Results of Intracoronary Stem Cell Therapy After Acute Myocardial Infarction

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To assess the effect of autologous bone marrow cell (BMC) therapy in patients with acute myocardial infarction in a rigorous double-blind, randomized, placebo-controlled trial. Patients with reperfusion >6 hours after symptom onset were randomly assigned in a 2:1 ratio to receive intracoronary BMC or placebo therapy 5 to 7 days after symptom onset. The patients were stratified according to age, acute myocardial infarction localization, and left ventricular (LV) function. Rigorous double-blinding was ensured using autologous erythrocytes for the placebo preparation that was visually indistinguishable from the active treatment. Serial cardiac magnetic resonance imaging studies were performed before study therapy and after 1, 3, and 6 months. The primary end point was the difference in the LV ejection fraction from baseline to 6 months. The secondary end points included changes in the LV end-diastolic and end-systolic volume indexes and infarct size. A total of 42 patients were enrolled (29 in the BMC group and 13 in the placebo group) in the integrated pilot phase. A mean of 381 × 10⁶ mononuclear BMCs were administered. The baseline clinical and cardiac magnetic resonance imaging parameters did not differ. Compared to baseline, the difference in LV ejection fraction for the placebo group versus BMC group was 1.7 ± 6.4% versus −0.9 ± 5.5% at 1 month, 3.1 ± 6.0% versus 1.9 ± 4.3% at 3 months, and 5.7 ± 8.4% versus 1.8 ± 5.3% at 6 months (primary end point; not significant). No difference was found in the secondary end points between the 2 groups, including changes in infarct size or LV end-diastolic and end-systolic volume indexes. In conclusion, in this rigorous double-blind, randomized, placebo-controlled trial, we did not observe an evidence for a positive effect for intracoronary BMC versus placebo therapy with respect to LV ejection fraction, LV volume indexes, or infarct size. © 2010 Elsevier Inc. All rights reserved. (Am J Cardiol 2010;105:804–812)

Conflicting results for intracoronary bone marrow cell (BMC) therapy for improvement of left ventricular (LV) ejection fraction (EF) in patients with acute myocardial infarction (AMI) have been reported. In the Reinfusion of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI), the LVEF improved significantly more in the BMC-treated patients compared to the placebo group.1 However, in other randomized trials, this could not be shown2–4 or was not maintained during follow-up.5,6 A randomized double-blind setting—often ethically rejected because of BMC aspiration and intracoronary placebo administration—has been implemented in 3 trials.1,3,7 Despite the double-blind setting, syringes for BMC or placebo therapy could be easily differentiated because of the non–cell-based placebo preparation. Using autologous erythrocytes for the placebo preparation, double blinding can be completely ensured. Different techniques for the evaluation of LVEF have been used, including echocardiography,2,8 single photon emission computed tomography,2,7 levocardiography,1,4,8 and cardiac magnetic resonance imaging (CMRI).2–6,9 Short-axis volumetry using CMRI is the reference standard for the assessment of LV volumes and LVEF. From previous trials, a high number of injected cells10,11 and application time of >5 days after AMI10,11 have been linked to improved outcomes in BMC-treated patients. Against this background, we performed a randomized, placebo-controlled, rigorous, double-blind study—the intracoronary Stem Cell therapy in patients with Acute Myocardial Infarction (SCAMI) study—including the intracoronary administration of a high cell number at days 5 to 7 after AMI with 4 serial CMRI studies within 6 months to determine whether BMC therapy is superior to placebo regarding improvement in the LVEF.

Methods

We screened patients aged 35 to 75 years with AMI. The inclusion criteria were AMI with 6 to 48 hours between symptom onset to successful percutaneous coronary intervention, a proximal lesion, creatine kinase level >1,000 U/L, and infarct size of >10% of the LV muscle mass, as determined by CMRI. The exclusion criteria were a history of myocardial infarction, cardiogenic shock, contraindications...
tions to CMRI or 12 months of dual antiplatelet therapy, a history of hematologic disease, chemotherapy, previous stem cell therapy or treatment with granulocyte colony-stimulating factor, severe renal dysfunction, or documented terminal illness. The study protocol conformed to the Declaration of Helsinki and was approved by the local ethics committee, Federal Office for Radiation Protection, and the Paul-Ehrlich Institute (the ClinicalTrials.gov number is NCT00669227). All patients gave written informed consent.

