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Intracoronary infusion of mononuclear cells from bone marrow or peripheral blood compared with standard therapy in patients after acute myocardial infarction treated by primary percutaneous coronary intervention: results of the randomized controlled HEBE trial

Alexander Hirsch[1†], Robin Nijveldt[2†], Pieter A. van der Vleuten[3†], Jan G.P. Tijssen[1], Willem J. van der Giessen[4], René A. Tio[3], Johannes Waltenberger[5], Jurrien M. ten Berg[6], Pieter A. Doevendans[7], Wim R.M. Aengevaeren[8], Jaap Jan Zwaginga[9,10], Bart J. Biemond[11], Albert C. van Rossum[2], Jan J. Piek[1*], and Felix Zijlstra[3], on behalf of the HEBE Investigators[‡]

[1]Department of Cardiology, Academic Medical Center, University of Amsterdam, PO Box 22660, 1100 DD Amsterdam, The Netherlands; [2]Department of Cardiology, VU University Medical Center, Amsterdam, The Netherlands; [3]Thorax Center, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [4]Thorax Center, Department of Cardiology, Erasmus University Medical Center, Rotterdam, The Netherlands; [5]Department of Cardiology, University Hospital Maastricht, Maastricht, The Netherlands; [6]Department of Cardiology, St Antonius Hospital, Nieuwegein, The Netherlands; [7]Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands; [8]Department of Cardiology, University Medical Center St Radboud, Nijmegen, The Netherlands; [9]Department of Experimental Immunohaematology, Sanquin Research, Amsterdam, The Netherlands; [10]Department of Immunohaematology and Blood transfusion, Leiden University Medical Center, Leiden, The Netherlands; and [11]Department of Haematology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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Aims Previous trials that investigated cell therapy as an adjunctive therapy after acute myocardial infarction (AMI) have shown conflicting results. We designed a randomized controlled trial to determine the effect of intracoronary infusion of mononuclear cells from bone marrow (BM) or peripheral blood in patients with AMI.

Methods and results In a multicentre trial, 200 patients with large first AMI treated with primary percutaneous coronary intervention were randomly assigned to either intracoronary infusion of mononuclear BM cells ($n = 69$), mononuclear peripheral blood cells ($n = 66$), or standard therapy (without placebo infusion) ($n = 65$). Mononuclear cells were delivered intracoronary between 3 and 8 days after AMI. Regional and global left ventricular myocardial function and volumes were assessed by magnetic resonance imaging before randomization and at 4 months, and clinical events were reported. The primary endpoint of the percentage of dysfunctional left ventricular segments that improved during follow-up did not differ significantly between either of the treatment groups and control: $38.6 \pm 24.7\%$ in the BM group, $36.8 \pm 20.9\%$ in the peripheral blood group, and $42.4 \pm 18.7\%$ in the control group ($P = 0.33$ and $P = 0.14$). Improvement of left ventricular ejection fraction was $3.8 \pm 7.4\%$ in the BM group, $4.2 \pm 6.2\%$ in the peripheral blood group when compared with $4.0 \pm 5.8\%$ in the control group ($P = 0.94$ and $P = 0.90$). Furthermore, the three groups did not differ significantly in changes in left ventricular volumes, mass, and infarct size and had similar rates of clinical events.

† Dr Hirsch, Nijveldt, and van der Vleuten contributed equally to this article.

‡ The names of the investigators and committee members are listed in supplementary material online, *Appendix*.

* Corresponding author. Tel: +31 20 5662609, Fax: +31 20 6962609, Email: j.j.piek@amc.uva.nl

Conclusion Intracoronary infusion of mononuclear cells from BM or peripheral blood following AMI does not improve regional or global systolic myocardial function in the HEBE trial.

Registration The Netherlands Trial Register #NTR166 (www.trialregister.nl) and the International Standard Randomised Controlled Trial, #ISRCTN95796863 (http://isrctn.org).

