J. of Cardiovasc. Trans. Res. (2010) 3:160–168
DOI 10.1007/s12265-009-9123-8

Efficiency of Intramyocardial Injections of Autologous Bone Marrow Mononuclear Cells in Patients with Ischemic Heart Failure: A Randomized Study

Evgeny Pokushalov · Alexander Romanov · Alexander Chernyavsky · Petr Larionov · Igor Terekhov · Sergey Artyomenko · Olga Poveshenko · Elena Kliver · Natalya Shirokova · Alexandr Karaskov · Nabil Dib

Received: 2 July 2009 / Accepted: 20 August 2009 / Published online: 24 September 2009

Abstract Intramyocardial transplantation of autologous bone marrow mononuclear cells (BMMC) is believed to be a promising method for the treatment of patients with chronic ischemic heart disease. The aim of this study was to evaluate long-term results of intramyocardial bone marrow cell transplantation in patients with severe ischemic heart failure. One hundred nine patients with chronic myocardial infarction and end-stage chronic heart failure were randomized into two groups: 55 patients received intramyocardial BMMC injection and 54 received optimal medical therapy. The NOGA system (Biosense-Webster) was used to administer 41±16×106 BMMC into the border zone of myocardial infarction. None of the patients developed periprocedural complications following BMMC injections. The injections led to improvement of CCS class (3.1±0.4 to 1.6±0.6 after 6 months and 1.6±0.4 after 12 months; p=0.001) and NYHA functional class (3.3±0.2 to 2.3±0.2 after 6 months and 2.5±0.1 after 12 months; p=0.006). Left ventricular ejection fraction increased significantly in the BMMC group (27.8±3.4% vs 32.3±4.1%; p=0.04) while it tended to decrease in the control group (26.8±3.8% to 25.2±4.1%; p=0.61). Summed rest score improved in the BMMC group after 12 months (30.2±5.6 to 27.8±5.1; p=0.032). The improvement of stress score was more noticeable (34.5±5.4 to 28.1±5.2; p=0.016). Neither stress nor rest score changed in patients numbers on medical therapy. In BMMC group 6 (10.9%) patients died at 12-month follow-up compared with 21 (38.9%) in control group (log-rank test, p=0.0007). Intramyocardial bone marrow cell transplantation to patients with ischemic heart failure is safe and improved survival, clinical symptoms, and has beneficial effect on LV function

Keywords Ischemic Heart Disease · Heart Failure · Bone Marrow Cells · Myocardial Perfusion

E. Pokushalov (✉) · A. Romanov · A. Chernyavsky · P. Larionov · I. Terekhov · S. Artyomenko · O. Poveshenko · E. Kliver · N. Shirokova · A. Karaskov
State Research Institute of Circulation Pathology,
Rechkunovskaya 15,
630055 Novosibirsk 55, Russia
e-mail: E.Pokushalov@gmail.com

N. Dib
University of California-San Diego,
San Diego, CA, USA

Introduction

At present, bone marrow cell transplantation is regarded as a potentially promising therapy used for the treatment of patients suffering from a chronic ischemic heart disease. Known clinical studies confirmed the safety of intramyocardial injections of autologous bone marrow mononuclear cells (BMMC) [1–5]. Moreover, they demonstrated that bone marrow cell transplantation reduced anginal symptoms, enhanced myocardial perfusion, and improved cardiac function. However, the effect of BMMC transplantation on patients with heart failure has not been demonstrated in larger studies [5].

The primary objective of the present study was to assess long-term follow-up results of modern medical therapy and intramyocardial bone marrow cell injections in patients with chronic ischemic heart failure.

Methods

Patient Population and Study Protocol

A total of 109 no option for revascularization consecutive patients with chronic myocardial infarction and end-stage chronic heart failure were randomized and enrolled in the study. Inclusion criteria comprised (1) a history of myocardial infarction >12 months before the enrolment and a fixed perfusion defect on Tc-99m tetrofosmin SPECT; (2) the class of clinical symptoms of heart failure; (3) non-revascularizable patient who is symptomatic on optimal medical therapy; and (4) left ventricular ejection fraction (LVEF) <35% as determined by two-dimensional echocardiography. The following exclusion criteria were applied: (1) eligibility for percutaneous coronary intervention, coronary artery bypass grafting, previous valve surgery, surgical remodeling of the left ventricle, or cardiac resynchronization therapy; (2) hemorrhagic symptoms; (3) severe renal and liver dysfunction; and (4) the history of malignancy.

The patients were randomly allocated to two groups, viz. BMMC group in which the patients were treated by BMMC injections combined with modern medical therapy (n=55) and control group in which patients were given medical therapy alone (n=54).