The study design is shown in Figure 1. The patients were randomly assigned in a 2:1 ratio to either the mononuclear BMC group or the placebo group by external randomization. The patients were stratified according to following criteria: age <60 or ≥60 years, anterior versus nonanterior AMI, and a reduction in LVEF (severe vs nonsevere) as visually assessed by echocardiography the day after percutaneous coronary intervention. Baseline CMRI was done the day before study therapy. All patients underwent BMC aspiration and intracoronary administration of the study therapy. CMRI and clinical evaluation for adverse events were repeated at 1, 3, and 6 months after AMI, including coronary angiography after 6 months.

With the patient under local anesthesia, the bone marrow was aspirated from the iliac crest into 20-ml syringes containing 500 IU heparin, 0.04 mg gentamicin, and 3,000 IU penicillin in 3-ml 0.9% sodium chloride. The aspirate was shipped at room temperature between the catheter laboratory and cell-processing laboratory within about 15 minutes.

The cells were morphologically checked to exclude any subclinical hematologic disease. Mononuclear cells were isolated with Ficoll density gradient centrifugation (Cambrex, Belgium, same charge as in REPAIR-AMI, 823 g, 20 minutes, without a brake), washed, and resuspended in 15 ml 0.9% sodium chloride with 2% human albumin. The placebo syringes also contained 15 ml 0.9% sodium chloride with 2% human albumin and autologous erythrocytes with a hematocrit of 0.1% without BMC. The placebo and active treatment could not be visually distinguished, ensuring rigorous double-blinding. The cell preparations were analyzed using a fluorescence-activated cell sorter. For the identification of populations with the dual platform lysis and wash method, we used directly conjugated antibodies against human CD34, CD45 (BD Biosciences Pharmingen, San Diego, California), CD133 (Miltenyi Biotec, Bergisch Gladbach, Germany), and vascular endothelial growth factor-R2 (R&D Systems, Minneapolis, Minnesota). The analyses were performed according to the protocol of the International Society of Hematotherapy and Graft Engineering. Viability testing was performed using the trypan blue method. Hematopoietic colony assays (burst-forming unit-erythroid and colony-forming unit granulocyte, erythrocyte, monocyte, megakaryocyte) and mesenchymal colony assays (colony-forming unit fibroblast) were performed using Methocult and MesenCult (StemCell Technologies, Cologne, Germany) according to the manufacturer’s instructions.

The colony-forming units were counted after 14 days.

Figure 1. Study design and enrollment. (A) Study design with no difference regarding bone marrow aspiration, administration of study therapy, and follow-up examinations between groups. (B) Enrollment and selection of study patients.
The reproducibility of the hematopoietic colony assays was verified by regular, successful participation in a global proficiency testing program (Stem Cell Technologies). Patients underwent serologic testing for hepatitis and human immunodeficiency virus infection. The study therapy was administered the same day of bone marrow aspiration using the stop-flow technique through an over-the-wire balloon catheter positioned within the stented segment.

CMRI was done using a 1.5 T Intera CV whole body magnetic resonance scanner (Philips Medical Systems, Best, The Netherlands). All data were acquired using a dedicated 5-element cardiac phased-array coil. Parallel imaging was used for all scans. The LV volumes, LVEF, and LV muscle mass were analyzed using a ViewForum workstation using short-axis volumetry, as described previously. To determine the LV function, a retrospective electrocardiographic-gated segmented k-space balanced turbo-field-echo sequence (steady-state-free-precession) without view sharing was used in short-axis views perpendicular to the true heart axis. The slice thickness was 10 mm without a gap. For whole coverage of the right and left ventricles, the slice number was individually adjusted. Depending
on the required field of view, the spatial resolution was between 1.7×1.8 mm and 2.3×1.8 mm in-plane. The following parameters were used: echo time 1.7 ms, repetition time 3.4 ms, parallel imaging (sensitivity encoding) factor 2. Papillary muscles were assigned to the myocardium. A late enhancement study was performed 15 minutes after infusion of 0.2 mmol/kg body weight gadolinium-diethylenetriaminepentaacetate (Magnevist, Schering, Germany) using a 3-dimensional spoiled turbo gradient echo sequence with a selective 180° inversion recovery prepulse in the short axis covering the whole left ventricle (20 to 22 5-mm slices). The infarct size was defined as late enhancement in relation to the LV muscle mass. Late enhancement was quantitatively assessed on a ViewForum Workstation. The analyses were done by 2 physicians with consensus reading.