Keywords Cell therapy • Myocardial infarction • Magnetic resonance imaging • Left ventricular function • Remodelling

Introduction

Major advances in treatment for acute myocardial infarction (AMI) over the past decades have translated into a considerable decline in mortality. However, an increasing number of patients suffer from symptoms of heart failure as a result of post-infarct ventricular remodelling.[1] In an attempt to address these problems, the use of cell therapy as an adjunctive therapy has been advocated.[2,3] Recent randomized trials that investigated the effect of intracoronary infusion of bone marrow (BM) cells after primary percutaneous coronary intervention (PCI) for AMI have shown conflicting results.[4–8] A considerable degree of heterogeneity has been observed among these trials[9] and this may in part be explained by differences in cell isolation protocols, timing of cell infusion, patient selection, and the imaging modalities used to measure the treatment effect.[7,10–12]

Several mechanisms by which cell therapy may enhance functional cardiac recovery have been suggested including cardiac and vascular regeneration. Alternatively, paracrine activities of the cells may be responsible for the functional recovery.[13–15] So far, there are no conclusive data showing that a particular cell population should be preferred. In most clinical studies, unselected mononuclear BM cells are used and progenitor or stem cells are only a small fraction of the infused BM cells. Because the paracrine function is considered as an important mechanism[13] and all mononuclear cells are capable of releasing vast amounts of growth factors and cytokines, it has been suggested that the potential beneficial effects can be attributed to the combined effects of all infused mononuclear cells, rather than the small progenitor cell subpopulation present in the BM.[14] These considerations constituted the rationale for a randomized controlled trial with three arms.[16] In addition to randomization to intracoronary infusion of mononuclear BM cells or standard therapy, patients were randomized to a third arm in which we infused unselected mononuclear cells isolated from the peripheral blood.

Methods

The HEBE trial was a multicentre, randomized, open trial with blinded evaluation of endpoints. Between August 2005 and April 2008, 200 patients with first ST-segment elevation myocardial infarction treated with primary PCI and stent implantation were enrolled in eight hospitals in The Netherlands. The design of the study has previously been published,[16] and prior to participation all centres had to participate in a pilot trial.[17] In summary, patients 30–75 years of age were eligible for inclusion if they met the following inclusion criteria: successful PCI within 12 h after onset of symptoms, ≥3 hypokinetic or akinetic left ventricular (LV) segments observed on echocardiography performed at least 12 h after PCI, and an elevation of creatine kinase (CK) or CK-MB >10 times the local upper limit of normal (ULN). Main exclusion criteria were haemodynamic instability, anticipated additional PCI, or coronary-artery bypass grafting within the next 4 months, severe comorbidity, and contraindications for magnetic resonance imaging (MRI).

The study complied with the principles set out in the Declaration of Helsinki. All patients gave informed consent. The study protocol was approved by the Institutional Review Boards of the participating centres.

Randomization and treatment

Baseline MRI was performed at least 2 days after PCI. After MRI, on Day 2–7, patients were randomly assigned in a 1:1:1 ratio to either intracoronary infusion of autologous mononuclear BM cells, intracoronary infusion of mononuclear peripheral blood cells, or standard therapy (without placebo infusion). Permuted-block randomization was performed with stratification according to site, with the use of a computerized voice-response system. After randomization, study processes were not blinded.

In the BM and peripheral blood group, cell harvesting was performed within 8 days after primary PCI. Either, 60 mL of BM was aspirated from the iliac crest under local anaesthesia or 150–200 mL of venous blood was taken. Bone marrow or peripheral blood was collected in a sterile container with heparin and send to one of the six participating cell-processing laboratories. In both groups, mononuclear cells were isolated by density gradient centrifugation using Lymphoprep™. After two washing steps, mononuclear cells were resuspended in 15–20 mL saline, supplemented with 4% human serum albumin and 20 I.E./mL sodium heparin.[12,17] The number of nucleated blood cells was measured and the number of $CD34^+$ cells and $CD14^+$ cells was determined according to the ISHAGE protocol.[18] A small sample of the BM cells were shipped to the Sanquin Research, Amsterdam where the clonogenic potential was tested in a semi-solid Colony Forming Unit-Granulocyte-Macrophage (CFU-GM) assay.[12] All participating laboratories are accredited stem cell laboratories. We validated our isolation protocol with regard to the quantity and quality of isolated cells by comparing it with processing protocols used in other clinical trials for cell therapy (for a summary of these results see Supplementary material online, *Table S1*).[12]

