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Determinants of functional recovery after myocardial infarction of patients treated with bone marrow-derived stem cells after thrombolytic therapy

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ABSTRACT

Objective To assess the determinants of functional recovery in patients with ST-elevation myocardial infarction (STEMI) treated initially with thrombolysis, followed by percutaneous coronary intervention and intracoronary injection of bone marrow-derived stem cells (BMC).

Design A randomised, placebo-controlled, double-blind study (substudy of FINCELL).

Setting Two tertiary cardiac centres.

Participants 78 patients with STEMI randomly assigned to receive either intracoronary BMC (n=39) or placebo (n=39) into the infarct-related artery.

Interventions Thrombolysis a few hours after symptom onset, percutaneous coronary intervention and intracoronary injection of BMC 2–6 days later.

Main outcome measures Efficacy of the BMC treatment was assessed by measurement of the change of global left ventricular ejection fraction (LVEF) from baseline to 6 months after STEMI. Various predefined variables (eg, the levels of certain natriuretic peptides and inflammatory cytokines) were analysed as determinants of improvement of LVEF.

Results In the BMC group, the most powerful determinant of the change in LVEF was the baseline LVEF (r=−0.58, p<0.001). Patients with baseline LVEF at or below the median (≤62.5%) experienced a more marked improvement in LVEF (+12.7±12.5% units, p<0.001) than those above the median (−0.8±6.3% units, p=0.10). Elevated N-terminal probrain natriuretic peptide (p<0.001) and N-terminal proatrial natriuretic peptide (p=0.052) levels were also associated with improvement in LVEF in the BMC group but not in the placebo group.

Conclusions The global LVEF recovers most significantly after intracoronary infusion of BMC in patients with the most severe impairment of LVEF on admission. The baseline levels of natriuretic peptides seem also to be associated with LVEF recovery after BMC treatment.

Trial registration ClinicalTrials.gov number, NCT00363324.

The intracoronary administration of autologous bone marrow-derived stem cells (BMC) has been suggested to restore myocardial damage in patients with acute ST-elevation myocardial infarction (STEMI). It has also been proposed that BMC enhance cardiac performance through an improvement of global left ventricular ejection fraction (LVEF) when administered after primary percutaneous coronary intervention (PCI) in patients after an acute STEMI.1−3 Despite the putative benefits of BMC administration, some studies have found the effect of BMC therapy to be rather modest or even absent.4−9 This may be partly due to the different baseline characteristics of study populations, such as the timing of BMC therapy after STEMI, dose and viability of BMC and infarct size. Inflammation after STEMI may also be one potential factor modifying the treatment effect. One previous study in patients treated with primary PCI followed by BMC administration suggested that the recovery of LVEF is greatest in those patients experiencing the most severe impairment in LVEF at baseline.5 However, other determinants to predict the treatment effect have not yet been fully studied.

We have previously shown that intracoronary BMC therapy results in a slight improvement of the global left ventricular function in STEMI patients treated with thrombolysis followed by PCI a few days later.10 The aim of the present study, which is part of the FINCELL trial, was to study the determinants of functional recovery after STEMI. Therefore, we examined several demographic and clinical factors that have previously been proposed to influence patient outcome. Our primary hypothesis was that elevated natriuretic peptides or the degree of inflammation early after STEMI would play some role in the prediction of the benefits of BMC therapy in addition to baseline LVEF.

PATIENTS AND METHODS

We examined a consecutive series of STEMI patients treated with thrombolytic therapy who were admitted to the University Hospital of Oulu (n=68) and University Hospital of Turku (n=12), Finland, between October 2004 and February 2007. Inclusion criteria were age less than 75 years, both ECG and enzymatic evidence of STEMI, and thrombolytic therapy initiated within 12 h after the onset of symptoms. Exclusion criteria were primary PCI, cardiogenic shock, rescue PCI due to chest pain, haemodynamic instability or lack of resolution of ST-segment elevations after thrombolysis, or a severe coexisting condition that would have interfered with the ability of the patient to comply with the protocol. Written informed consent was obtained from patients within 2 days after thrombolytic therapy. The study protocol conformed to...
the Declaration of Helsinki and was approved by the Ethical Committee of the Northern Ostrobothnia Hospital District.

**Study design**

The day of thrombolysis of acute STEMI was defined as day 0. On days 1–2, patients were randomly assigned, in a double-blinded fashion and in a 1:1 ratio, to either the BMC group or the placebo group, as described previously. Bone marrow aspiration, collection and preparation of cells were performed locally in both study centres in the morning preceding the PCI, which was performed 2–6 days after thrombolysis.