The primary end-point of the study was the efficacy of the intramyocardial injection of autologous bone marrow mononuclear cells, measured by change in myocardial perfusion defects at rest and under pharmacological stress between baseline and 6 and 12 months follow-up SPECT. The secondary end-points at 12 months follow-up were: the safety of the intramyocardial BMMC therapy, quality of life, Canadian Cardiac Society (CCS) angina class, New York Heart Association (NYHA) functional class, LV function (from the results of echocardiography and level of brain natriuretic peptide (BNP)), life-threatening arrhythmias, mortality between two groups, and NOGA change in voltage as assessed by NOGA follow-up endocardial mapping (unipolar voltage). Randomization was done using an electronic system. Clinical characteristics of the patients are presented in Table 1. The study protocol was approved by the hospital ethics committee, and written informed consent was obtained from all patients.

The baseline assessment included clinical evaluation, angina (Canadian Cardiovascular Society score) and quality of life (Minnesota Living with Heart Failure Questionnaire (MLwHF) score). All patients underwent a 6-min walking test to evaluate their exercise capacity, 24-h Holter monitoring to assess ventricular arrhythmias, two-dimensional echocardiography to assess LV function, and single photon emission computed tomography (SPECT) to assess perfusion and ischemia and laboratory analyses (complete blood count, blood chemistry, C-reactive protein, creatine kinase, troponin T serum, and BNP levels).

Clinical and laboratory evaluation of all patients supplemented by 24-h Holter monitoring was performed 1, 3, 6, and 12 months after initiation of the study. NYHA functional class, angina (CCS score), and quality of life (MLwHF) were assessed; walking test, two-dimensional echocardiography, and SPECT were carried out after 3, 6, and 12 months. Electroanatomical mapping with the NOGA system (Biosense-Webster) and SPECT were performed in all patients of the BMMC group 6 and 12 months after the onset of the study.

Bone Marrow Aspiration and Cell Isolation

At the day of the injection procedure, bone marrow was aspirated from the iliac crest under local anesthesia by the standard technique. Mononuclear BMC were isolated by Ficoll density gradient centrifugation (1.077; Ficoll-Plaque Plus, Amersham Pharmacia Biotech). Three washing steps were performed and the cells were resuspended in heparinized saline for further use. Cell viability was tested by Trypan Blue (exclusion method) and estimated at more than 98% for each transplant.

Electroanatomical Mapping and Bone Marrow Cell Injection

Isolation of mononuclear cells was accompanied by non-fluoroscopic mapping with the NOGA system via femoral artery access and retrograde aortic approach using a 7-Fr NOGAStar catheter (2 mm tip, two electrodes, interelectrode distance 0.5 mm; Biosense-Webster). Areas with unipolar voltage (UV) <6.9 mV were assumed to be infarction zones [6] if they corresponded to the areas with a perfusion defect on Tc-99m tetrofosmin SPECT. In other words, an area of interest located by technetium-99m tetrofosmin SPECT was delineated in detail by means of NOGA mapping; it included ischemic but viable myocardium (unipolar voltage ≥6.9 mV, bipolar voltage ≥1.5 mV) [5–8]. Immediately before injection, the catheter was positioned perpendicularly to endocardium with excellent loop stability and the extension of the needle to induce premature ventricular contraction [9,10]. Ten successive intramyocardial injections (roughly 0.2 ml each) were administered into the infarction border zone. Second electroanatomical mapping was performed 6 and 12 months after the first by the same method.

The total duration of the procedure (mapping and injection) averaged 59±19 min and that of fluoroscopy 11±6 min. The electromechanical map had 90±22 points on the average. Ten 0.2-ml injections of BMMC were administered into each ischemic segment of all patients.

Table 1 Clinical characteristics of the patients

	BMMC group (n=55)	Control group (n=54)
Age (years)	61±9	62±5
Male (%)	48 (87)	46 (85)
Time since MI (years)	9±8	8±5
No. of major coronary arteries narrowed≥50%		
1	2 (3%)	3 (5%)
2	1 (2%)	3 (5%)
3	52 (95%)	48 (90%)
Previous PCI	20 (36%)	16 (29%)
Previous CABG	39 (71%)	41 (76%)
Previous ICD	4 (7%)	3 (6%)
Hypertension	28 (51%)	32 (59%)
Diabetes mellitus	5 (9%)	6 (11%)
Hyperlipidemia (total cholesterol>5 mmol/L)	53 (96%)	52 (96%)
6-mWT, m	185±39	197±34
NYHA, FC	3.3±0.2	3.5±0.1
CCS, FC	3.1±0.4	3.5±0.5
LVEF, %	27.8±3.4	26.8±3.8
EDV LV, ml	243±32	239±38
ESV LV, ml	146±39	149±43
Number of MI	2.8±0.6	2.9±0.7
MLwHF, points	65.3±21	63.2±23

Values are mean±SD or percentage of patients

6-mWT 6-min walk test, *NYHA FC* New York Heart Association functional class, *CCS FC* Canadian Cardiac Society functional class, *LVEF* left ventricular ejection fraction, *EDV* end-diastolic volume of left ventricle, *ESV* end-systolic volume of left ventricle, *Number of MI* quantity of myocardial infarction, *MLwHF* Minnesota life with Heart Failure Questionnaire score

The mean number of BMMC injected to each patient was $41\pm16\times10^6$. Cell viability was 98±1% and the $CD34^+$ cell fraction was 2.5±1.6%.

