Influence of bone marrow stem cells on left ventricle perfusion and ejection fraction in patients with acute myocardial infarction of anterior wall: randomized clinical trial

Impact of bone marrow stem cell intracoronary infusion on improvement of microcirculation

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Aims
Randomized trial to assess change in left ventricle ejection fraction (LVEF) and myocardial perfusion in patients with acute myocardial infarction (AMI) of anterior wall treated with bone marrow stem cells (BMSCs), compared with control group—from baseline in the acute phase up to 12 months of follow-up.

Methods and results
Forty-five patients were randomized 2:1 to BMSC group (n = 31) or to control group (n = 14). Bone marrow stem cells were administered into infarct-related artery (IRA) at 4–6 day after primary PCI. Groups were followed up with Tc-99m-MIBI SPECT, radionuclide ventriculography (EF-RNV), echocardiography (ECHO), and spiroergometric stress test. Coronary angiography was repeated after 6 months. EF-RNV did not differ significantly in both groups, but trend towards increase in EF at 6 months and its maintenance after 12 months was noticed in the BMSC group. At rest study, perfusion index (PI) of region supplied with blood by IRA distal to its previous occlusion (PI-IRA) improved significantly in the BMSC group at 6 months: PI-IRA at 4–6 days vs. PI-IRA at 6 months (3.00 ± 0.97 vs. 2.65 ± 0.64; P = 0.017). At 12 months, PI-IRA at rest was 2.66 ± 0.55; P = 0.07. The difference between BMSC and control groups at rest study in PI-IRA was not observed. At dipyridamole study (PI-dip), perfusion in the BMSC group was better compared with controls at 6 months (2.26 ± 0.44 vs. 2.47 ± 0.40; P = 0.033) and at 12 months (2.34 ± 0.55 vs. 2.52 ± 0.42; P = 0.014), also for region supplied with blood by IRA (PI-IRA-dip: at 6 months 2.63 ± 0.77 vs. 3.06 ± 0.46; P = 0.021 and at 12 months 2.71 ± 0.63 vs. 3.15 ± 0.51; P = 0.001). Results of LVEF, LVEDV, LVESV in ECHO and results of spiroergometric stress test did not differ significantly between groups. Major adverse cardiac events occurred more often in the control group (P = 0.027).

Conclusion
In our study, BMSC intracoronary transplantation in patients with anterior AMI did not result in increase in EF. Slight improvement of myocardial perfusion was noticed in the BMSC group. This finding may indicate better microcirculation enhanced by BMSCs, but small number of patients allow for hypothesis rather than final statement.

Keywords
Stem cells • Angiogenesis • Myocardial regeneration • Acute myocardial infarction
Introduction

Bone marrow stem cells transplantation may influence the course of acute myocardial infarction (AMI), leading to a better perfusion and function of left ventricle after AMI and may diminish its post-myocardial remodelling.1–3 Bone marrow stem cells may be able to multiply and differentiate into new blood-vessel cells itself or to enhance mobilization of resident cardiac stem cells mainly in a paracrine manner.4–6 Transdifferentiation of BMSC into new cardiomyocytes is less probable.7 Results of clinical trials are confusing. There are studies by Strauer et al.,8 Stamm et al.,9 TOPCARE-AMI,10 and REPAIR-AMI11 in favour of BMSCs. The results of Janssens et al. study,12 ASTAMI,13 and BOOST trial with 18 months of follow-up14 and REGENT trial (Hotline, ESC Congress August–3 September 2008) turned out to be disappointing. Here, we present the results of our study on the assessment of left ventricle perfusion and ejection fraction (EF) in patients with their first AMI of anterior wall who were treated with intracoronary BMSC infusion.

Methods

Study design and protocol

A single centre, clinical randomized trial was conducted between June 2003 and June 2006 in the First Department of Cardiology, Poznań University of Medical Sciences, in collaboration with the Department of Hematology and Department of Endocrinology. The study protocol, complied with the Declaration of Helsinki, was approved by the local Ethics Committee. Safety monitoring board was informed of adverse events.