Cell infusion was performed at the same day of harvesting in all but one patient in whom infusion was done the following day. Cells were infused into the infarct-related artery through the central lumen of an over-the-wire balloon catheter in three sessions of 3 min of coronary occlusion, interrupted by 3 min of coronary flow. The protocol specified administration of heparin and nitroglycerine prior to coronary angiography. The level of CK-MB and/or CK was measured at 6-h intervals during the first 24 h after cell infusion.

Magnetic resonance imaging

Magnetic resonance imaging was performed at baseline and repeated after 4 months. Patients were studied on a clinical 1.5 or 3.0 T scanner (193 and 7 patients, respectively). Magnetic resonance imaging acquisition and analyses involved a standardized protocol published previously.[16,17] In short, contiguous short-axis slices were acquired every 10 mm covering the whole LV using a segmented steady-state free precession pulse sequence. Late gadolinium enhancement (LGE) images were obtained 10–15 min after administration of a gadolinium-based contrast agent (Dotarem, Guerbet; 0.2 mmol/kg) using a 2D segmented inversion recovery gradient-echo pulse sequence, with slice position identical to the cine images.

Left ventricular volumes and mass were measured on the cine images and indexed for body-surface area. Left ventricular ejection fraction (EF) was calculated. Infarct size was determined on the LGE images as previously described using a standardized and predefined definition of hyperenhancement.[16,19] For analysis of regional myocardial function, each short-axis slice was divided in 12 equi-angular segments to calculate wall thickening (in millimetre) of each segment by subtracting end-diastolic from end-systolic wall thickness. Myocardial segments were considered dysfunctional if segmental wall thickening was <3 mm, based on the mean wall thickening of 4.4 ± 0.7 mm (mean $\pm$ 2 SD) in a group of 10 healthy volunteers (age 50–75 years).[20] Improved wall thickening of a segment at follow-up was defined as >1.5 mm improvement in segmental wall thickening between baseline and follow-up and complete recovery was defined as segmental wall thickening ≥ 3.0 mm at follow-up.

Endpoint measures

The primary endpoint was the change in regional myocardial function in dysfunctional segments at baseline defined as the percentage of dysfunctional segments with improved segmental wall thickening at 4 months. Secondary endpoints included changes in absolute segmental wall thickening in dysfunctional segments, changes in global LVEF, volumes, mass, and infarct size, and changes in regional myocardial function stratified by transmural extent of infarction. All MRI analyses were performed in a core laboratory using a standardized protocol.[16,17] To assess clinical status and adverse events, patients were seen at the outpatient clinic at 1 and 4 months after randomization. Recurrent myocardial infarction associated with cell delivery was defined as an increase in CK-MB levels of at least three times the ULN within 24 h after delivery. A clinical event committee independently adjudicated all potential clinical events.

Statistical analysis

We estimated enrolment of 60 patients in each study group to achieve a power of 90%, with a two-sided significance level of 0.05, to detect a 6% difference in change in global LVEF between active treatment and control, assuming a standard deviation of 10%. It was assumed that up to 10% of patients would not have paired MRI studies and therefore a total of 200 patients were required. The decision about the sample size was based upon the consideration that the power of this study for the primary endpoint would at least match the power for the secondary endpoint of the change in global LVEF.[16]

All analyses were performed on the basis of the intention-to-treat principle. Categorical data are presented as frequencies (percentage) and continuous data as mean $\pm$ SD (unless stated otherwise). The prespecified primary analysis consisted of separate comparisons of the endpoints between the two active treatment groups and control. For the comparison of changes in MRI variables between groups, analysis of covariance was used including treatment group as the main factor and each baseline variable as a covariate. Paired Student's *t*-test was used to compare baseline and follow-up values within each study group.