**Cell preparation and administration**

A total of 80 ml bone marrow was aspirated into heparin-treated syringes from the posterior iliac crest under local anaesthesia. Mononuclear cells were isolated from the aspirate using density gradient centrifugation on Ficoll–Hypaque. After being washed twice with heparinised physiological saline, the mononuclear cells were suspended in 10 ml medium containing 5 ml of the patient’s own serum and heparinised physiological saline. Then the BMC suspension was filtered through 100 μm nylon mesh (BD Falcon Cell Strainer; BD Biosciences, Erembodegem, Belgium) and subjected to quality-control procedures, that is, microbial culture for sterility and flow cytometry analysis for CD34+ cell counting in the accredited laboratory of the Oulu University Hospital, which is subjected to both outside and inside quality control. The BMC separation procedure took approximately 3 h and the intracoronary injection of the cells was performed within 3 h after the procedure. The placebo medium contained physiological saline. The validity of the cell preparation system was assessed as described previously.

PCI of the culprit coronary lesion supplying the infarct area was performed by standard techniques with the implantation of paclitaxel drug-eluting stents for all patients. After stenting, the medium containing the BMC or placebo medium was injected intracoronarily by using intermittent balloon inflation in the stent at the time of injection.

**Blood sampling and biochemical determinations**

All the patients underwent blood sampling 0–10 days after the onset of symptoms. The mean baseline sampling time was 3.5 days after STEMI. The final sample was taken at the control visit 6 months after the index event. Serum was prepared by allowing the blood to clot for 30 minutes followed by centrifugation at 2000×g for 10 minutes. The serum was stored at −20°C until analysed. Blood samples for plasma extraction were collected on ethylenediamine tetraacetic acid tubes on ice, immediately spun and the plasma was stored at −70°C until analysed.

The concentration of high-sensitivity C-reactive protein (CRP) was determined from serum and the concentration of troponin-I (TnI) from plasma samples using an Innolot Acio! analyser. The immunofluorometric Innolot Acio! ultrasensitive CRP assay and Innolot Acio! second generation cardiac TnI assay (Innolot Diagnostics, Turku, Finland) were used. The analytical sensitivities of the assays were 0.003 mg/l and 0.007 mg/l, respectively. The concentrations giving coefficient of variation of 10% were 0.15 mg/l and 0.04 mg/l for the assays, respectively.

Serum IL-6 levels were analysed using a sandwich ELISA (Quantikine High Sensitivity Immunoassay; R&D Systems Inc, Minneapolis, Minnesota, USA). The sensitivity of the assay was 0.059 pg/ml.

The plasma concentrations of N-terminal proatrial natriuretic peptide (NT-proANP) and N-terminal probrain natriuretic peptide (NT-proBNP) were determined with radioimmunoassay utilising antisera directed to NT-proANP46–79 and NT-proBNP10–29, as described in detail previously.

**Measurement of LVEF**

Left ventricular angiograms were performed at the time of baseline cardiac catheterisation and PCI and repeated with identical standard projections at 6 months after STEMI. An experienced investigator in a central core laboratory quantitatively analysed the left ventricular angiograms using the Philips Integris BH5000 system (Philips Medical System, Netherland BV, The Netherlands), unaware of the patient’s treatment assignment. Left ventricular volumes and LVEF were calculated with the use of the biplane area-length method including the left ventricular outflow tract in the measurements.

**Statistical analysis**

The Kruskal–Wallis test was applied to determine the differences in the natriuretic peptide and inflammatory marker levels between the two treatment groups (BMC/placebo) at baseline. A change in the LVEF measured by left ventricular cineangiography was used as an index of functional recovery. Spearman’s correlation analysis was used to determine the predictors of the absolute change in LVEF in the stem cell and placebo group. The most important clinical variables (age, gender, body mass index, active smoking, diabetes, previous angina, time from acute myocardial infarction (AMI) to thrombolysis, infarct-related vessel, severity of coronary artery disease, time from AMI to cell injection and LVEF) and laboratory variables (number of CD34+ cells, levels of TnI, CRP, IL-6, NT-proANF and NT-proBNP at baseline) were included in the correlation analysis. The association of certain laboratory values with the change in LVEF were further analysed with a linear regression model. The possible interaction between inflammatory markers or natriuretic peptides and the treatment group were added to the regression model to investigate whether the inflammatory markers or natriuretic peptides and the change in LVEF had different associations in the two treatment groups. Logarithmic transformation was performed for laboratory measures, and the baseline LVEF was squared to correct the skewness of the distributions. For further analyses, patients were divided into subgroups based on their median value of LVEF. The absolute change of LVEF in different subgroups was analysed using a univariate analysis of variance model.