SPECT Imaging

SPECT imaging (using 500 MBq technetium-99m tetrofosmin) was done according to the 2-day stress/rest protocol. Pharmacological stress with intravenous administration of adenosine (0.14 mg $kg^{-1}min^{-1}$ for 6 min) or dobutamine (up to a maximum dose of 40 µg $kg^{-1}min^{-1}$ for 15 min) was used to obtain stress images. The stress imaging procedure at 6- and 12-month follow-up was carried with the same type of agent as at baseline. Medications did not differ between the baseline and follow-up examinations. Image interpretation was by consensus of two readers blinded to the type of the study (baseline or follow-up) and clinical data. A semiquantitative 20-segment scoring system was used, with a range of scores from 0 to 4 (0=normal activity, 4=no activity). These scores were added to yield summed stress and summed rest scores [11].

Statistical Analysis

Results are expressed as mean values (SD) for continuous parameters or as numbers and percentages for categorical ones. Continuous variables were compared by repeated-measures ANOVA; T test and Wilcoxon–Mann–Whitney tests. Categorical variables were compared by Pearson's chi-square test or Fisher's exact test. Multivariable logistic regression was also used to determine the independent relationship. Survival curves were calculated and plotted using the Kaplan–Meier method with the log-rank test. A value of $p<0.05$ was considered to be statistically significant.

Results

The study included 109 patients with chronic ischemic heart disease and end-stage chronic heart failure. Clinical characteristics of the patients in both groups were not significantly different (Table 1). Also, they received virtually identical medical therapy (nitrates in 100%, angiotensin-converting enzyme inhibitors in 94%, diuretics in 100%, oral anticoagulants in 100%, or β-blockers in 91%). The type and the dose of medication did not change during the 12-month follow-up (excluding nitrates doses).

Serious Adverse Events and Survival

None of the 55 patients in the first group given intramyocardial injections of bone marrow cells developed periprocedural complications. Two-dimensional echocardiography did not reveal postprocedural pericardial

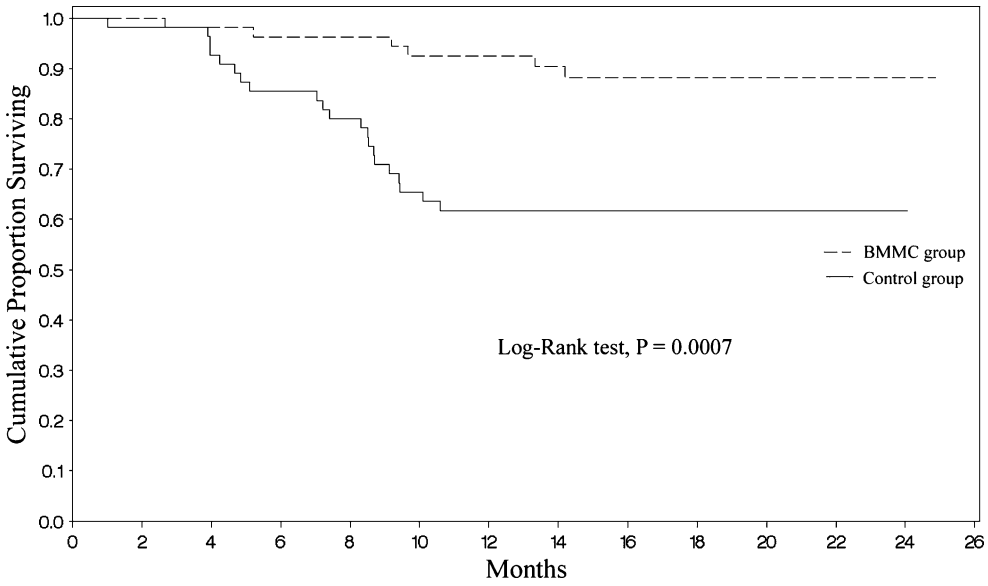

Fig. 1 Kaplan–Meier curves survival in both groups

effusion. Laboratory characteristics, such as creatine kinase activity (98±36 vs 108±51 U/L; p=0.72) and peak troponin T level (0.16±0.24 vs 0.18±0.21 μg/L; p=0.64) remained unaltered. No new arrhythmias were recorded during 24 h of consecutive electrocardiographic monitoring immediately, 3, 6, and 12 months after the BMMC injection procedure. An implantable cardioverter-defibrillator (ICD) was implanted to two patients with ventricular tachycardia prior to cell injections. All the patients were discharged from the hospital on day 3 after the injection procedure.

BMMC group 6 (10.9%) patients died at 12-month follow-up compared with 21 (38.9%) in control group (log-rank test, p=0.0007; Fig. 1).