The study design (Figure 1) consists of 62 consecutive patients admitted to our department with the first AMI of anterior wall between June 2003 and June 2006, of which 54 were eligible, and 45 gave written informed consent. Total number of primary PCI in our department between June 2003 and June 2006 was 1794. The patients were randomized in a two-to-one ratio using prepared envelopes with group assignment either to a group receiving BMSCs (n = 31) or to a control group (n = 14). Patients were assigned to BMSC or control group by means of restricted randomization (permuted blocks randomization). The block size was 6 and the number of block was chosen using a computer random number generator. Patients having numbers 1–4 were allocated to the treatment group, whereas patients having numbers 5 or 6 were allocated to the control group (2:1 ratio). The inclusion criteria were: first AMI of anterior wall with ST elevation within 12 h from symptom onset, age between 35 and 70, and the absence of critical stenosis in other than left anterior descending (LAD), which was infarct-related artery (IRA). The exclusion criteria were: previous MI, insulin-dependent diabetes mellitus, cardiogenic shock, pulmonary oedema, renal or hepatic dysfunction, and other severe disease or suspected inability to comply with the study protocol. Patients underwent immediate primary angioplasty with bare metal stent implantation. They were eligible for the study when the IRA patency was obtained with thrombolysis in myocardial infarction (TIMI) 2 or 3 flow. The study was not blinded for the patients. Patients from the BMSC group underwent bone marrow cells aspiration from the pelvic bones between 4 and 5 days. Repeated coronary angiography was performed in both groups at 5–6 days, and BMSCs were administered via IRA in the BMSC group. Echocardiography (ECHO), Tc-99m-MIBI SPECT myocardial rest perfusion study, and radionuclide ventriculography (EF-RNV) were performed at 4–6 days. ECHO and Tc-99m-MIBI SPECT rest and dipyridamole studies were repeated after 3, 6, and 12 months. Radionuclide ventriculography was carried out after 6 and 12 months. Coronary angiography was repeated after 6 months. Investigators assessing RNV, SPECT, TIMI flow, corrected TIMI frame count (CTFC), myocardial blush grade (MBG), ECHO, and cardiopulmonary exercise testing were blinded to the group assignment.

Initially, we planned to gain 80% power to detect a significant difference in LV-EF estimated 4–5% (EF-RNV) and Δ0.3 Pl of LV myocardial perfusion between baseline and 12 months after the acute phase at a two-sided significance level of 5%. Therefore, in order to reach that, we planned to recruit about 50–60 patients in each group within 2 years. Unfortunately, although we managed to prolong the recruitment period to 3 years, during that period only 62 patients admitted with acute AMI of anterior wall to our centre fulfilled the rigid inclusion criteria initially and only 45 of 54 eligible patients did agree to participate into this experimental study. Thus, we were forced to accept that small final number of patients.

Outcomes

The primary outcome of our study was the change in left ventricle function from baseline (acute phase of AMI) up to 12 months of follow-up, analysed at 3, 6, and 12 months. Therefore we measured:

1. left ventricle perfusion: with Tc-99m-MIBI SPECT rest and dipyridamole studies;
2. left ventricle EF: with radionuclide ventriculography.

Additionally, as secondary outcomes, we assessed changes in left ventricle end-systolic volume, left ventricle end-diastolic volume, and left ventricle wall motion score index (WMSI) with ECHO, changes in results of cardiopulmonary exercise testing, and the occurrence of major adverse cardiac events (MACE): death, AMI, and need for revascularization.

Bone marrow collection and preparation

Bone marrow from the pelvic bones was collected to phosphate-buffered saline (PBS) with heparin (50 U/mL) from 31 patients under local anaesthesia. Total volume of aspirates ranged from 50 to150 mL, mean 80 mL. The material was diluted 1:2 with PBS and then centrifuged on Ficoll gradient (400 g, 10 min, 18 °C). Mononuclear cells were removed from the interphase between the red cells and the Ficoll. After washing in PBS with heparin (200g, 10 min, 18 °C), cells were resuspended in a few millilitres of X-vivo 15 medium (BioWhittaker, USA) with 2% heat-inactivated autologous plasma. The cells were counted automatically using Micros 60 (ABX, Germany). The haematopoietic cells were identified by three-colour immunofluorescence using FACs scan flow cytometry (Becton-Dickinson, USA).

