Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial

Stefan Janssens, Christophe Dubois, Jan Bogaert, Koen Theunissen, Christophe Deroose, Walter Desmet, Maria Kalantzi, Lieven Herbots, Peter Sinnaeve, Joseph Dens, Johan Maertens, Frank Rademakers, Steven Dymarkowski, Olivier Gheysens, Johan Van Cleemput, Guy Bormans, Johan Nuyts, Ann Belmans, Luc Mortelmans, Marc Boogaerts, Frans Van de Werf

Summary

Background The benefit of reperfusion therapies for ST-elevation acute myocardial infarction (STEMI) is limited by post-infarction left-ventricular (LV) dysfunction. Our aim was to investigate the effect of autologous bone marrow-derived stem cell (BMSC) transfer in the infarct-related artery on LV function and structure.

Methods We did a randomised, double-blind, placebo-controlled study in 67 patients from whom we harvested bone marrow 1 day after successful percutaneous coronary intervention for STEMI. We assigned patients optimum medical treatment and infusion of placebo (n=34) or BMSC (n=33). Our primary endpoint was the increase in LV ejection fraction and our secondary endpoints were change in infarct size and regional LV function at 4 months' follow-up, all assessed by MRI. We assessed changes in myocardial perfusion and oxidative metabolism with serial 1-[^{11}C]acetate PET. Analyses were per protocol. This study is registered with clinicaltrials.gov, number NCT00264316.

Findings Mean global LV ejection fraction 4 days after percutaneous coronary intervention was 46·9% (SD 8·2) in controls and 48·5% (7·2) in BMSC patients, and increased after 4 months to 49·1% (10·7) and 51·8% (8·8; OR for treatment effect 1·036, 95% CI 0·961–1·118, p=0·36). Compared with placebo infusion, BMSC transfer was associated with a significant reduction in myocardial infarct size (BMSC treatment effect 28%, p=0·036) and a better recovery of regional systolic function. Myocardial perfusion and metabolism increased similarly in both groups. We noted no complications associated with BMSC transfer and all but one patient in the BMSC group completed the 4 months' follow-up.

Interpretation Intracoronary transfer of autologous bone marrow cells within 24 h of optimum reperfusion therapy does not augment recovery of global LV function after myocardial infarction, but could favourably affect infarct remodelling.

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Department of Cardiology (S Janssens MD, C Dubois MD, W Desmet MD, L Herbots MD, P Sinnaeve MD, J Dens MD, F Rademakers MD, J Van Cleemput MD, A Belmans MSc, F Van de Werf MD), **Department of Radiology** (J Bogaert MD, M Kalantzi MD, S Dymarkowski MD), **Department of Nuclear Medicine** (C Deroose MD, O Gheysens MD, G Bormans PhD, J Nuyts PhD, L Mortelmans MD), **and Department of Haematology** (K Theunissen MD, J Maertens MD, M Boogaerts MD), **Gasthuisberg University Hospital, University of Leuven, Leuven, Belgium; Centre for Transgene Technology and Gene Therapy, Interuniversity Institute of Biotechnology, University of Leuven, Leuven, Belgium** (S Janssens); **and Stem Cell Institute Leuven (SCIL), Leuven, Belgium** (S Janssens, C Dubois, K Theunissen, L Mortelmans, M Boogaerts)

Correspondence to:
Dr Stefan Janssens, Department of Cardiology, Gasthuisberg University Hospital, 49 Herestraat, B-3000 Leuven, Belgium
Stefan.Janssens@uz.kuleuven.ac.be

Introduction

Even after early coronary reperfusion, salvage of ischaemic myocardium is incomplete and loss of viable myocardium initiates a process of adverse left-ventricular (LV) remodelling,[1] which compromises clinical outcome.[2,3] Autologous bone marrow-derived or circulating progenitor cells might aid LV function recovery,[4–10] but underlying mechanisms are unclear and prominent cardiomyocyte transdifferentiation has only been reported under selected experimental conditions.[11,12] Findings of early non-randomised clinical investigations[7] indicate feasibility, safety, and enhanced functional recovery after infusion of autologous human bone marrow-derived stem cells (BMSC) into the infarct-related artery. Results of a randomised open-label study[13] indicate improvement of LV systolic function but not of LV remodelling after transfer of BMSC. However, in the absence of trials in which the control group reproduces the exact conditions of the group to which cells are transferred, including bone marrow aspiration and a placebo intracoronary injection, the true benefit of cell transfer cannot be fully appreciated.

Our aim, therefore, was to investigate the effect of autologous BMSC versus placebo transfer on LV functional and structural recovery after myocardial infarction.

Methods

Patients

Between May, 2003, and November, 2004, we did a randomised, double-blind, placebo-controlled exploratory study. We included patients, aged 18–75 years, with acute myocardial infarction with cumulative ST-segment elevation of 6 mm or more, successful epicardial reperfusion after percutaneous coronary intervention with stent replacement, and significant LV dysfunction (hypokinesia or akinesia, involving more than half of the anterior, septal, or inferior wall, using angiography, or involving three contiguous segments or more out of 17, using echocardiography). To avoid dilution of any treatment effect, resulting from aborted infarctions, we excluded patients who presented within 2 h of symptom onset. We also excluded those who had had a previous coronary artery bypass graft, or who had pulmonary oedema, cardiogenic shock, or major co-morbidities.