Table 2 Comparison of baseline and follow-up values for BMMC and control group

	Baseline		3month		6month		12month		p value[a]
	BMMC (n=55)	Control (n=54)	BMMC (n=54)	Control (n=53)	BMMC (n=53)	Control (n=46)	BMMC (n=49)	Control (n=33)	
CCS, FC	3.1±0.4	3.5±0.5	2.4±0.6	3.5±0.6	1.6±0.6#*	3.4±0.6	1.6±0.4#*	3.5±0.4	0.0001
NYHA, FC	3.3±0.2	3.5±0.1	2.4±0.2	3.5±0.8	2.3±0.2#*	3.8±0.1	2.5±0.1#*	3.9±0.1‡	0.0001
LVFE, %	27.8±3.4	26.8±3.8	28.1±5.6	26.5±5.8	32.8±6.2‡*	26.2±6.1	32.3±4.1‡*	25.2±4.1	0.026
ESV LV, ml	146±39	149±43	137±41	143±37	119±31‡*	156±46	113±37‡*	152±31	0.038
EDV LV, ml	243±32	239±38	251±38	243±34	242±27	241±42	245±34	252±49	0.82
BNP, pg/ml	907±391	859±413	515±286#*	1,045±586	418±273#*	972±521	–	–	0.001
Creatinine, mg/dL	1.22±0.8	1.32±1.1	1.1 ±0.9	1.62±0.9	1.04±0.7	1.58±0.8	–	–	0.022
Angina episodes/day	2.8±4.1	2.7±3.9	1.9±3.2	2.7±4.2	1.0±1.5#*	2.8±4.6	0.6±1.2#*	2.8±4.2	0.001
Sublingual nitrates, tablets/day	1.8±2.8	1.9±2.7	0.8±1.6	1.9±3.1	0.5±1.2#*	2.1±2.8	0.5±1.3#*	2.1±2.9	0.029
Change in NYHA class in relation to baseline									
No change, n (%)			22 (40.7)	49 (92.5)	17 (32.1)	43 (93.5)	15 (30.6)	29 (87.9)	
Improvement by one class, n (%)			25 (46.3)	4 (7.5)	29 (54.7)	3 (6.5)	28 (57.1)	4 (12.1)	
Improvement by two classes, n (%)			7 (13)	0	7 (13.2)	0	6 (12.3)	0	

6-mWT 6-min walk test, *NYHA FC* New York Heart Association functional class, *CCS FC* Canadian Cardiac Society functional class, *MLwHF* Minnesota life with Heart Failure Questionnaire score, *BNP* brain natriuretic peptide, *LVEF* left ventricular ejection fraction, *ESV* end-systolic volume, *EDV* end-diastolic volume

#p<0.01 vs baseline; ‡p<0.05 vs baseline; *p<0.01 vs control group

[a] p values reflect comparison of the differences between BMMC and control groups over time

Clinical Outcomes

Patients who received BMMC group improved their New York Heart Association class (3.3±0.2 at baseline to 2.4±0.2 at 3 months, 2.3±0.2 at 6 months, and 2.5±0.1 at 12 months; p=0.006; Table 2). The distance covered by the patients during a 6-min walking test increased significantly from 185±39 m at baseline to 359±69 m after 12 months (p=0.0085; Fig. 2a). The frequency of daily angina episodes during the 6-month period dropped from 2.8±4.1 to 1.0±1.5 and was 0.6±1.2 after 12 months (p=0.022). Accordingly the frequency of daily intake of sublingual nitrates decreased from 1.8±2.8 tablets/day at baseline to 0.5±1.2 tablets/day after 6 months and to 0.5±1.3 tablets/day after 12 months (p=0.043). CCS angina class improved from 3.1±0.4 at baseline to 2.4±0.6 after 3 months, 1.6±0.6 after 6 months, and 1.6±0.4 after 12 months (p=0.001). The MLwHF score decreased significantly in the BMMC group compared to baseline (22.4±6 points vs. 65.3±21 points; p=0.0082) and control group (59.9±16; p=0.01; Fig. 2b).

Conversely, the symptoms of heart failure in patients of the control group deteriorated: NYHA scores were 3.5±0.1 initially, 3.8±0.1 at 6 months, and 3.9±0.1 after 12 months (p=0.064). CCS angina class did not change (3.5±0.5 at baseline and 3.4±0.6 and 3.5±0.4 after 6 and 12 months, respectively; p=0.82). No changes in the quality of life were reported within 6 and 12 months (p=0.34).

Of all baseline and follow-up laboratory values, only BNP and serum creatinine varied between the control (n=46) and BMMC groups (n=53) at follow-up. After 6 month BNP levels were significantly lower in BMMC group (418±273 pg/ml) as compared with the control group (972±521 pg/ml; p=0.048). There was a trend