Furthermore, several baseline characteristics were examined for potential impact on the primary endpoint. Subgroups were defined according to age, infarct-related artery, time to reperfusion, LVEF, LV end-diastolic volume, infarct size, and the presence of microvascular obstruction at baseline MRI. Treatment effects were explored across subgroups with tests for interaction. These analyses were not prespecified but were exploratory in nature and based on results from previous cell therapy trials. Regression analyses and analysis of covariance were used to assess correlations between baseline variables and outcomes.

We also assessed the association between extent of transmural infarction and treatment for changes in regional myocardial function in dysfunctional segments. Because regional function in different segments within one patient is strongly related, outcomes were analysed using multilevel analyses (linear and logistic regression) with three levels: segments within slices and slices within patients.[20,21]

Because the study was not powered for clinical outcomes, clinical event rates are presented for descriptive purposes only and no statistical comparisons were done. All *P*-values are two-sided and statistical significance was set at $P < 0.05$. Statistical analysis was done with the Statistical Package for Social Sciences software (SPSS 16.0 for Windows) and MLwiN, version 1.02.0002, Centre for Multilevel Modeling, London, UK.

Results

Enrolment and baseline characteristics

A total of 200 patients were enrolled in the study and underwent baseline MRI at a median time of 3 days after primary PCI [interquartile range (IQR) 2–4]. After MRI, 69 patients were assigned to the BM group, 66 to the peripheral blood group, and 65 to the control group. Intracoronary infusion was not performed in three patients assigned to the BM group. One patient withdrew consent, in one the BM aspiration was unsuccessful, and in one the infarct-related artery was occluded on control angiography prior to cell delivery. In the peripheral blood group, intracoronary delivery was performed in all but one patient who refused cell delivery (*Figure 1*). The three groups were well matched with respect to baseline and procedural characteristics (*Table 1*). Overall, the mean age was 56 ± 9 years, 85% of the patients were men, median time from onset of symptoms to reperfusion was 3.3 h (IQR 2.3–4.5), and 90% had thrombolysis in myocardial infarction flow grade 3 after primary PCI.

Cell harvesting and intracoronary infusion

Intracoronary cell infusion was performed between 3 and 8 days after PCI with a median of 6 days in the BM group and 5 days in the peripheral blood group. The median time from cell harvesting to cell infusion was 6.3 h (IQR 5.7–6.9) in the BM group and 6.3 (IQR 5.8–7.0) in the peripheral blood group. The total number of cells was comparable in the BM and peripheral blood group ($296 \pm 164 \times 10^6$ vs. $287 \pm 137 \times 10^6$), see also *Table 1*. The number of cells did not differ between the stem cell laboratories within the BM and peripheral blood group ($P = 0.94$ and $P = 0.66$) (see Supplementary material online, *Table S2*). In the BM