We obtained written informed consent from all patients. The ethics review board of the Gasthuisberg University Hospital, Leuven, Belgium, approved the

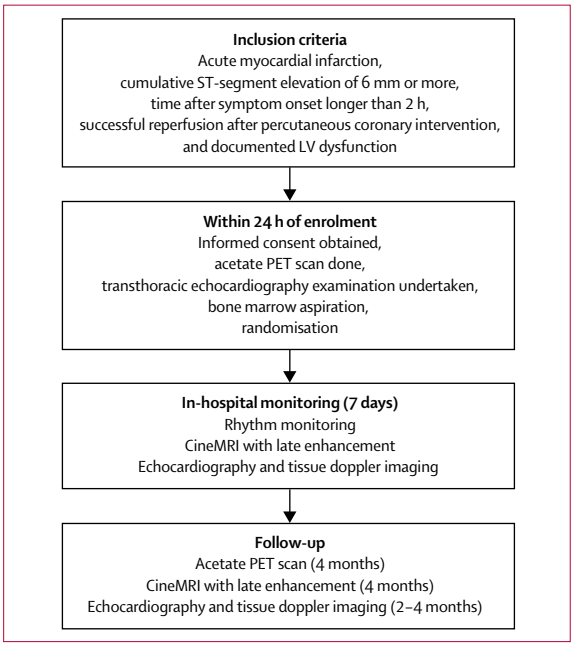

Figure 1: **Study design**

protocol, and the study was done in accordance with the Declaration of Helsinki.

Procedures (figure 1)

We assigned patients, according to a computer-generated randomisation list, in a one-to-one ratio, using sequentially numbered sealed envelopes provided by the Leuven Coordinating Centre for Clinical Trials to BMSC or placebo infusion. We used a block size of four without further stratification. Within 24 h of reperfusion and before bone-marrow harvest, we did a qualifying baseline echocardiographic examination of global and regional LV function (Vivid7, GE Medical Systems, Vingmed, Horten, Norway) and baseline 1-[^{11}C]acetate PET.

We harvested bone marrow from every patient under local anaesthesia (posterior iliac crest punctures by a staff haematologist) and isolated BMSC by Ficoll density gradient centrifugation. 4–6 h after harvest, we washed the final cell or placebo preparation twice and resuspended it in three unlabelled syringes, all containing 3·3 mL saline supplemented with 5% autologous serum. Placebo solution consisted of 0·9% sodium chloride, containing 5% autologous serum. We stored BMSC from control patients in the Bone Marrow Transplantation Laboratory's cell bank. We counted the number of nucleated cells in the final preparation with an automated haemocytometer and assessed nucleated cell viability by trypan blue exclusion. We characterised the final 10 mL cell suspension by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA) with directly conjugated antibodies against haematopoietic progenitor cells (CD34+, BD Biosciences), endothelial progenitor cells (CD133+, Miltenyi Biotec, Bergisch-Gladbach, Germany), human CD45, CD117, CD73 (BD Biosciences), CD90 (Immunotech, Marseille, France), CD105 (Research Diagnostics Inc, Flanders, NJ, USA), and matched isotype controls (all BD Biosciences). We injected the reconstituted final cell or placebo preparation in three fractions over 2–3 min, using a perfusion catheter (Maverick, Boston Scientific, Natick, MA, USA), during three low-pressure stop-flow inflations in the stent. We repeated the procedure three times with a 3-min reperfusion period between injections to reduce the likelihood of ischaemia to a minimum. After completion of cell transfer, we injected contrast medium into the infarct-related artery to ascertain vessel patency. We ascertained TIMI (thrombolysis in myocardial infarction) frame count[14] before and after percutaneous coronary intervention and before and after cell or placebo transfer.

We did cardiac MRI (1·5T-Intera, Philips, Best, Netherlands) at 4 (range 3–5) days and after 4 months. We did all studies with commercially available cardiac MRI software, electrocardiographic triggering, and cardiac-dedicated surface coils. We identified the myocardial area at risk with a T2-weighted short-tau inversion-recovery (STIR) fast spin-echo MRI.[15,16] We assessed global and regional LV function with breath-hold cineMRI (with the steady-state free-precession gradient-echo sequence) in the cardiac short axis, vertical axis, and horizontal long axis. In the cardiac short axis direction, the left ventricle was completely encompassed by contiguous 8-mm thick slices. We assessed regional LV function by measuring systolic wall thickening in the infarct region, the border zone (30º adjacent sectors), and remote myocardium.

We defined microvascular obstruction on late-enhanced images taken early—ie, within 2–5 min after injection of 0·15 mmol/kg of gadopentetate dimeglumine (Gd-DTPA)—as a dark, subendocardial zone in the area at risk with variable transmurality. We defined infarct area as the zone of bright signal on late-enhanced images—ie, 10–20 min after contrast injection—by inversion-recovery gradient-echo technique.