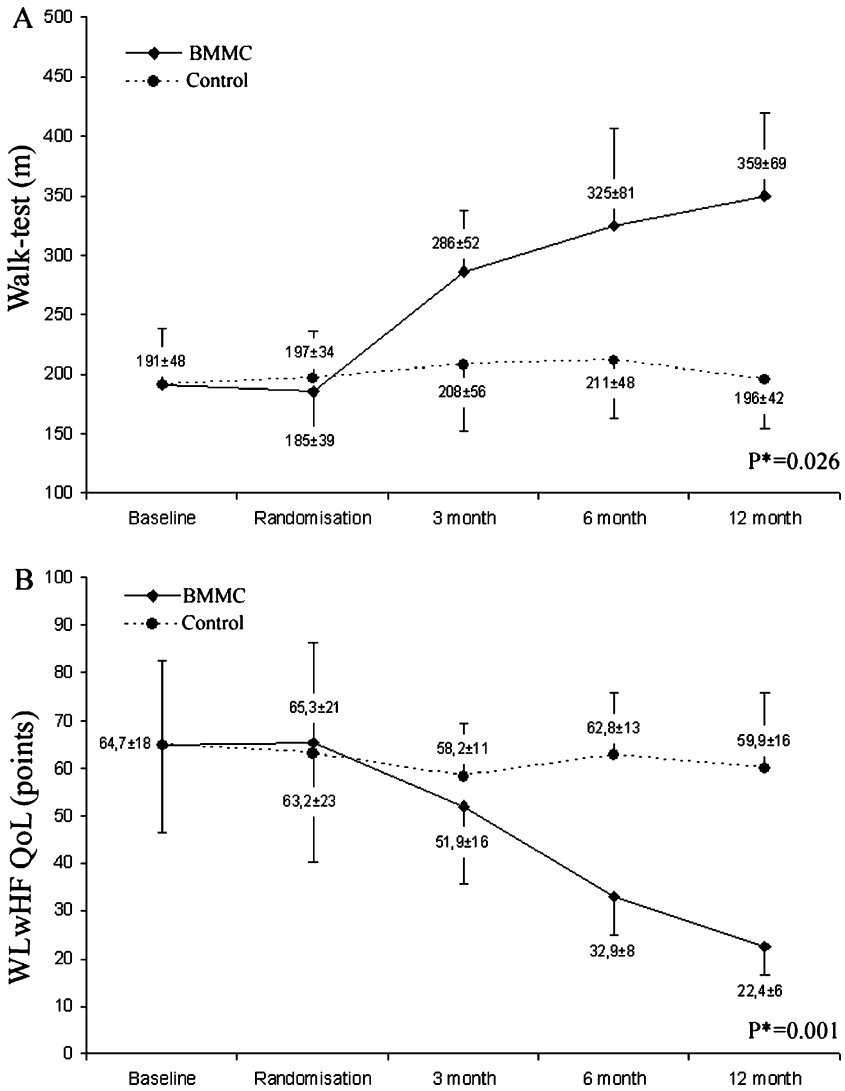

Fig. 2 The distance walked in 6 min and the quality of life score (assessed using the Minnesota Living with Heart Failure questionnaire) after 3, 6, and 12 months of follow-up in both study groups. Values are expressed as mean±SD. p* values reflect comparison of the differences between BMMC and control groups over time

toward increased difference of serum creatinine at follow-up between the two groups, with higher levels in the control group (p=0.06).

The Cox proportional hazard model detected no significant correlation between different patients variables and positive clinical outcome.

Left Ventricular Function

On the average, LVEF improved from 27.8±3.4% to 32.3±4.1% (p=0.04; Table 2). EDV remained unaltered. In the control group, LVEF tended to decrease (from 26.8±3.8% to 25.2±4.1%; p=0.61).