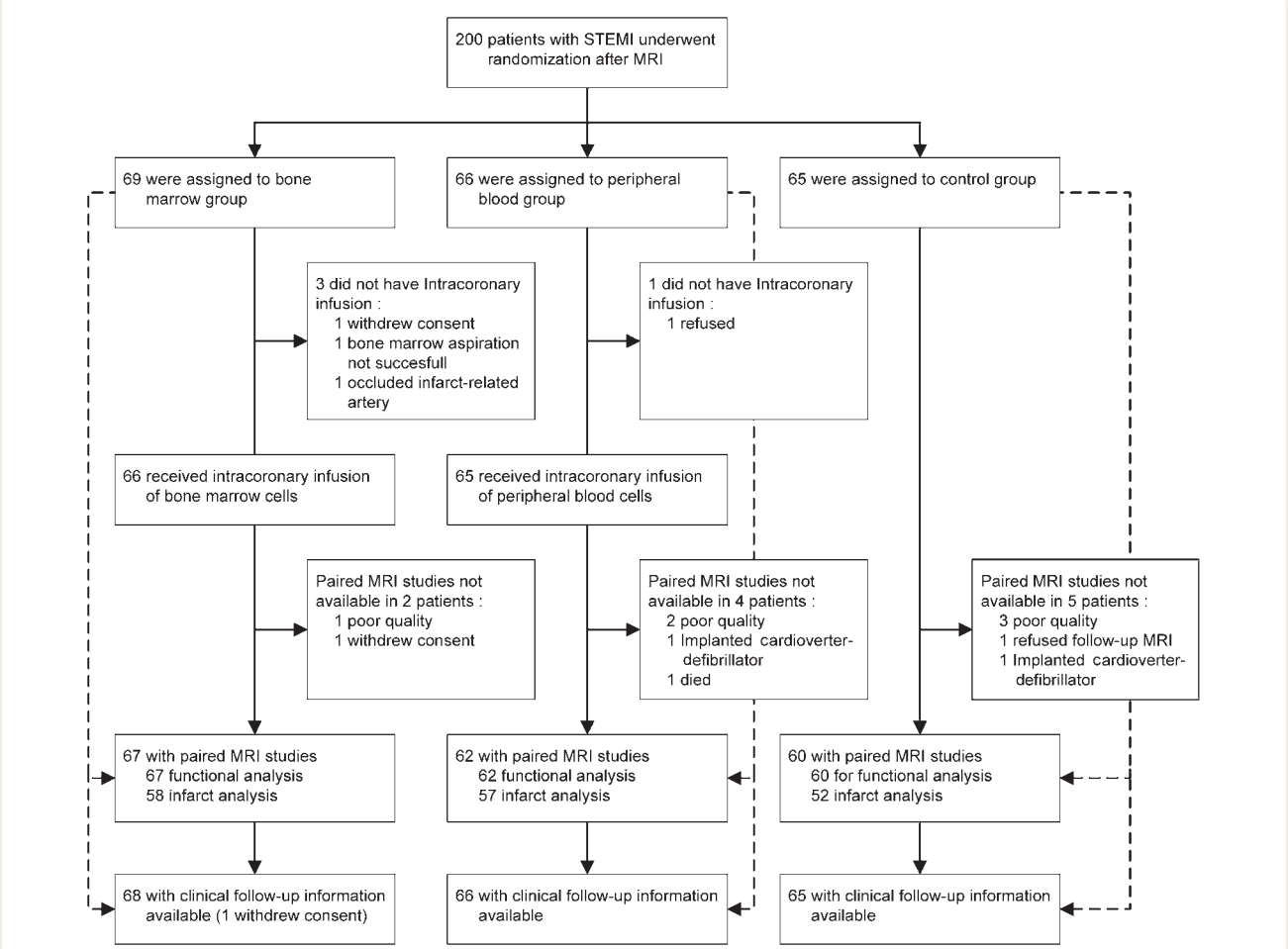

Figure 1 Trial profile. STEMI, ST-segment elevation myocardial infarction; MRI, magnetic resonance imaging.

group the CFU-GM capacity was 345 ± 250 colonies per 10^5 cells ($n = 59$). No complications of cell harvesting were noted in either group.

The adverse events related to the catheterization for cell delivery are summarized in *Table 2*. Three patients in the peripheral blood group developed a recurrent myocardial infarction related to the cell delivery procedure: in one patient this was due to coronary spasm after cell infusion, in the second an occlusion of a small side branch occurred, and in the third no cause was identified.

Left ventricular function, volumes, and infarct size

Paired cine MRI images for functional analysis were available for 67 patients in the BM group, 62 in the peripheral blood group, and 60 in the control group. Paired images for infarct analysis were available for 58, 57, and 52 patients, respectively, due to missing or poor quality of the LGE images (*Figure 1*). There were no differences in MRI parameters between the three groups at baseline. Among all patients baseline LV end-diastolic volume was 98.4 ± 15.4 mL/m^2 and LV end-systolic volume was 57.0 ± 15.1 mL/m^2. This resulted in a mean LVEF of 42.6 ± 8.8%.