We analysed all MRI studies on an off-line workstation (ViewForum, Philips Electronics). For assessment of global and regional LV function and calculation of LV mass, we traced endocardial and epicardial borders in end-diastolic and end-systolic short-axis slices. We calculated LV end-diastolic and end-systolic volumes (LVEDV and LVESV) and indexed them to body-surface area. We corrected volumes at end systole for longitudinal ventricular shortening.

We also semiquantitatively assessed regional thickening, using a 17-segment model.[17,18] We graded transmural extent of late hyperenhancement within each segment, according to the following classification: 0–25%, 26–50%, 51–75%, and more than 75% hyperenhancement. We defined segmental functional recovery as

an increase from hypokinetic to normokinetic, from akinetic to hypokinetic or normokinetic, or from dyskinetic to akinetic, hypokinetic, or normokinetic. JB and MK independently analysed all images and were unaware of treatment allocation.

We used routine echocardiography with standard parasternal and apical views (Vivid7, M3S probe) to measure cardiac dimensions, mitral annulus displacement (index of systolic longitudinal shortening), and wall thickness at baseline—ie, after successful percutaneous intervention and before injection of BMSC or placebo. These measurements confirmed systolic dysfunction after index percutaneous coronary intervention and before randomisation, and represent true baseline functional values. In the context of an exploratory study of a potential new therapy for myocardial infarction, we serially repeated measurements during follow-up of patients to assess changes in global and regional systolic function as well as in diastolic function. Hence, we made conventional doppler measurements of early and late diastolic transmitral flow and the ratio between the two, deceleration time, duration of the late diastolic flow, and pulmonary venous curves. We did regional tissue doppler analysis with the 17-segment polar map, according to published recommendations of the American Society of Echocardiography[18] and computed end-systolic strain as an index of regional longitudinal shortening. We did tissue doppler imaging of the lateral mitral annulus from the apical four-chamber view for assessment of diastolic function (peak early diastolic myocardial velocity).

We did PET studies with a whole-body PET scanner (CTI Siemens, Knoxville, TN, USA). Before each study, we did a rectilinear scan (2 min) to position the heart within the field of view and a transmission scan (15 min) for photon attenuation correction. After bolus injection of a tracer dose of 740 Mbq of 1-[^{11}C]acetate, we took an emission scan by recording 22 dynamic frames per patient with a total acquisition time of 25 min. We calculated absolute blood flow (mL/min/g) and oxidative metabolism (1/min) with a three-compartment model[19] and report normalised flow and metabolic values for infarcted segments, as defined by late enhancement MRI.

Our predefined primary endpoint was the increase from baseline in global LV ejection fraction at 4 months' follow-up. Secondary endpoints were changes from baseline in infarct size and regional LV function at 4 months' follow-up.

Statistical analysis

Prespecified sample-size calculations, based on an expected treatment difference for the primary endpoint of 4–5% with a common SD of 6·5%, indicated that 34 patients in each group needed to be enrolled to detect a 4·5% treatment difference with a 5% two-sided significance and 80% power.

We explored underlying treatment mechanisms with PET, echocardiography, and tissue doppler analysis, and limited our subgroup analyses to prespecified clinically relevant variables (time to reperfusion <6 h *vs* $\geqslant 6$ h, infarct size at baseline $<20\%$ *vs* $\geqslant 20\%$ of LV mass, and presence *vs* absence of microvascular obstruction).

Continuous variables are presented as mean (SD) or median (IQR). Categorical data are presented by frequencies and percentages. For the analysis of MRI, echocardiography, and PET variables, we did an ANCOVA to assess differences between the treatment groups at 4 months, adjusted for baseline values. The ANCOVA included the values at month 4 as dependent variables and the associated baseline values and a factor for treatment as independent variables. We estimated treatment effects by computing the differences (BMSC *vs* control) between the adjusted means and their corresponding 95% CIs. For the log-transformed data, we backtransformed the obtained results, so that the treatment effect is expressed as a ratio between treatments. For regional functional recovery, we assessed the association between infarct transmurality and treatment with the probability of improved contractility in dysfunctional segments with logistic regression, including a random intercept to account for the non-independence of the data. The model included treatment and the extent of transmurality as fixed factors and the extent of transmurality as a continuous variable. We tested the interaction with treatment to verify whether the effect of treatment was affected by the extent of transmurality. Treatment effects are estimated by odds ratios (OR) and corresponding 95% CIs.

All tests were two-sided and assessed at the 5% significance level. Because of the exploratory nature of this study, we made no adjustment to the significance level to account for multiple testing. All analyses were done with SAS version 8.02, and results are presented for the per-protocol analysis.

This study is registered with clinicaltrials.gov, number NCT00264316.