The following antibodies were used: CD34-FITC, CD133-PE, CD45-APC. Mononuclear cells (1 × 10^6 in 1 mL) were placed in Teflon bags (Vuelife, Cell Genix, USA) and overnight cultivated (5% CO2, 37 °C, 100% humidity) in X-vivo 15 medium with 2% heat-inactivated autologous plasma. The next day, cells were harvested and washed three times with heparinized PBS. Viability with the use of trypan blue test was 94 ± 3%. Microbiological tests of the clinically used cell preparations were negative.

Tc-99m-MIBI SPECT and EF-RNV

Myocardial perfusion SPECT study was performed with the use of Tc-99m-MIBI at 4–6 days at rest. ECHO and Tc-99m-MIBI SPECT rest and dipyridamole studies were repeated after 3, 6, and 12 months. Radionuclide ventriculography was carried out after 6 and 12 months. Coronary angiography was repeated after 6 months. Investigators assessing RNV, SPECT, TIMI flow, corrected TIMI frame count (CTFC), myocardial blush grade (MBG), ECHO, and cardiopulmonary exercise testing were blinded to the group assignment.
protocol with rest and dipyridamole studies were carried out. Rest studies were performed using 740 MBq (20 mCi) of Tc-99m-MIBI (technetium generator by Amersham Health, MIBI by Polatom, Poland). On the other day, pharmacological stress was performed using dipyridamole (Curantyl, Berlin-Chemie, Germany) infused at a rate of 0.56 mg/kg body weight over 4 min (‘low dose’), followed by the injection of 740 MBq (20 mCi) of Tc-99m-MIBI 2 min later. Acquisition of SPECT images started 60–90 min after the tracer injection. Images were obtained by the dual-head Varicam gamma camera (Elscint). Semi-quantitative evaluation of the images was based on the assessment of Tc-99m-MIBI uptake in the left ventricle divided into 16 myocardial segments and scored in a five-point scale by two independent observers blinded to group assignment; 5, no uptake; 4, severe defect; 3, moderate defect; 2, slight impairment of uptake; and 1, normal isotope uptake in a segment. Perfusion index (PI) was then calculated in the same manner as WMSI (PI = \sum \text{points}/16). The PI was calculated after 4–6 days at rest (PI-rest 4-6 day); after 3, 6, and 12 months. Wall motion score index was calculated in a four-point scale, LV was divided in 16 myocardial segments model.15

To assess contractility and perfusion of the infarcted and ischaemic region, WMSI and PI were calculated separately for segments supplied with blood by IRA distal to its previous occlusion; WMSI-IRA and PI-IRA, respectively.

**Echocardiography**

Left ventricular end-diastolic and end-systolic volumes (LVEDV and LVESV) were measured, LVEF, and WMSI were calculated at 4–6 days, 3, 6, and 12 months. Wall motion score index was calculated in a four-point scale, LV was divided in 16 myocardial segments model.15

To assess contractility and perfusion of the infarcted and ischaemic region, WMSI and PI were calculated separately for segments supplied with blood by IRA distal to its previous occlusion; WMSI-IRA and PI-IRA, respectively.

**Coronary angiography and myocardial perfusion**

Coronary angiography was performed on admission, at 4–6 days of AMI and after 6 months. During the first study, PCI with stent implantation was carried out. During second coronary angiography and after

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**Figure 1** Flow chart of the study design.
6 months, LAD patency was checked. TIMI flow, CTFC, and MBG were calculated as described elsewhere

Bone marrow stem cell administration

The BMSC material was divided into three to four portions, containing 3–4 mL cell suspension each. Then the material was administered via IRA to the infarcted zone with the use of a stop-flow technique through an over-the-wire-balloon catheter as described by Strauer et al.8

Cardiopulmonary exercise testing

Patients performed maximal treadmill exercise test according to modified Bruce protocol at 20–30 days after AMI and according to Bruce protocol after 3, 6, and 12 months. The peak oxygen consumption (peak VO2), carbon dioxide production (VCO2), and minute ventilation (VE) were measured by breath-by-breath technique, using Sensor Medics, model Vmax29. A standard 12-lead ECG was continuously recorded. Peak VO2 was determined as an average value during last 20 s of exercise, and expressed in mL/kg/min, in L/min, and as the percentage of predicted peak oxygen consumption. Anaerobic threshold was defined by V-slope analysis. VO2 at anaerobic threshold (VO2 AT), oxygen pulse (pulse O2), and VE/VCO2 slope were analysed.