The primary end point was the difference in the LVEF from baseline to 6 months, as measured by CMRI. The study was designed with 80% power to detect a significant difference in LVEF at a 1-sided significance level of 2.5%. A clinically important difference was defined as an absolute difference in LVEF at a 1-sided significance level of 2.5%. The analyses were done by 2 physicians with consensus reading.

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### Results

During the enrollment period, 155 patients, aged 35 to 75 years, with AMI successfully reperfused >6 hours after symptom onset by stent implantation, were screened.
A total of 42 patients were randomized, underwent bone marrow aspiration and received the study therapy. The median delay to percutaneous coronary intervention from symptom onset was 14.3 hours. Of the 42 patients, 29 were randomly assigned to receive BMC therapy and 13 to receive placebo. Both groups were well-matched with respect to the baseline clinical data and procedural parameters (Table 1).

A total of 128 ± 14 ml (median 130, interquartile range 124 to 136) of bone marrow was aspirated. The cell morphology was normal. Intracoronary injection of the study therapy was performed a median of 6.1 days (interquartile range 5.5 to 7.3) after the onset of AMI and a median of 6.1 hours (interquartile range 5.7 to 6.9) after BMC aspiration. In the BMC group, a mean of $3.81 \times 10^6$ mononuclear BMCs were administered. The cell characteristics are listed.
Figure 3. Primary and secondary end points. (A) Absolute values for LVEF, LV end-diastolic volume index (LVEDVI), LV end-systolic volume index (LVESVI), and infarct size. (B) Difference between baseline and 1, 3, and 6 months of follow-up. Upper and lower edges of each box plot indicate twenty-fifth and seventy-fifth percentiles; “whiskers,” the tenth and ninetieth percentiles; solid horizontal line, the median; and dotted line, the mean. All outliers shown as individual data points. Numbers are mean $\pm$ SD.
in Table 2. Drug therapy did not differ between the placebo and BMC groups at hospital discharge or at 1, 3, and 6 months of follow-up (Figure 2).

Of the 42 patients, 2 died of noncardiac causes, 1 in the placebo group after 158 days from metastatic ovarian cancer and 1 in the BMC group after 121 days from pneumonia. Neither patient underwent the 6-month CMRI study, and neither was integrated into the analysis of CMRI data. With consequent dual antiplatelet therapy, no myocardial infarction, stent thrombosis, or urgent target vessel revascularization occurred. Two patients in the BMC group were hospitalized because of heart failure 38 and 69 days after AMI. At 6 months, restenoses had occurred in 3 (25%) of 12 placebo patients and 7 (25%) of 28 BMC patients. The combined clinical end point of death, myocardial infarction, and rehospitalization because of heart failure occurred in 1 (8%) of 13 placebo patients and 3 (10%) of 29 BMC patients ($p = 0.79$). The period between the CMRI studies did not differ between the 2 groups (Table 1). We noted no difference in the baseline CMRI parameters, including LVEF, LV end-diastolic volume index, LV end-systolic volume index, and infarct size (Figure 3). The baseline late enhancement mass was not significantly different at 50.2 ± 17.1 g for placebo and 60.1 ± 36.3 g for BMC treated patients. Microvascular obstruction was present in 24 (57%) of the 42 patients.

The primary end point—the difference in LVEF between the baseline CMRI study and the 6-month CMRI study—was 5.7 ± 8.4% in the placebo group and 1.8 ± 5.3% in the BMC group. Statistical analysis revealed a 1-sided $p$ value of 0.88, with subsequent early study termination owing to the prespecified stopping rules. We noted no significant treatment-related differences in LVEF, LV end-diastolic volume index, and LV end-systolic volume index between the control and BMC patients at 1, 3, and 6 months follow-up, as was the case for differences in those parameters between the baseline and 3-month follow-up CMRI studies. In both groups, the LV end-diastolic volume index increased by approximately 10 ml and the LV end-systolic volume index remained almost stable. No difference was found between the 2 groups in infarct size during follow-up (Figure 3).