Role of the funding source

Ours was an academia-initiated exploratory phase II study. There was no external sponsor involved in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Figure 2 shows the trial profile. Of 69 patients who agreed to participate, we randomised 67. One patient in the BMSC group died from haemorrhagic shock and 66 completed the 4-month follow-up. The baseline characteristics of these patients did not differ greatly between groups (table 1). Median time from symptom onset to percutaneous coronary intervention was less

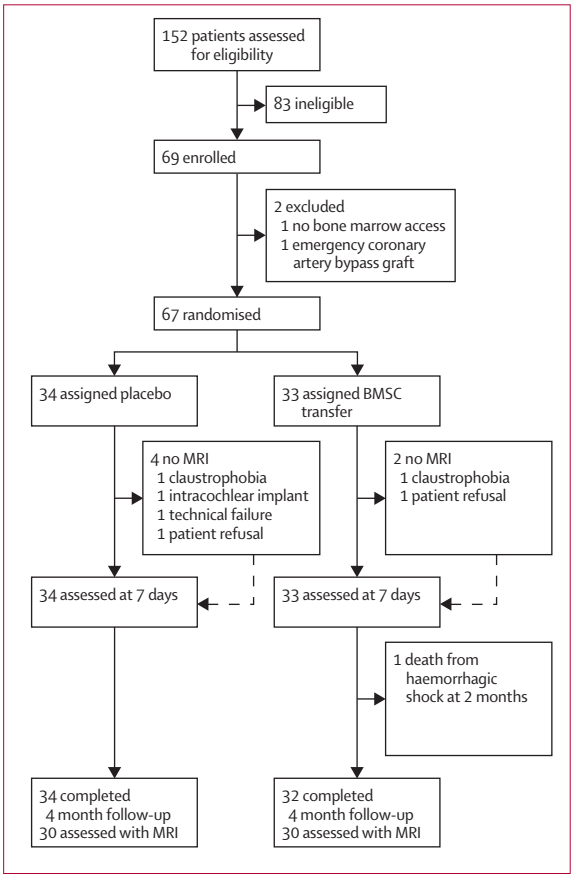

Figure 2: **Trial profile**

	Control (n=34)	BMSC (n=33)
Age (years) (mean, SD)	57·9 (10)	55·8 (11)
Men	28 (82%)	27 (82%)
Body-mass index (kg/m²) (mean, SD)	26·8 (3·2)	26·1 (4·1)
Hyperlipidaemia	18 (53%)	14 (42%)
Diabetes mellitus	4 (12%)	3 (9%)
Hypertension	14 (41%)	6 (18%)
Current smoker	20 (59%)	17 (51%)
Family history of coronary artery disease	15 (44%)	15 (45%)
Previous PCI/myocardial infarction	0/1	5/3
Infarct related artery		
Right coronary	13 (38%)	12 (36%)
Left coronary	21 (62%)	21 (64%)
Kilip class		
1	33 (97%)	30 (91%)
2	1 (3%)	3 (9%)
Time to PCI (h) (median, IQR)	4·1 (3·1–8·3)	3·7 (2·5–7·6)
TIMI flow before PCI		
Grade 0 or I	24 (71%)	20 (61%)
Grade II	3 (9%)	6 (18%)
Grade III	7 (21%)	7 (21%)
Corrected TIMI frame count (mean, SD)	40·6 (7·4)	41·4 (18·1)
LV ejection fraction (%) (mean, SD)		
Angiography	55 (11·6)	56 (11·8)
Transthoracic echocardiography	53 (7·8)	56 (7·1)
TIMI flow after PCI		
Grade II	2 (6%)	4 (12%)
Grade III	32 (94%)	29 (88%)
Corrected TIMI frame count (mean, SD)	22·9 (14·1)	22·9 (9·9)
Maximum serum creatine kinase (U/L) (mean, SD)	2344 (1464)	2255 (1336)
Maximum serum cardiac-specific isoenzyme (U/L) (mean, SD)	198 (132)	202 (115)
Maximum serum troponin I (μg/L) (mean, SD)	88 (61)	79 (52)
Admission systolic blood pressure (mm Hg) (mean, SD)	136 (20)	134 (24)
Admission diastolic blood pressure (mmHg) (mean, SD)	79 (14)	80 (18)
Pre-PCI thrombolysis	3 (9%)	6 (19%)
Peri-PCI glycoprotein IIb/IIIa inhibitors	21 (62%)	25 (76%)
Medication at discharge		
Aspirin	33 (97%)	33 (100%)
Clopidogrel	34 (100%)	33 (100%)
β blockers	33 (97%)	31 (94%)
Angiotensin-converting enzyme inhibitors	32 (94%)	32 (97%)
Angiotensin receptor blockers	3 (9%)	1 (3%)
Statins	32 (94%)	31 (94%)
Oral anticoagulants	1 (3%)	2 (6%)

Data are number (%) unless otherwise indicated. PCI=percutaneous coronary intervention. Previous infarctions were uniformly small (less than one third of current CKmax), nonSTEMI (n=1 control), or located in a different perfusion territory (n=2, BMSC).