All variables are expressed as means±standard deviation, or medians with interquartile range with skewed data. All tests were two-sided. Analyses were performed using the SAS system, version 9.1 for Windows.

**RESULTS**

**Patient characteristics and procedural results**

A total of 80 patients, 40 in each group, was randomly assigned into the study. One patient randomly assigned into the BMC group in fact was not administered the cells and was excluded because of normal coronary angiography, and one patient was excluded from the placebo group due to the failure of PCI to open the totally occluded target vessel. The characteristics of the remaining 79 patients in both groups have been described previously. Adequate contrast opacification of left ventricular angiograms both at baseline and at 6 months were available for 56 patients in each group. The two groups were well matched with respect to all baseline characteristics, including their pharmacological therapy at the time of discharge from hospital and at 6 months after STEMI. In brief, the mean age of the patients was 60±10 years in the BMC group and 59±10 years in the placebo
group. The median time delay after symptom onset to thrombosis was 2 h in both groups. No procedural complications occurred related to the bone marrow puncture.

**Natriuretic peptide and inflammatory marker levels**

We have previously reported that in the FINCELL study there was no difference between the two treatment groups in the clinical or demographic parameters at baseline.\(^\text{10}\) Here we show that baseline levels of NT-proANP, NT-proBNP, IL-6 or the maximum levels of TnI and CRP did not differ between the stem cell and the placebo group either (table 1).

### Determinants of the change of ejection fraction

The associations of several demographic, clinical and laboratory values with the absolute change in LVEF (from baseline to 6 months, dLVEF) were tested with Spearman’s correlation analysis (table 2). There was a strong association between the baseline LVEF and dLVEF (r = –0.58, p < 0.001) and between the baseline NT-proBNP level and dLVEF (r = 0.53, p = 0.050) in the BMC group but not in the placebo group. Also the effect of the time delay from STEMI to thrombosis was statistically significant in the BMC group (r = 0.38, p = 0.024).

According to the correlation analysis, the level of NT-proBNP appeared to be the only laboratory value associated with dLVEF. However, the dLVEF was so strongly dependent on the baseline value of LVEF that it was reasonable to adjust the further analyses with it. A linear regression model was applied to assess whether baseline IL-6, NT-proANP, or NT-proBNP, in addition to baseline LVEF, were predictors of dLVEF in the BMC and placebo groups. A test of interaction was included in the model to compare the associations between the two treatment groups.

In the adjusted model, a strong interaction was found between dLVEF and baseline NT-proBNP (p < 0.001, figure 1A) and dLVEF and baseline NT-proANP (p = 0.052, figure 1B) in the BMC group but not in the placebo group. No significant association was found between IL-6 and dLVEF in either group (figure 1C).

In a further analysis, patients were divided into two subgroups according to the baseline LVEF. Patients with baseline LVEF at or below the median (≤62.5%) had a significantly greater improvement of the global LVEF (+12.7±12.5 %/units, p < 0.001) after BMC therapy than patients with baseline LVEF above the median (–0.8±6.5 %/units, p = 0.12). In the placebo group, the improvement in global LVEF did not differ between patients with LVEF below and patients with LVEF above the median (figure 2).

### DISCUSSION

The main finding of the present study was that the baseline LVEF is the most important determinant of left ventricular functional recovery of patients with AMI who have been treated with BMC. The recovery in global LVEF was most marked in those patients who had experienced the most severe impairment of LVEF on admission. This result is in line with two previous studies.\(^\text{1,5}\) We also found that the levels of natriuretic peptides NT-proANP and NT-proBNP are associated with the LVEF recovery after stem cell treatment. However, the degree of acute inflammation measured by IL-6 and CRP levels and the extent of AMI, as assessed by the maximum TnI level, may not be as important determinants of the functional recovery of LVEF as the baseline LVEF and natriuretic peptides.

As described earlier, the FINCELL main study showed that the intracoronary administration of BMC is associated with a slight improvement in global LVEF.\(^\text{10}\) However, the mechanism of action remains unclear. It has been suggested that several critical phases such as homing, engraftment, survival, differentiation and paracrine action of the stem cells are to some extent dependent on the inflammatory environment.\(^\text{17–20}\) It is also known that the level of circulating CD34+ endothelial progenitor cells predicts future cardiovascular events,\(^\text{21}\) and that bone marrow-derived CD34+ cells may be important for cardiovascular repair.\(^\text{22}\) The number of CD34+ cells in the injected BMC fraction as well as the serum levels of inflammatory markers could thus be considered important factors when assessing the determinants of the functional recovery after BMC therapy. However, no correlation was detected in the present study between the number of CD34+ cells and the improvement in LVEF. This result is in line with three previous trials.\(^\text{9,23–24}\)

However, it has also been suggested that significant effects on LVEF may only be achieved when administered doses are higher than 10⁶ mononuclear cells.\(^\text{25}\) In the present study, the number of BMC injected was 4.02×10⁶ cells and the variation in cell dose was very small. In addition to the small number of participants, it is possible that the correlation between cell dose and LVEF recovery was not detected in this study because of the small variation in cell dose.