**Discussion**

In the present randomized, rigorous double-blind, placebo-controlled trial, we did not find a positive effect for intracoronary BMC therapy compared to placebo administration with respect to LVEF, the LV volume indexes, or infarct size in patients with AMI and successful percutaneous coronary intervention >6 hours after symptom onset. We would like to stress some important issues that were different from those of previous trials. First, using autologous erythrocytes in the placebo preparation, the administration of the study therapy was absolutely double-blinded. Previous trials used placebo preparations without cells in which the content of the syringes could be easily distinguished between the active treatment and the placebo. Second, for the evaluation of the primary end point, we used CMRI. With short-axis CMRI, volumetry LVEF can be determined most exactly. In contrast to several previous trials that used echocardiography, echocardiography, single photon emission computed tomography, or the area-length method, despite the use of CMRI.

Short-axis volumetry by CMRI was also performed by Janssens et al using 0.9% sodium chloride containing 5% autologous serum as placebo and in a subgroup of REPAIR-AMI using 10 ml of X-VIVO 10 medium (Cambrex, Belgium), including 2 ml of autologous serum for placebo. Consistent with our results, Janssens et al did not observe a difference in LVEF, LV end-diastolic volume index, or LV end-systolic volume index after 4 months. The median interval between symptom onset and percutaneous coronary intervention was 5 hours. The LVEF difference was 2.2 ± 7.3% in the placebo and 3.4 ± 6.9% in the BMC group. In contrast to Janssens et al, we did not observe a difference in infarct size at any follow-up CMRI study. In a subgroup of REPAIR-AMI, including 54 patients, the difference in the increase in LVEF observed with levocardiography was confirmed by short-axis CMRI volumetry, with 3.6 ± 6.8% in the BMC group and 0.6 ± 6.2% in the placebo group. This is in contrast to our observed LVEF improvement of 1.8 ± 5.3% in the BMC and 5.7 ± 8.4% in the placebo group, although we injected a greater number of cells, which has been linked to better LVEF improvement. Furthermore, cell preparation was performed in analogy to the REPAIR-AMI study, the stop-flow technique was used for readministration, and the study therapy was given a median of 6.1 days after AMI, which represents the optimal point according to a subgroup analysis of REPAIR-AMI and a recent meta-analysis. In contrast to REPAIR-AMI, with a median of 4.5 hours between symptom onset and percutaneous coronary intervention, the delay in our study was longer, with a median of 14.3 hours. Furthermore, the infarct size was larger in our patients, the frequency of microvascular obstruction was substantially greater, and cell readministration was faster.