Table 1: **Baseline characteristics**

than 5 h for both groups, and one fifth of patients displayed TIMI III epicardial flow on the first injection of the infarct-related artery, in part because of successful prehospital thrombolysis. The slight imbalance in visual TIMI II flow grade before percutaneous coronary intervention did not translate in different corrected TIMI frame counts (table 1). After percutaneous coronary intervention, a slightly greater proportion of patients in the control group had TIMI III flow grade, although corrected TIMI frame counts were again nearly identical in both groups. Finally, degree of LV dysfunction after primary percutaneous coronary intervention was comparable between groups (table 1), and similar to values reported in previous intervention trials.[20]

With respect to cell transfer, centrifugation and resuspension reduced cell volume from the bone marrow harvest (mean 130 mL, SD 22) to a final volume of 10 mL, which we injected into the infarct-related coronary artery and which contained 304×10^6 (SD 128×10^6) nucleated cells and 172×10^6 (72×10^6) mononuclear cells. FACS analysis revealed $2{\cdot}8\times10^6$ ($1{\cdot}7\times10^6$) CD34+, $2{\cdot}0\times10^6$ ($1{\cdot}3\times10^6$) CD133+, $0{\cdot}2\times10^6$ ($0{\cdot}15\times10^6$) CD90+/Thy-1+, $2{\cdot}5\times10^6$ ($2{\cdot}0\times10^6$) CD105+/endoglin, $7{\cdot}0\times10^6$ ($3{\cdot}9\times10^6$) CD117+/c-kit, and 32×10^6 (20×10^6) CD73+ cells. Repeated balloon occlusion in the stented culprit lesion during cell or placebo injection did not impair TIMI frame count or trigger an increase in concentrations of troponin.[20]

Paired MRI was available for 30 control and 30 BMSC patients (figure 2). Global LV ejection fraction increased over time in both groups (OR for treatment effect 1·036, 95% CI 0·961–1·118, p=0·36: figure 3). Changes in LVEDV and LVESV over time did not differ (table 2). The lack of an additional BMSC effect on LV ejection fraction was consistent across clinically relevant subgroups (time

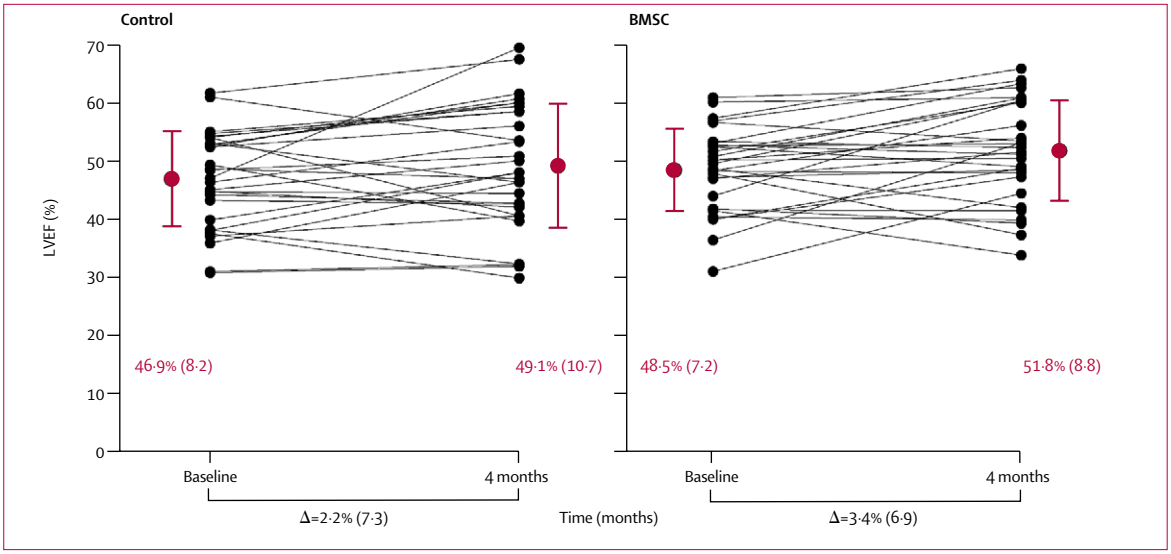

Figure 3: **Treatment effect of BMSC transfer on global LV ejection fraction (LVEF)**

to percutaneous coronary intervention, infarct location, and infarct size). Microvascular obstruction, prevalent in more than half of patients, irrespective of cell transfer (n=19 controls, n=17 BMSC), precluded LV function recovery (mean ejection fraction at 4 months 47% [SD 9%] *vs* 46% [8] at baseline) and was associated with adverse remodelling (LVEDV 162 mL [33] at day 4 *vs* 175 mL [43] at follow-up, p=0·014). We identified a significant correlation between microvascular obstruction and changes in LVEDV (r=0·41; p=0·02 Spearman correlation coefficient) and in LVESV (r=0·49; p=0·004), which was not affected by treatment assignment.

With respect to secondary endpoints, mean myocardial area at risk was 38·2 g (SD 22·9) and 33·4 g (18·0) in control and BMSC patients, corresponding to 30% (15) versus 31% (16) of LV mass, respectively. The infarcted area comprised a mean of 51% (25) of the area at risk in controls and 57% (25) in BMSC patients. Infarct size decreased over time in controls from 22 g (16) to 15 g (9) and, for a similar area at risk, significantly more after BMSC transfer (from 21 g [14] to 10 g [8], representing a 28% treatment effect, 95% CI 3–47, p=0·036). A separate analysis without the four patients who had had a previous acute coronary syndrome did not affect the noted changes in global LV function or infarct size after cell transfer (data not shown).