Statistical analysis

Continuous variables are presented as mean values with standard deviation, or median with average deviation, where mentioned. In Tables 1 (clinical characteristics of groups) and 2 (characteristics of myocardial perfusion), continuous variables were compared with the use of Mann–Whitney U test, and categorical variables were compared using the χ2 test or Fisher’s exact test. Unequal N HSD Tukey test was defined by V-slope analysis.

Table 1 Characteristics of the patients and of acute myocardial infarction

<table>
<thead>
<tr>
<th></th>
<th>BMSC group</th>
<th>Control group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Men/women</td>
<td>27.4</td>
<td>12.2</td>
<td>0.932</td>
</tr>
<tr>
<td>Age</td>
<td>49.9 ± 8.4</td>
<td>50.9 ± 9.3</td>
<td>0.833</td>
</tr>
<tr>
<td>Hypertension</td>
<td>15 (48%)</td>
<td>9 (64%)</td>
<td>0.256</td>
</tr>
<tr>
<td>NID diabetes mellitus/IGT</td>
<td>2/1 (9%)</td>
<td>0/2 (14%)</td>
<td>0.220</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>24 (77%)</td>
<td>10 (71%)</td>
<td>0.709</td>
</tr>
<tr>
<td>BMI</td>
<td>27.7 ± 4.1</td>
<td>26.0 ± 3.5</td>
<td>0.312</td>
</tr>
<tr>
<td>Smoking</td>
<td>23 (74%)</td>
<td>11 (78%)</td>
<td>0.665</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>19 (61%)</td>
<td>11 (78%)</td>
<td>0.166</td>
</tr>
<tr>
<td>ST-segment elevation sum before PCI*</td>
<td>17.8 ± 11.8</td>
<td>18.0 ± 9.5</td>
<td>0.588</td>
</tr>
<tr>
<td>ST-segment elevation sum after PCI*</td>
<td>9.5 ± 4.8</td>
<td>10.8 ± 9.0</td>
<td>0.403</td>
</tr>
<tr>
<td>Maximal CPK*</td>
<td>2648 ± 1556</td>
<td>3105 ± 1100</td>
<td>0.364</td>
</tr>
<tr>
<td>Time from pain to balloon (min)*</td>
<td>290 ± 234</td>
<td>190 ± 212</td>
<td>0.248</td>
</tr>
<tr>
<td>Proximal LAD lesion</td>
<td>16 (51%)</td>
<td>10 (71%)</td>
<td>0.375</td>
</tr>
</tbody>
</table>

*Data are presented as median ± average deviation; P-value was estimated using Mann–Whitney U test.

Table 2 Parameters of myocardial perfusion

<table>
<thead>
<tr>
<th></th>
<th>BMSC group (n = 31)</th>
<th>Control group (n = 14)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary angiography during acute phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIMI after PCI</td>
<td>III-27, II-4</td>
<td>III-11, II-3</td>
<td>0.391</td>
</tr>
<tr>
<td>CTFC after PCIa</td>
<td>21.0 ± 11.8</td>
<td>24.0 ± 6.9</td>
<td>0.362</td>
</tr>
<tr>
<td>MBG after PCI</td>
<td>III-13, II-11, I-4, O-3</td>
<td>III-2, II-3, I-8, O-1</td>
<td>0.024b</td>
</tr>
</tbody>
</table>

*aData are presented as median ± average deviation; P-value was estimated using Mann–Whitney U test.

*bStatistical difference found between groups; discussed in: MBG and CTFC influence on improvement in SPECT study.

Results

Clinical characteristics

Clinical characteristics did not reveal any significant differences between the BMSC group and the control group (Table 1).

