% after 48 h of implantation. Compared to other methods, that wash-out to other organs is extremely high, even to 95% in 48h [34, 35]. Carefull attention in this study was paid to the appearance of new arrhythmias [12, 13]. There were no associated arrhythmias recorded in the BMMNC group of patients in this study.

There is still a number of open questions for actual and future clinical studies: does bone marrow stem cell implantation improve left ventricular function in all patients with heart failure; which group of patients can achieve the most benefit from the procedure; what is the optimal timing for conducting procedure after MI; what is the optimal number and population of cells to achieve most benefit from the procedure; is the intramyocardial transepicardial route best for cell administration; should it be performed simultaneously with CABG for all ischemic cardiomyopathy patients?

Conclusion

Implantation of stem cells during coronary artery bypass grafting surgery is a safe and feasible procedure that does not aggravate any additional risk of cardiac surgery. Intramyocardial stem cell implantation during coronary ar-

tery bypass grafting in comparison to coronary artery bypass grafting alone, improves functional capacity of patients with ischemic cardiomyopathy and reduces long-term cardiac mortality. However, larger randomized trials should confirm effectiveness of intramyocardial bone marrow mononuclear cells therapy during coronary artery bypass grafting operation.

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