Infarct remodelling was associated with improved regional systolic wall thickening in the infarct zone over time (table 2), but this difference was not significant. In a more detailed, predefined segmental analysis of regional LV function recovery, according to infarct transmurality, we noted that the greater the infarct transmurality, the smaller the improvement in contractility, but significantly less so in BMSC than in control patients (p for interaction=0·038). The effect of treatment on the probability of improved regional function showed a predominant interaction in the most severely affected segments (51–75% and 76–100%; figure 4). We noted improved function in these segments in 19 BMSC-treated patients and in 11 controls (p=0·074 for the difference).

	Baseline		4 months		Difference		Treatment effect*	p
	Control (n=30)	BMSC (n=30)	Control(n=30)	BMSC (n=30)	Control (n=30)	BMSC (n=30)		
LVEDV index (mL/m²)	83·1 (14·7)	81·2 (14·0)	85·9 (19·5)	84·1 (20·8)	2·8 (15·0)	2·8 (15·2)	0·997 (0·915 to 1·086)	0·95
LVESV index (mL/m²)	44·4 (12·3)	42·2 (10·5)	45·0 (17·9)	41·0 (15·5)	0·6 (11·6)	−1·1 (11·2)	0·980 (0·861 to 1·115)	0·76
Global LVEF (%)	46·9 (8·2)	48·5 (7·2)	49·1 (10·7)	51·8 (8·8)	2·2 (7·3)	3·4 (6·9)	1·036 (0·961 to 1·118)	0·36
LV mass index (g/m²)	64·5 (15·8)	57·0 (11·0)	58·7 (11·1)	50·9 (9·6)	-5·8 (11/9)	−6·1 (6·8)	0·931 (0·864 to 1·003)	0·06
Late contrast enhancement (g)	22·3 (16·1)	20·6 (14·3)	14·7 (9·3)	10·3 (8·0)	-7·9 (8·5)	−10·2 (7·9)	0·717 (0·530 to 0·971)	0·036
Systolic wall thickening in infarct area (%)	21·8 (19·21)	23·6 (17·9)	23·7 (18·9)	29·3 (21·7)	1·9 (21·4)	5·7 (24·4)	4·99 (−5·3 to 15·3)	0·35
Systolic wall thickening in border zone (%)	32·7 (15·4)	36·6 (18·9)	38·4 (21·1)	40·8 (17·2)	5·7 (18·8)	4·2 (22·6)	−0·84 (-10·5 to 8·9)	0·87

LVEF=LV ejection fraction. Data in first six columns are mean (SD). *Expressed as ratios (BMSC/CONTROL) of adjusted means for all variables (ANCOVA) with 95% CIs, except for wall thickening where expressed as differences (BMSC−CONTROL) in adjusted means (ANCOVA) with corresponding 95% CIs.

Table 2: **LV volume and mass indices, global and regional LV function, and late contrast enhancement 4 days after intracoronary infusion and at 4 months' follow-up**

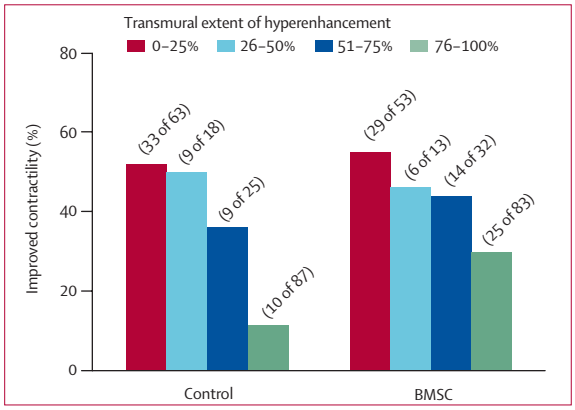

Figure 4: **Relation between transmural extent of hyperenhancement at baseline and likelihood of increased contractility in all dysfunctional segments at 4 months' follow-up**

In the study population, baseline systolic dysfunction before treatment assignment and cell transfer was fairly uniform (table 1). After 2 months, longitudinal shortening was significantly greater in BMSC-treated patients than in controls, with enhanced average mitral ring displacement (OR for treatment effect 1·121, 95% CI 1·049–1·199, p=0·0014). Also, end-systolic strain in the infarcted (2·356, 0·107–4·605, p=0·047) and remote zones (1·506, 0·300–2·712, p=0·017) was significantly greater after BMSC transfer. We noted no significant treatment-related differences in LV volumes, wall thickness, or in diastolic function variables between control (n=34) and cell-transfer patients (n=32). After 4 months, there was a residual trend for enhanced anterior wall ring displacement (from a mean of 11·2 mm [SD 4·1] at baseline to 13·0 mm [3·6] at 4 months' follow-up in control patients, and from 11·5 mm [3·5] to 14·3 mm [3·1] in BMSC patients; OR for treatment effect 1·106, 95% CI 1·000–1·223, p=0·054) and for enhanced end-systolic strain in the infarct area after BMSC transfer (from –4·55% [3·6] at baseline to –9·97% [6·4%] at 4 months' follow-up in control patients and from –4·99% [4·23] to –13·1 [6·0] in BMSC patients; OR for treatment effect 2·962, 95% CI –0·260 to 6·185, p=0·077). Taken together, these serial echocardiography measurements suggest a transient effect of BMSC transfer on systolic LV function variables. However, these findings are preliminary.