Characteristics of bone marrow cells

Mean collected bone marrow volume was 80 ± 30 mL, total cell number 2.34 ± 1.2 x 109, bone marrow final volume 12.25 ± 2.05 mL, mononuclears 0.410 ± 0.18 x 109, CD34+ cells count 3.89 ± 1.45 x 106, CD133+/CD45+ cells count 0.96 ± 0.6 x 106, and CD133+/CD45− cells count 0.15 ± 0.1 x 106.

Tc-99m-MIBI SPECT results—PI of left ventricle and of segments supplied with blood by infarct related artery (PI-IRA): Table 4: results of ECHO—LVEF, LVEDV, LVESV, WMSI and WMSI-IRA; Figure 2: EF-RNV and indeces of perfusion (PI-rest, PI-dip, PI-IRA dipyridamole during 12 month follow-up; variables were compared with use of ANCOVA—N HSD Tukey test; Figures 4–7: correlation between EF-RNV and indeces of perfusion (PI-rest, PI-dip, PI-IRA rest, PI-IRA dip, respectively) after 12 months was analyzed with method of linear regression; Figure 8: freedom from combined MACE (death, AMI, need for revascularization) was assumed with Kaplan-Meier analysis. Statistical significance was assumed at a value of P < 0.05; all tests were two-sided. Analysis was performed with the use of StatSoft, Inc. (2004) STATISTICA data analysis software system, version 7.1, www.statsoft.com.
Parameters of myocardial perfusion
Myocardial blush grade after PCI in acute phase and CTFC at 4–6 days differed significantly between groups, but neither parameter differed significantly after 6 months (Table 2).

Radionuclide imaging
Radionuclide imaging was done after 12 months in 30 patients from the BMSC group and 14 controls. Results of patients with critical restenosis or progression of atherosclerosis defined as >70% stenosis in coronary angiography between 6 and 12 months were excluded from final analysis (final analysis: BMSC group n = 27, control group n = 12).

Ejection fraction measured by radionuclide ventriculography
There were no differences between groups in EF. However, trend towards increase in EF at 6 months and its maintenance after...
Figure 2 Ejection fraction measured by radionuclide ventriculography.

Figure 3 PI-IRA dipyridamole during 12 months of follow-up (improvement in BMSC group vs. control group).
**Figure 4** Correlation between EF-RNV and PI-rest after 12 months.

**Figure 5** Correlation between EF-RNV and PI-dip after 12 months.
Figure 6 Correlation between EF-RNV and PI-IRA-rest after 12 months.

Figure 7 Correlation between EF-RNV and PI-IRA-dip after 12 months.
12 months was noticed in the BMSC group. In the control group, EF tended to decrease (Figure 2), but this was insignificant.

Scintigraphy

There were no significant differences in PI of the whole left ventricle at rest study between groups (PI-rest; Table 3). However, at dipyridamole study (PI-dip; Table 4), the results were better in the BMSC group compared with the control group (at 6 months: 2.26 ± 0.44 vs. 2.47 ± 0.40; P = 0.033 and at 12 months: 2.34 ± 0.55 vs. 2.52 ± 0.42; P = 0.014). In particular, differences were observed for segments supplied with blood by IRA (PI-IRA; Table 3). PI-IRA at rest study was better in the BMSC group at 6 months compared with the control group (2.65 ± 0.64 vs. 2.93 ± 0.47; P = 0.04). However, the difference was not significant at 12 months (2.66 ± 0.53 vs. 2.89 ± 0.39; P = 0.105).

Within the BMSC group, PI-IRA at rest study improved significantly at 6 months (3.00 ± 0.79 at 4–6 days vs. 2.73 ± 0.58 at 6 months; P = 0.017). At 12 months, PI-IRA was 2.66 ± 0.55; P = 0.076. We did not observe significant improvement of myocardial perfusion within the control group at any period of follow-up, neither at rest, nor at dipyridamole study. At 6 and 12 months at dipyridamole study (PI-IRA dip, Figure 3), a significant difference was found between groups. PI-IRA dip in the BMSC group vs. controls was 2.63 ± 0.77 vs. 3.06 ± 0.46; P = 0.021 at 6 months and 2.71 ± 0.63 vs. 3.15 ± 0.51; P = 0.001 at 12 months.