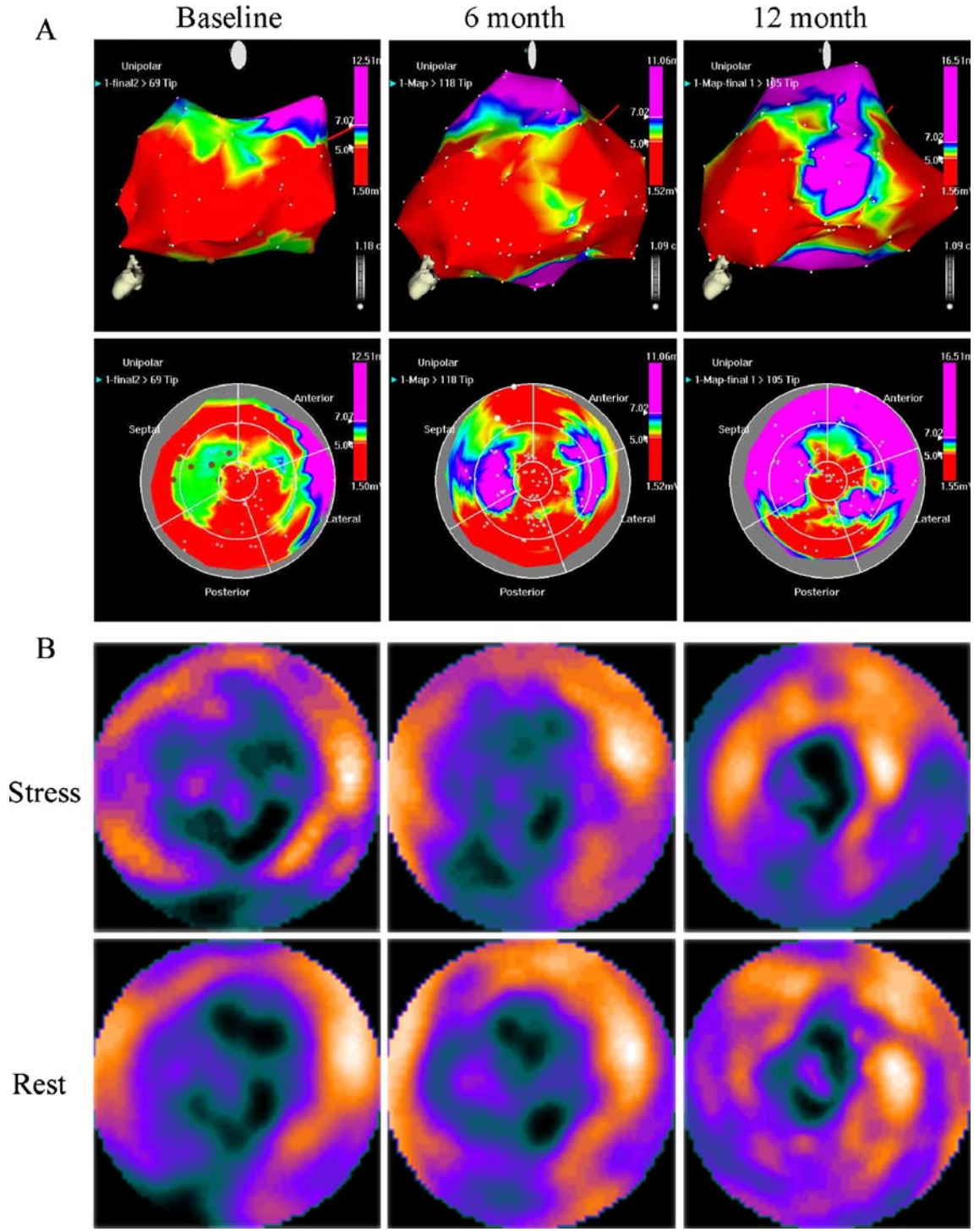

Fig. 3 LV mapping (UV) and Tc-99m tetrofosmin computed tomographic images at baseline and at 6 and 12 months follow-up in the patient of BMMC group

Myocardial Perfusion

In the BMMC group, after 6 months, 39 (72.9%) of the 55 patients showed improved myocardial perfusion in the segments into which BMMC were injected. An example of enhanced myocardial perfusion following BMMC injections is presented in Fig. 3b. No change of myocardial perfusion was apparent in 12 (21.8%) patients; it deteriorated in three others. The number of segments with improved perfusion after BMMC therapy were 3.4±2.7 for stress and 2.9±2.0 for rest.

The summed rest score improved within 6 months (30.2±5.6 to 27.2±5.8) and underwent no further change after 12 months (27.8±5.1; p=0.032; Fig. 4). The improvement of the summed stress score was more pronounced than that of the rest score (34.5±5.4 vs 27.6 ±4.9 after 6 months and 28.1 ±5.2 after 12 months; p=0.016). In contrast, neither stress nor rest score changed appreciably in control patients.

Electroanatomical Mapping: Electrical Activity

All patients of the BMMC group underwent the electroanatomical mapping after 6 and 12 months. The total number of points and their distribution (number of points in segments) were not significantly different from the respective initial values and after 6 and 12 months were the same. Total UV increased significantly from 9.1±2.4 mV to 12.4±2.1 mV after 6 month and also improve to 14.2±3.2 after 12 month (p=0.026; Fig. 3a), largely due to increased UV in the segments which BMMC were administered into the infarction border zone (5.6±1.2 mV initially and 9.8± 1.8 mV after 6 months, 11.2±2.1 mV after 12 month; p=0.006). In BMMC-free segments, UV remained virtually unaltered (14.3±3.2 mV and 15.6±2.8 mV; p=0.19).

Discussion

This study was designed to evaluate the effect of intramyocardial transplants of autologous bone marrow mononuclear cells on the clinical condition of patients with ischemic heart failure. The following results were obtained:

- Intramyocardial administration of bone marrow cells to patients with chronic ischemic heart disease and marked left ventricular dysfunction was safe and feasible. It was well tolerated by the patients and induced no periprocedural complications.
- Transplantation of autologous bone marrow cells resulted in the substantial improvement of survival, anginal symptoms, and LV function. Significantly fewer patients given cell injections required re-hospitalization for the treatment of decompensated heart failure than in the control group. Survival among recipients of BMMC injections was also significantly higher and hospital admissions were less
- Cell transplant improved myocardial perfusion in ischemic segments.
- No increase in extent of scar tissue was observed after intramyocardial injections of bone marrow cells.