We analysed 30 control patients and 26 BMSC patients with PET, and paired analysis was available in 28 control and 22 BMSC-treated patients. The paired analysis indicated a similar increase for myocardial flow (mean 12·3% [SD 11·2] *vs* 11·5% [10·4], p=0·71) and metabolism (18·4 [8·5] *vs* 21·0 [12·4], p=0·71) in infarcted segments of control and BMSC patients. Patients with larger myocardial infarctions had a greater increase in metabolic activity after cell transfer than after placebo infusion (28·8% [6·9] in BMSC, n=9, *vs* 15·9% [7·9] in controls, n=9; OR for treatment effect 1·205, 95% CI 1·063–1·366, p=0·012).

With respect to clinical outcomes, during hospital stay, we noted no differences in treatment-related tachyarrhythmia on Holter monitoring (supraventricular arrhythmia, n=6 control and n=5 BMSC; non-sustained ventricular tachycardia, n=3 control). We detected no late potentials in any patient. All patients were discharged on post-infarction treatment, including aspirin, clopidogrel, β blockers, and angiotensin converting enzyme inhibitors (table 1). One control patient developed an acute in-stent thrombosis after 2 months and was successfully treated with a drug-eluting stent. One control and two BMSC patients developed recurrent angina, requiring dilatation of an in-stent stenosis. During follow-up, one control patient was diagnosed with lung adenocarcinoma and one BMSC patient with squamous larynx carcinoma. Both were heavy smokers.

Discussion

The findings of this randomised double-blind controlled trial indicate that intracoronary BMSC transfer does not further enhance global LV functional recovery, beyond improvements obtained by contemporary reperfusion therapy. Furthermore, LV volumes did not differ between the group treated and the group that received placebo at baseline and at 4 months' follow-up. In controls, LV ejection fraction increased over time, consistent with placebo-controlled pharmacological reperfusion trials[21] and large mechanical intervention trials.[2,22] Cell transfer did not greatly increase LV ejection fraction, refuting our primary hypothesis that in timely reperfused myocardial infarction BMSC transfer would significantly augment functional recovery. Although subgroup analysis should be considered cautiously in view of the size and exploratory nature of our study, lack of treatment effect on global functional recovery was not affected by infarct size, transmurality, or time from symptom onset to reperfusion.

By contrast, for a similar myocardial area at risk, reduction of infarct volume over 4 months, as measured by serial contrast-enhanced MRI, was greater in BMSC patients than in controls. Although the primary endpoint was not met, this additional reduction in infarct volume over and beyond the effect noted in controls suggests a potentially interesting biological effect of bone marrow cells on infarct remodelling. To start exploring its functional relevance, we did detailed segmental MRI analysis, and noted enhanced recovery of regional function in infarct regions 4 months after cell transfer, especially in the most severely infarcted segments. These findings are consistent with increased longitudinal shortening (strain), as measured with tissue doppler imaging, and with enhanced oxidative metabolism, as measured with PET, in patients with large myocardial infarctions, but need to be confirmed.

The possibility of protective or regenerative capacity of autologous progenitor cells, devoid of autoimmune

complications, has spurred early pilot trials of autologous cell transfer in patients with chronic angina[23,24] and acute myocardial infarction.[7,25–27] With initial clinical studies primarily focusing on safety and feasibility of cell transfer, specific benefits beyond those offered by reperfusion therapy were incompletely understood.[28] Our trial, designed to address some of these questions, differs from the BOOST trial,[13] where 30 of 60 randomised patients underwent bone marrow aspiration under general anaesthesia and a second catheterisation with repeated intracoronary injections under stop-flow conditions. Both procedures can induce cytokine release and therefore affect subsequent infarct healing and functional recovery.[29,30] We therefore did bone marrow aspirations and intracoronary injections of BMSC or placebo solution during repeated stop-flow conditions in all patients.