Correlation between radionuclide ventriculography and the PI after 12 months for the left ventricle and for segments supplied with blood by IRA was similar in both groups and did not differ significantly (Table 5). However, subgroup of patients with EF > 50% and better myocardial perfusion (PI ≤ 2.2) consisted mainly of patients from the BMSC group. PI-rest and RNV correlation: 10 patients (37%) from the BMSC group vs. 3 patients (25%) from the control group (P = 0.486; Figure 4); PI-dip and RNV correlation: 9 patients (33%) from the BMSC group vs. 2 patients

**Table 5** Matrix of partial correlation between perfusion index vs. group and EF-RNV at 12 months: model of slope homogeneity

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Predictive variable</th>
<th>Predictive variable</th>
<th>Predictive variable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EF-RNV 12 months</td>
<td>Group</td>
<td>Interaction EF-RNV 12 months*group</td>
</tr>
<tr>
<td>PI-rest 12 months</td>
<td>−0.558; P = 0.002</td>
<td>−0.051; P = 0.793</td>
<td>0.025; P = 0.896</td>
</tr>
<tr>
<td>PI-dipyridamole 12 months</td>
<td>−0.593; P = 0.0007</td>
<td>−0.089; P = 0.646</td>
<td>0.091; P = 0.638</td>
</tr>
<tr>
<td>PI-IRA rest 12 months</td>
<td>−0.653; P = 0.0001</td>
<td>−0.084; P = 0.628</td>
<td>−0.093; P = 0.666</td>
</tr>
<tr>
<td>PI-IRA-dipyridamole 12 months</td>
<td>−0.510; P = 0.0005</td>
<td>0.115; P = 0.533</td>
<td>−0.060; P = 0.755</td>
</tr>
</tbody>
</table>

Figure 8 Kaplan–Meier analysis: freedom from combined major adverse cardiac events (death, AMI, need for revascularization).
Kaplan–Meier analysis (no patient from control group (P = 0.292; Figure 6); PI-IRA dip and RNV correlation: 6 patients (22%) from the BMSC group vs. no patient from control group (P = 0.151; Figure 7).

Echocardiography

After 3 months of follow-up, WMSI of the whole left ventricle was better in the BMSC group vs. the control group (1.53 ± 0.20 vs. 1.53 ± 0.29; P = 0.042), but the difference in WMSI was not significant after 6 and 12 months (Table 4). No significant differences between groups were found after 3, 6, and 12 months of follow-up in LVEF, LVEDV, and LVESV (Table 4).

Cardiopulmonary exercise stress testing results

Both groups gained similar results in maximal workload; BMSC vs. control group: at 3 weeks 7.9 ± 2.4 vs. 8.5 ± 3.5 METs; at 3 months 11.1 ± 4.7 vs. 9.9 ± 4.4 METs; at 6 months 11.2 ± 5.1 vs. 10.4 ± 3.3 METs; and at 12 months 10.7 ± 4.2 vs. 10.9 ± 4.0 METs (NS). The peak oxygen consumption in BMSC vs. control group was: at 3 weeks 19.7 ± 7.4 vs. 18.4 ± 8.2 mL/kg/min; at 3 months 23.9 ± 9.4 vs. 22.3 ± 10.5 mL/kg/min; at 6 months 24.2 ± 5.2 vs. 22.0 ± 7.2 mL/kg/min and at 12 months 22.2 ± 7.4 vs. 21.8 ± 6.2 mL/kg/min (NS). Serious arrhythmia in both groups was not found during stress testing.

Adverse events

There was one sudden death in the BMSC group 7 months after AMI (autopsy was not done). One AMI and one hospitalization due to unstable angina occurred in each group. At six months, in three patients from the BMSC group (9.6%) and in four controls (28.5%), angiographic restenosis was found and PCI of IRA was performed. Between 6 and 12 months, three patients from the BMSC group and two patients from the control group needed revascularization due to clinical symptoms and progression of atherosclerosis in another than IRA coronary artery. Combined MACE (death, AMI, need for revascularization) occurred more often in the control group (P = 0.027), as it was shown by Kaplan–Meier analysis (Figure 8).