Some patients failed to benefit from the effect of BMMC transplantation. Most of them experienced a decrease of CCS angina class and improvement of QOL, but the frequency of enhanced perfusion revealed by SPECT was much lower. Moreover, only half of the patients responded by an increase of EF despite reduced NYHA functional class. In certain patients, marked improvement of QOL was the sole positive manifestation of the effect of the treatment unaccompanied by beneficial changes of other characteristics. Taken together, these findings suggest the necessity of a deeper insight into the mechanism of action of cells

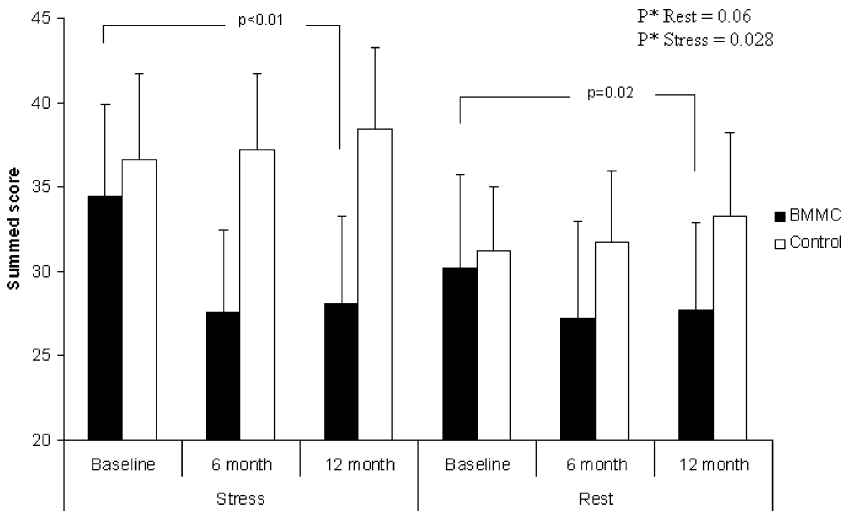

Fig. 4 Semiquantitative summed scores before and 6 and 12 months after therapy both group. Values are expressed as mean±SD. p* values reflect comparison of the differences between BMMC and control groups over time

transplanted into the myocardium. The present study leaves unsolved the mystery of beneficial effect on the ischemic myocardium. Its results partly agree with the hypothesis of angiogenesis activation by bone marrow cells, probably due to the production of angiogenic cytokines and upregulation of endogenous cytokine expression resulting in improved myocardial perfusion and function [12]. It is worthwhile to note that the scar tissue volume in the myocardium did not increase after administration of bone marrow cells and was therefore unrelated to the improvement of the angina class.

This study included patients in a very poor clinical state who had no chance of conventional revascularization (PCI or/and CABG). Such patients are traditionally managed by medicamentous therapy that, for all the advantages of modern pharmaceuticals, fails to improve quality and quantity of life and leaves patients with angina of effort and heart dysfunction. This directly leads to the high frequency of unscheduled re-hospitalization. Heart transplantation is hampered by the lack of donors, high risk, and cost. Hence, there is the necessity to search for adequate therapeutic modalities applicable in this category of patients. Cellular therapy opens up new prospects. Several experimental studies have demonstrated restoration of cardiac function after the introduction of cells of different types into the heart tissue [13]. Clinical studies of Perin et al. confirmed that transendocardial cell implantation appreciably moderates manifestations of heart failure and angina pectoris and improves left ventricular function. Beneficial effect of cellular therapy may persist as long as 12 months. It is worth to note that five of the 14 patients on the waitlist did not eventually need heart transplantation [14]. A similar result of intramyocardial cell implantation was reported by other research groups for ‘no-option’ patients with refractory angina and severe myocardial ischemia [1,15–19]. However, those studies had serious drawbacks, such as the small number of patients, short follow-up period, and lack of randomization. In the present study, we tried to compensate for these shortcomings and obtained similar results. Although most clinical data, electroanatomical mapping, left ventricular function, and myocardial perfusion were analyzed by two researchers who were unaware of other clinical and imaging materials, positive effect of placebo cannot be ruled out. It was a serious limitation of the study. Therefore, the present findings need confirmation in randomized, double blind placebo-controlled studies comprising a large cohort of patients with chronic myocardial infarction and severely depressed LV function. Nevertheless, even the available data suggest the value of cellular therapy as an intermediate treatment.

NOGA mapping may be used to optimize the application of cellular therapy in clinical practice. Exact electrical and mechanical mapping allows the best implantation site to be selected and bone marrow cells introduced within its small limits. In this way, maximum cellular therapy benefits may be attained. Repeated NOGA mapping in the present study revealed improvement of both electrical activity and contractility of myocardium in the treated patients not only at implantation sites but also in the adjoining myocardial regions.

To conclude, intramyocardial bone marrow cell transplantation in patients with chronic ischemic heart disease and marked left ventricular dysfunction is safe and improves clinical outcome. Cell injections into ischemic segments promote perfusion without inducing additional scarry areas. Unfortunately, there is currently no other alternative to the adequate treatment of patients with manifest ischemic heart failure producing the minimal number of negative effects and giving patients a real chance to benefit from this therapy. Our findings can be expected to stimulate new clinical studies aimed at elucidating the role of intramyocardial bone marrow cell transplantation in patients with chronic ischemic heart disease.