Our main finding does not confirm the reported 6% gain in LV ejection fraction noted for the cell transfer group in BOOST.[13] Several factors could account for this discrepancy. First, median times from symptom onset to reperfusion were 8·0–9·8 h in BOOST versus 3·7–4·1 h in this study. Earlier reperfusion, and by inference smaller absolute infarct sizes, could mitigate potential cell-mediated effects on functional recovery. However, relative infarct sizes averaged 17% of LV mass, well above the 10% threshold deemed necessary to explore novel ancillary therapies for patients with acute myocardial infarction.[31] In addition, angiographic LV ejection fractions in our patients were similar to values reported in two other randomised trials[2,20] of primary coronary intervention for myocardial infarction, emphasising the representative nature of our population. Second, the recent observation that after intracoronary transfer only 1·3–2·6% of ^{18}F-FDG-labelled unselected bone marrow mononuclear cells are detected in the infarcted myocardium,[32] reduces the likelihood of a large-scale regenerative process whereby progenitor cells home to jeopardised myocardium and transdifferentiate into cardiac cells, capable of generating active force development in scar tissue. Consistent with these findings, we did not detect replacement of scar tissue by viable myocardium across the wall in the BMSC group (serial quantitative MRI data not shown) and we did not detect greater improvement of perfusion or metabolic indices after BMSC transfer as measured by PET. Also the limited homing and residence of BMSC after intracoronary injection could account in part for only a transient improvement in systolic function variables as measured by serial echocardiography. Finally, microvascular obstruction occurred irrespective of treatment assignment in more than half of patients, despite restored epicardial coronary flow and normalised corrected TIMI frame counts. Its importance in hindering functional recovery and predicting outcome has been recognised.[33,34] Although our study was not powered to examine the interaction of this complication with cell transfer, both the increase in LV ejection fraction and reduction in infarct size were greatly enhanced after cell transfer in patients without microvascular obstruction, suggesting an important target for future clinical investigation.

Although insufficient homing of cells, among other factors, could account for the lack of proper transdifferentiation and global functional recovery, our study suggests a biological effect on infarct remodelling and regional systolic function. Potential mechanisms for enhanced infarct remodelling include paracrine anti-apoptotic effects of transferred cells, survival of hibernating myocardium with limitation of infarct expansion, or improved angiogenesis, as suggested by findings of preclinical studies.[35–38]

Our study had several limitations. In view of the sample size, we have avoided formal subgroup analyses. Questions about responsible cell type,[39] optimum dose, and timing of cell transfer still need to be addressed. We did cell transfer 24 h after percutaneous coronary intervention—ie, before the first MRI study at day 4—and cannot, therefore, exclude potential cell-mediated effects that arose before then, which nevertheless seem unlikely in view of similar echocardiography measurements of LV function in both groups at day 1 (before cell or placebo transfer) and at day 7 (hospital discharge, data not shown). Because myocardial interstitial oedema after recanalisation of the infarct-related artery persists for at least a week,[15] the exact timing of cell transfer within this window is unlikely to have a predominant effect. By contrast, progressive increase of microvascular obstruction within the first 48 h after reperfusion[40] and limited homing after 5 days[32] might favour early cell transfer. At the American Heart Association Scientific Sessions in November, 2005, the results of two randomised trials on the use of autologous bone marrow-derived mononuclear cell transfer 5 days after acute myocardial infarction were reported and indicated either no improvement in LV function as assessed by MRI at 6 months (ASTAMI[41]) or a significant 2·5% increase in LV ejection fraction as assessed by LV angiography at 4 months (REPAIR-AMI[42]). The reasons for the different results are unclear. Future studies need to carefully consider the choice of imaging modalities, and address the number and phenotype of infused bone marrow cells, as well as differential homing characteristics and repair capacity, depending on the age of patients, time of cell transfer, and degree of LV dysfunction. Finally, whether effects noted after 4–6 months (or sooner in some trials) are sustained over time needs to be (and is being) studied. In this respect, the observation of similar LV ejection fractions at 16 months' follow-up in control and BMSC patients studied in BOOST is noteworthy.[43]

In summary, we noted no incremental effect of autologous BMSC on global LV functional recovery in patients with timely reperfused myocardial infarction. In view of the complexity and cost of BMSC transfer,

the intervention in its present form does not offer sufficient benefit to be incorporated in reperfusion therapy for myocardial infarction with moderate reduction in global LV function. Whether noted changes in infarct remodelling will have greater and clinically relevant benefit in patients without hindering microvascular obstruction, with larger-sized infarcts and greater LV dysfunction or later presentation, needs to be investigated. The documented safety profile in this and in previous studies should facilitate efforts to identify best cell transfer conditions and target populations.

Contributors

S Janssens contributed to study design, patients' enrolment, clinical follow-up of patients, data analysis, statistical analysis, and writing of the report. C Dubois contributed to patients' enrolment, intracoronary transfer of bone marrow, and follow-up of patients. J Bogaert contributed to study design, MRI data acquisition, and analysis. M Kalantzi and S Dymarkowski contributed to MRI data acquisition and analysis. K Theunissen contributed to study design and bone-marrow aspirations. J Maertens contributed to bone-marrow aspirations. C Derooze, O Gheysens, G Bormans, and J Nuyts contributed to acquisition and analysis of PET studies. L Herbots contributed to echocardiographic studies. F Rademakers contributed to echocardiographic and MRI analysis and to writing of the report. P Sinnaeve, J Van Cleemput, and J Dens contributed to patients' enrolment and intracoronary transfer of bone marrow. W Desmet contributed to intracoronary transfer of bone marrow and to writing of the report. A Belmans contributed to statistical analysis and L Mortelmans contributed to study design and to PET studies. M Boogaerts contributed to study design and BMSC data analysis and F Van de Werf contributed to study design, data analysis, and writing of the report.

Conflict of interest statement

S Janssens is a basic clinical investigator for the Fund of Scientific Research Flanders and is holder of a named chair financed by AstraZeneca. All other authors declare that they have no conflict of interest.

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