Myocardial blush grade and corrected thrombolysis in myocardial infarction frame count influence on improvement in SPECT study

In univariate analysis, the significant difference between groups were: MBG after PCI in acute phase, CTFC at 4–6 days after BMSC/placebo infusion i.e., PI-IRA-dip after 12 months, and improvement in PI-IRA rest at Tc-99m-MIBI study in the BMSC group within 12 months. Therefore, we investigated the hypothesis whether MBG after PCI in acute phase results and CTFC at 4–6 days after BMSC/placebo infusion i.e. results might be related to the improvement in SPECT results at Tc-99m-MIBI study at any time between 4–6 days, 3, 6, and 12 months. ANCOVA did not show any of these variables to be statistically significant.

Discussion

Owing to a small number of patients randomized to our trial, our results require a caution in drawing final conclusion. We did not observe improvement in EF after intracoronary BMSC transplantation. The results of our study indicate improvement of myocardial perfusion after BMSC i.c. transplantation, but not significant increase in EF during 1 year follow-up compared with the control group. We did not find significant differences in detailed ECHO parameters during 12 months of follow-up either, except for transient improvement in WMSI at 3 months’ observation. Exercise capacity and tolerance, MVO₂ and ischaemia results were similar in both groups.

Meta-analysis of 10 clinical trials on BMSC i.c. therapy in the setting of AMI²⁹ (n = 698, follow-up 6 months) revealed that patients receiving BMSC had significant although small improvement in LVEF [3.0% (95% CI 1.9–4.1); P < 0.001] and lower MACE rate: recurrent MI (P = 0.04), trend towards reduced death, rehospitalization for CHF, and repeat revascularization. Particularly in REPAIR-AMI,³¹ better improvement in EF in the BMSC group was found, but these populations were not homogenous. In REPAIR-AMI, 76% of patients presented with LAD as IRA in the placebo group, whereas it was only 64% in the BMSC group (P = 0.09). Inferior or lateral wall AMI are less prone to remodeling. In the placebo group, 21% of patients suffered from DM, whereas in the BMSC group it was only 12% (P = 0.07), and at discharge, 16% of patients from the placebo group were treated with aldosterone antagonists, whereas it was only 5% in the BMSC group (P = 0.01). Janssens et al. in their study³² observed comparable increase in EF after 4 months in both groups (from 48.5 ± 7.2 to 51.8 ± 8.8% in the BMSC group vs. 46.9 ± 8.2 to 49.1 ± 10.7% in the placebo group; NS). In REPAIR-AMI, 80% of patients from both groups, and in Janssens et al. study, > 72% of the BMSC group and 62% of the placebo group were treated with GP IIb/IIIa blocker, mainly with abciximab, which is not only a GP IIb/IIIa blocker, but also a potent monoocyte inhibitor reducing no reflow. In addition, one-fifths of patients in both groups from Janssens et al. study presented with TIMI 3 in IRA on admission, due to previous thrombolysis. These findings, although unintended, might have influenced the results.

We emphasize that in our study, the population is homogenous, only with anterior wall MI, like in ASTAMI.³³ In ASTAMI, there were no significant differences between groups in EF, LVEDV, or infarct size, and no correlation between the increase in EF and the number of mononuclear cells injected.