Two randomized, not blinded, studies also used CMRI short-axis volumetry. In the BOne marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial, the difference in LVEF at 6 months was 0.7% in the control group and 6.7% in the BMC population. Parallel to our data, no differences were found in the LV end-diastolic volume index, LV end-systolic volume index, or infarct size between the 2 groups at 6 months or 18 months. The initial difference in LVEF did not persist during long-term follow-up. With peripheral BMCs harvested after granulocyte colony-stimulating factor mobilization, Kang et al demonstrated, in a randomized, not blinded, study, a LVEF difference of 5.1 ± 9.1% in the active treatment group compared to −0.2 ± 8.6% in the control group. The infarct size, as determined by late enhancement, even increased in the control group between the baseline and 6-months CMRI studies. The common finding in patients after AMI is a reduction in the infarct size, which was shown by serial scintigraphy in 626 patients from 24.6% to 8.0% at 6 months. Also, in REPAIR-AMI, the infarct size did not change in the placebo group, with 33.1 g at baseline and 32.9 g at 12 months of follow-up. The missing decrease in infarct size in REPAIR-AMI and in Myocardial Regeneration and Angiogenesis in Myocardial Infarction With G-CSF and Intra-Coronary Stem Cell Infusion-3-DES
(MAGIC Cell-3-DES)\textsuperscript{17} might, in part, explain the missing increase in LVEF in the placebo\textsuperscript{1,9} or control group.\textsuperscript{17} The scintigraphic study by Ndrepepa et al\textsuperscript{18} showed a 5.7 ± 11.3% increase in the LVEF within 6 months, confirming our assumption and the results of the placebo group. In a double-blind trial of patients with AMI treated with granulocyte colony-stimulating factor, with >6 hours between symptom onset and percutaneous coronary intervention, the increase in the LVEF in the placebo arm was 5.3 ± 9.8% by CMRI.\textsuperscript{19} Including the changes in LV volumes, these results almost perfectly match the results of our placebo group. In the Autologous Stem-Cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial, 100 patients with anterior AMI were randomized to BMC therapy versus a control group without placebo treatment.\textsuperscript{2} LVEF was determined using single photon emission computed tomography, echocardiography, and CMRI. With either technique, the change in LVEF did not differ between the 2 groups. A >10% difference in the mean LVEF was found between single photon emission computed tomography and CMRI. The values derived from CMRI were within the range of our data. Using CMRI in the ASTAMI trial, the improvement in LVEF was found among the 3 randomized treatment groups (4.3 ± 7.1% vs 1.2 ± 7.5%), paralleling our results. The different results between ASTAMI\textsuperscript{2} and REPAIR-AMI\textsuperscript{1} have been linked to different cell preparation techniques.\textsuperscript{20} Our cell preparation was performed in analog to REPAIR-AMI. The number of cells administered in ASTAMI was lower than the number in REPAIR-AMI. The number of administered mononuclear cells in our trial was even greater than in REPAIR-AMI. Recently, the results of the HEBE trial, including 200 patients, were presented at the American College of Cardiology. Using CMRI at baseline and after 4 months, no difference in the improvement of LVEF was found among the 3 randomized treatment groups (bone marrow 3.8%, peripheral blood 4.2%, standard therapy 4.0%). In the Myocardial Regeneration by Intracoronary Infusion of Selected Population of Stem Cells in Acute Myocardial Infarction (REGENERATION) trial,\textsuperscript{4} 200 patients were randomized to a nonblinded control group, unselected BMC group, or CD34\textsuperscript{+} CXCR4\textsuperscript{+} cells. The cells were also prepared by Ficoll density gradient centrifugation and readministered the same day. No differences were found in LVEF, LV end-diastolic volume, or LV end-systolic volume among the groups. On multivariable logistic regression analysis, an interval from symptom onset to primary percutaneous coronary intervention greater than or equal to the median was a significant predictor of LVEF improvement. The median delay was 6 hours, fitting exactly with our study population, in which no benefit to BMC therapy was shown in our randomized, absolutely double-blind, placebo-controlled trial.

Although all trials of BMC have focused on the improvement of LVEF to date, this end point has been questioned.\textsuperscript{21} With the loss of viable myocardium, a process of adverse remodeling is initiated, leading to chamber dilation and contractile dysfunction. However, even using the LV end-diastolic volume index as a marker of postinfarction remodeling, we did not observe any relevant difference between the BMC and placebo groups at any point. The LV end-diastolic volume index increased in both groups by about 10 ml/m\textsuperscript{2} between the baseline and 6-month CMRI studies. An increase in the LV end-diastolic volume index has been demonstrated for patients with and without cell therapy,\textsuperscript{1,5,6} and a reduction was described in ASTAMI for both populations\textsuperscript{2} and in Transplantation Of Progenitor Cells And Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI), considering only cell-treated patients.\textsuperscript{13} Only in REPAIR-AMI, the increase in LV end-diastolic volume was statistically significantly greater in the placebo group as compared to the BMC population using CMRI at 4 and 12 months.\textsuperscript{3} Using levocardiography, this was not demonstrated for the total population.\textsuperscript{1} Statistically, no difference was found in the LV end-diastolic volume in ASTAMI\textsuperscript{2} (with the reduction more pronounced in the BMC patients), the study by Janssens et al\textsuperscript{3} (a similar increase in both groups), BOOST,\textsuperscript{5,6} or REGENT\textsuperscript{4} (increase more pronounced in BMC patients).

Our trial was limited by the number of included patients. However, the stopping rules were required by the ethics committee. No independent CMRI laboratory was available; however, double-blinding is still maintained.

8. Huikuri HV, Kervinen K, Niemelä M, Ylitalo K, Säily M, Koistinen P, Sainio Jäinen EK, Ukkonen H, Pietilä M, Airaksinen JK, Knutti J, Mikkakallio TH. Effects of intracoronary injection of mononuclear bone marrow cells on left ventricular function, arrhythmia risk profile, ...