References

1. Tse, H. F. Kwong, Y. L. Chan, J. K. Lo, G. Ho, C. L. & Lau, C. P. (2003). Angiogenesis in ischemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet, 361*, 47–49.
2. Fuchs, S. Satler, L. F. Kornowski, R. et al. (2003). Catheter-based autologous bone marrow myocardial injection in no-option patients with advanced coronary artery disease. *Journal of the American College of Cardiology, 41*, 1721–1724.
3. Perin, E. C. Dohmann, H. F. Borojevic, R. et al. (2003). Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation, 107*, 2294–2302.
4. Perin, E. C. Dohmann, H. F. Borojevic, R. et al. (2004). Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. *Circulation, 110*(suppl II), II-213–II-218.
5. Beeres, S. Bax, J. Dibbets, P. Stokkel, M. Zeppenfeld, K. et al. (2006). Effect of intramyocardial injection of autologous bone marrow—derived mononuclear cells on perfusion, function, and viability in patients with drug-refractory chronic ischemia. *Journal of Nuclear Medicine, 47*, 574–580.
6. Perin, E. C. Silva, G. V. Sarmento-Leite, R. Sousa, A. L. Howell, M. et al. (2002). Assessing myocardial viability and infarct transmurality with left ventricular electromechanical mapping in patients with stable coronary artery disease: Validation by delayed-enhancement magnetic resonance imaging. *Circulation, 106*, 957–961.
7. Wrobleski, D. Houghtaling, C. Josephson, M. E. Ruskin, J. N. & Reddy, V. Y. (2003). Use of electrogram characteristics during sinus rhythm to delineate the endocardial scar in a porcine model of healed myocardial infarction. *Journal of Cardiovascular Electrophysiology, 14*, 524–529.
8. Marchlinski, F. E. Callans, D. J. Gottlieb, C. D. & Zado, E. (2000). Linear ablation lesions for control of unmappable ventricular tachycardia in patients with ischemic and nonischemic cardiomyopathy. *Circulation, 101*, 1288–1296.

9. Ben-Haim, S. A. Osadchy, D. Schuster, I. Gepstein, L. Hayam, G. et al. (1996). Nonfluoroscopic, in vivo navigation and mapping technology. *Nature Medicine, 2*, 1393–1395.
10. Gepstein, L. Hayam, G. & Ben-Haim, S. A. (1997). A novel method for nonfluoroscopic catheterbased electroanatomical mapping of the heart. In vitro and in vivo accuracy results. *Circulation, 95*, 1611–1622.
11. Berman, D. S. Hachamovitch, R. Kiat, H. Cohen, I. Cabico, J. A. et al. (1995). Incremental value of prognostic testing in patients with known or suspected ischemic heart disease: A basis for optimal utilization of exercise technetium-99m sestamibi myocardial perfusion single-photon emission computed tomography. *Journal of the American College of Cardiology, 26*, 639–647.
12. Liu, Y. Guo, J. Zhang, P. et al. (2004). Bone marrow mononuclear cell transplantation into heart elevates the expression of angiogenic factors. *Microvascular Research, 68*, 156–160.
13. Orlic, D. Hill, J. & Arai, A. (2002). Stem cells for myocardial regeneration. *Circulation Research, 91*, 1092–1102.
14. Silva, G. Perin, E. Dohmann, H. Borojevic, R. Silva, S. et al. (2004). Catheter-based transendocardial delivery of autologous bone-marrow-derived mononuclear cells in patients listed for heart transplantation. *Texas Heart Institute, 31*, 214–219.
15. Dohmann, H. Silva, S. Souza, A. Rossi, M. Takiya, C. & Borojevic, R. (2007). Bone-marrow mononuclear cell therapy of severe ischemic heart failure. *C. R. Biologies, 330*, 543–549.
16. Fuchs, S. Satler, L. Kornowski, R. Okubagzi, P. Weisz, G. et al. (2003). Catheter-based autologous bone marrow myocardial injection in nooption patients with advanced coronary artery disease: A feasibility study. *Journal of the American College of Cardiology, 41*, 1721–1724.
17. Fuchs, S. Kornowski, R. Weisz, G. Satler, L. Smits, P. et al. (2006). Safety and feasibility of transendocardial autologous bone-marrow cell transplantation in patients with advanced heart disease. *American Journal of Cardiology, 97*, 823–829.
18. Beeres, S. Bax, J. Dibbets-Schneider, P. Stokkel, M. Fibbe, W. et al. (2006). Sustained effect of autologous bone marrow mononuclear cell injection in patients with refractory angina pectoris and chronic myocardial ischemia: Twelve-month follow-up results. *American Heart Journal, 152*, 11–16.
19. Izawa, H. Kondo, T. Usui, A. Yamamoto, K. Shintani, S. et al. (2006). Clinical protocol for angiogenesis by intramyocardial injection of autologous bone marrow mononuclear cells in patients with severe coronary artery disease. *Circ. J, 70*, 1180–1183.