The impact of several different demographic and clinical parameters on the recovery of LVEF was tested in this study. Also in the BOOST study\(^\text{7}\) a subgroup analysis achieved by dividing the patient population into different demographic categories was performed. However, these investigators only compared the BMC treatment effect between the BMC group and the control group but did not assess whether there was any difference in the BMC treatment effect between the demographic subgroups. According to the BOOST study, four factors, that is, female sex, age greater than 58 years, low number of traditional risk factors (diabetes, hypercholesterolaemia, hypertension and current smoking), and more than 8 h time delay from STEMI to primary PCI resulted in a greater improvement in the LVEF after BMC treatment. In the present study, we tested the same variables but did not observe any similar associations with the change in global LVEF. There are some salient differences between the present study and previous reports. All previous randomised trials have used primary PCI in the initial treatment of patients\(^\text{4,5,7–9}\) compared with thrombolysis in the present study. In some studies BMC delivery has occurred during the very early phase after AMI.\(^\text{5,7,8}\) Furthermore, the time of storage of BMC has been longer in some of these studies.\(^\text{7,8}\) Here, BMC delivery occurred shortly after cell aspiration. This may partly explain the larger impact of the BMC therapy on global left ventricular function in the present study in those patients with impaired baseline left ventricular function compared with the results achieved in previous reports. Our results are in line with the TOPCARE-AMI trial in which Schächinger et al\(^\text{1}\) analysed

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**Table 1 Natriuretic peptide and inflammatory marker levels in the two treatment groups**

<table>
<thead>
<tr>
<th>Marker</th>
<th>BMC group baseline measurement</th>
<th>Placebo group baseline measurement</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT-proANP (pmol/l)</td>
<td>1343 (601–5293)</td>
<td>1558 (443–5588)</td>
<td>0.78</td>
</tr>
<tr>
<td>NT-proBNP (pmol/l)</td>
<td>331 (41–1287)</td>
<td>321 (41–1182)</td>
<td>0.68</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>6.2 (1.9–41)</td>
<td>8.9 (1.4–18)</td>
<td>0.32</td>
</tr>
<tr>
<td>max CRP (mg/l)</td>
<td>26.5 (6–190)</td>
<td>23 (3–157)</td>
<td>0.45</td>
</tr>
<tr>
<td>max TnI (µg/l)</td>
<td>10.7 (1.2–154)</td>
<td>5.5 (0.2–198)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

Baseline measurement data are shown as median and range. p Values for between-group differences were determined by the Kuskal–Wallis test.
several demographic factors but noted that baseline LVEF was the only significant univariate predictor of improvement in LVEF during a 4-month follow-up. The results of the REPAIR-AMI trial indicated that BMC infusion was more effective when performed more than 6 days after the reperfusion therapy. However, we did not find any correlation between the treatment effect and the time delay from thrombolysis to cell injection.

The NT-proBNP level measured at hospital admission is known to be closely associated with mortality both in the acute phase and at long-term follow-up after myocardial infarction treated with primary PCI. In the present study, the baseline levels of natriuretic peptides also predicted the change in LVEF in the BMC group so that the higher the levels of natriuretic peptides, the more marked the improvement in LVEF. These results suggest that BMC therapy may produce more beneficial effects in those patients with more marked haemodynamic deterioration after AMI.

There is an elevation in blood cytokine levels after myocardial infarction attributable to the local inflammatory reaction in the myocardium. It has been postulated that the proinflammatory cytokine IL-6 could regulate collagen formation and remodelling of the left ventricle after AMI. The level of IL-6 is known to be associated in long-term survival after STEMI, that is, patients with high inflammatory marker levels during the acute phase of myocardial infarction have a worse prognosis than patients with low levels. Even though the baseline IL-6 level correlated with baseline LVEF in the present study, it was not related to the absolute change in LVEF in either treatment group. This result suggests that, after all, there may not be an interaction between the recovery of left ventricular dysfunction after BMC therapy and the inflammation evoked by STEMI.