There is evidence from studies on BMSCs in mice¹ and from studies in vitro with human adipose-derived stem cells²⁰ that these stem cells may differentiate into myocytes independent of cell fusion. After i.c. administration of adipose-derived stem cells in porcine model of AMI, only engraftment of these cells around vasculature was confirmed.²² The hypothesis against BMSC differentiation into cardiomyocytes in humans after i.c. administration is supported by results of BOOST trial, where cardiac MRI showed that BMSCs did not result in the reduction of late contrast enhancement at 6 and 18 months. The difference in EF improvement between groups in BOOST trial was significant after 6 months but not after 18 months.¹⁴
Some differences in the perfusion pattern were observed in the BMSC group compared with the control group. At Tc-99m-MIBI SPECT myocardial perfusion rest study, we observed improvement of PI and PI-IRA in the BMSC group compared with controls. In the BMSC group, at dipyridamole SPECT study, PI-dip and PI-IRA-dip did not worsen, as it was observed in the control group. The possible explanation is better microcirculation in the BMSC group. As we have excluded patients with restenosis or significantly stenosed another vessel from final scintigraphic analysis in our study, therefore the protocol with dipyridamole let us to some extent assess the response of microcirculation reserve to adenosine. Better results of myocardial perfusion study in the BMSC group (both at rest and at dipyridamole study) confirm the hypothesis that BMSCs may react mainly through the enhancement of neovascularization. We should be very careful in the interpretation of Tc-99m-MIBI SPECT results, because we used a semi-quantitative method. However, in the KAT trial, the same scintigraphic method was used to assess myocardial perfusion in patients with chronic CAD treated with PCI only and with PCI and VEGF. Tc-99m-MIBI rest and dipyridamole imaging studies improved significantly in the VEGF group compared with the control group with PCI only.

The concept that BMSCs may promote vascular repair was postulated also by the authors of i.c. Doppler sub-study of REPAIR-AMI24 and in previously mentioned porcine model of AMI and adipose-derived stem cells. In our study, a significant difference between groups in MBG after PCI in acute phase and CTFC at 4–6 days after BMSC infusion in favour of the BMSC group might have contributed to better results of SPECT study in the BMSC group. However, analysis of covariance ruled out the hypothesis that SPECT results at rest and after dipyridamole in the BMSC group or in the control group were affected by MBG in the acute phase and CTFC at 4–6 days.

In our observation, the subgroup of patients with better EF (EF-RNV ≥ 50%) and myocardial perfusion (PI < 2.2) consisted mainly of patients from the BMSC group. Particularly, when PI-IRA at rest and PI-IRA at dipyridamole study and RNV correlation were analyzed, only patients from the BMSC group have been found in that subgroup. This observation may confirm the hypothesis of better perfusion within the IRA region due to BMSC administration. We are aware of the fact that due to a small number of patients included into our study, we can only speculate on BMSC influence on microcirculation enhancement. Therefore, we think that this very interesting hypothesis should be tested in other trials with BMSCs in AMI patients in order to draw final statistical conclusion.

Comparison of two methods: suspension in Ficol and storage in X-vivo 10 medium plus serum as in the REPAIR-AMI, and suspension in Lymphoprep and storage in NaCl plus plasma as in ASTAMI, showed better results of migratory capacity of BMSCs and blood flow recovery after ischaemia in REPAI-AMI method. In REPAIR-AMI, a larger number of stem cells, compared with ASTAMI, were injected. Despite being similar to REPAIR-AMI method and the number of cells, in our study we have been unable to find significant increase in EF in the BMSC group compared with controls, although the trend towards better EF improvement or its maintenance in the BMSC group was noticed.

Coronary infusion of BMSCs was safe; in 29 patients, we did not observe any complications. One patient had fever and chills 2 h after infusion, and in one patient we observed sudden increase in blood pressure, with no further complications in both cases. We did not find increased rate of restenosis or progression of atherosclerosis in coronary angiography performed 6 months after AMI in the BMSC group. Lower rate of combined MACE in the BMSC group, found in Kaplan–Meier analysis ($P = 0.027$), suggests additional positive influence of BMSC therapy.

**Study limitations**

We planned to recruit about 100–120 patients within 2 years (estimated sample size 50–60 patients in each group to assess 4–5% difference in ejection fraction and ΔPI = 0.3 of left ventricle perfusion). However, our inclusion criteria restricted to anterior wall AMI in order to have homogenous population let us recruit only 45 patients who agreed from 54 eligible within 3 years, even though we prolonged the recruitment period up to 3 years.

Although further follow-up is conducted, the results may be influenced by restenosis or/and progression of atherosclerosis in both groups.

Owing to technical limitations, we were able to calculate only semi-quantitative analysis of Tc-99m-MIBI SPECT, though performed by two observers independent and blinded to group assignment. However, the same semi-quantitative method was used also in the KAT trial and many other studies on myocardial ischaemia.

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