The most widely used laboratory marker for the size of the infarction injury is the plasma level of TnI. In the present study the maximum level of TnI correlated with baseline LVEF but did not associate with the absolute change in LVEF after stem cell treatment. In addition, TnI did not correlate with markers of inflammation such as CRP and IL-6.

### Table 2  Association of baseline demographic characteristics and laboratory values with the absolute change in global LVEF

<table>
<thead>
<tr>
<th></th>
<th>BMC group</th>
<th>Placebo group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman’s r</td>
<td>p Value</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.072</td>
<td>0.67</td>
</tr>
<tr>
<td>Gender</td>
<td>0.076</td>
<td>0.66</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.033</td>
<td>0.86</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>-0.12</td>
<td>0.49</td>
</tr>
<tr>
<td>Current smoking</td>
<td>-0.032</td>
<td>0.86</td>
</tr>
<tr>
<td>Previous angina</td>
<td>-0.056</td>
<td>0.74</td>
</tr>
<tr>
<td>Time from AMI to thrombolysis (h)</td>
<td>0.38</td>
<td>0.024</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>-0.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Severity of CAD (number of vessels 1/2/3)</td>
<td>0.079</td>
<td>0.65</td>
</tr>
<tr>
<td>Infarct-related vessel (LAD/Cx/RCA)</td>
<td>-0.084</td>
<td>0.63</td>
</tr>
<tr>
<td>Number of injected CD34+ cells</td>
<td>0.15</td>
<td>0.39</td>
</tr>
<tr>
<td>Time from AMI to cell/placebo injection (h)</td>
<td>-0.064</td>
<td>0.71</td>
</tr>
<tr>
<td>Baseline NT-proANP (pmol/l)</td>
<td>0.093</td>
<td>0.39</td>
</tr>
<tr>
<td>Baseline NT-proBNP (pmol/l)</td>
<td>0.33</td>
<td>0.050</td>
</tr>
<tr>
<td>Baseline IL-6 (gg/ml)</td>
<td>0.13</td>
<td>0.45</td>
</tr>
<tr>
<td>Maximum value of CRP (mg/l)</td>
<td>-0.036</td>
<td>0.85</td>
</tr>
<tr>
<td>Maximum value of TnI (gg/l)</td>
<td>-0.11</td>
<td>0.56</td>
</tr>
</tbody>
</table>

AMI, acute myocardial infarction; BMC, bone marrow-derived stem cell; CAD, coronary artery disease; CRP, C-reactive protein; Cx, circumflex coronary artery; LAD, left anterior descending coronary artery; LVEF, left ventricular ejection fraction; NT-proANP, N-terminal proatrial natriuretic peptide; NT-proBNP, N-terminal probrain natriuretic peptide; RCA, right coronary artery; TnI, troponin-I.
inflammation. These results suggest that even if myocardial infarction causes strong inflammatory reactions, which appear to be independent of the size of the infarction injury, the injected stem cells do not interfere with these processes. However, these observations do not exclude the concept that the positive effect of stem cell therapy after AMI is based on the paracrine effects of injected stem cells, but their mechanism of action is more likely to be haemodynamic rather than inflammatory.

LIMITATIONS

The small sample size is an obvious limitation of the present study. Further studies with a larger sample size will be needed to confirm these preliminary findings. Particularly in the subgroup analyses the number of patients is relatively small from the statistical point of view, but from a clinical point of view these preliminary data may guide further research in this area. In this respect, the data provided by the subgroup analyses should be interpreted as descriptive rather than confirmatory. Another significant limitation of this study was the size of the infarction injury, which was quite small in all of the patients (mean baseline LVEF 59% in the BMC group and 62% in the placebo group). Despite these limitations, the present study may help in guiding the design of future BMC studies among patients with AMI. Studies including patients with lower baseline LVEF and elevated natriuretic peptides will perhaps provide more insights into the crucial factors associated with the benefits of BMC therapy.

CONCLUSIONS

The results of the present study indicate that measurement of the levels of NT-proANP or NT-proBNP in addition to baseline LVEF could be useful in finding those patients who could benefit from stem cell therapy after acute STEMI. All these factors reflect the severity of the haemodynamic and neurohumoral reactions evoked by myocardial damage, that is, the stronger the neurohumoral reaction, the better the response to stem cell therapy. Nonetheless, despite the observed prognostic value of these biochemical markers, baseline LVEF remained the strongest determinant of functional recovery of the left ventricle after BMC therapy.

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Competing interests None.

Ethics approval This study was conducted with the approval of the Ethical Committee of the Northern Ostrobothnia Hospital District.

Patient consent Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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