The mean percentage of dysfunctional segments at baseline was 53.3 ± 19.6% in the BM group, 57.5 ± 19.6% in the peripheral blood group, and 56.2 ± 18.4% in the control group. At 4 months, 38.6 ± 24.7% of the dysfunctional segments showed improved segmental wall thickening in patients treated with mononuclear BM cells, compared with 36.8 ± 20.9% in the peripheral blood group, and 42.4 ± 18.7% in the control group. This resulted in non-significant differences between either of the treatment groups and control ($P = 0.33$ and $P = 0.14$, *Table 3*). Improvement of the LVEF was 3.8 ± 7.4% in the BM group, 4.2 ± 6.2% in the peripheral blood group when compared with 4.0 ± 5.8% in the control group ($P = 0.94$ and $P = 0.90$, *Figure 2*). There were also no significant differences in the changes in absolute segmental wall thickening in dysfunctional segments, and changes in LV volumes, mass, and infarct size between the BM, peripheral blood, and control group (*Table 3*).

In the BM and peripheral blood group, there was no significant correlation between the change in the LVEF and the total number of injected cells ($r = 0.05$, $P = 0.66$, and $r = -0.07$, $P = 0.62$, respectively). Also no significant correlation was observed between the change in the LVEF and the time from primary PCI to cell delivery. For the BM group, the increase in the LVEF was 5.0 ± 9.2% in patients treated ≤4 days after PCI ($n = 18$), 4.6 ± 6.3% in

Table 1 Baseline characteristics of the patients

Characteristic	Bone marrow group ($n = 69$)	Peripheral blood group ($n = 66$)	Control group ($n = 65$)
Age (years)	56 ± 9	57 ± 9	55 ± 10
Male gender (%)	58 (84)	56 (85)	56 (86)
Body mass index (kg/m^2)	26 ± 3	26 ± 4	27 ± 3
Risk factors (%)			
Diabetes mellitus	3 (4)	7 (11)	2 (3)
Known hypertension	27 (39)	13 (20)	17 (26)
Family history of coronary heart disease	33 (48)	30 (45)	33 (51)
Hypercholesterolaemia	17 (25)	14 (21)	15 (23)
Current cigarette smoking	37 (54)	31 (47)	37 (57)
Angiography and infarct treatment			
Time from symptom onset to PCI (h)	3.5 (2.4–5.1)	3.0 (2.1–4.8)	3.4 (2.3–4.2)
Infarct-related artery (%)			
Left anterior descending artery	42 (61)	46 (70)	40 (62)
Left circumflex artery	14 (20)	5 (8)	5 (8)
Right coronary artery	13 (19)	15 (23)	20 (31)
Multivessel disease (%)	12 (17)	21 (32)	16 (25)
TIMI flow grade post-PCI (%)			
Grade 1	1 (1)	1 (2)	0
Grade 2	8 (12)	5 (8)	6 (9)
Grade 3	60 (87)	60 (91)	59 (91)
Type of stent(s) used (%)			
Bare metal	62 (90)	60 (91)	57 (88)
Drug eluting	7 (10)	6 (9)	8 (12)
Number of stents (median, range)	1 (1–2)	1 (1–3)	1 (1–4)
Size of stent (mm)	3.4 ± 0.4	3.4 ± 0.4	3.5 ± 0.4
Length of stent (mm)	18 (15–28)	20 (18–28)	23 (18–28)
Platelet glycoprotein IIb/IIIa inhibitors (%)	49 (71)	47 (71)	43 (66)
Intra-aortic balloon pump (%)	3 (4)	4 (6)	4 (6)
Maximum serum creatine kinase MB or creatine kinase (×ULN)	37 (22–63)	38 (26–64)	42 (24–67)
Cell infusion[a]			
Days after primary PCI	6 (4–7)	5 (4–6)	—
Number of injected cells (×10^6)			
Mean	296 ± 164	287 ± 137	—
Median	258 (165–384)	270 (204–340)	—
CD34$^+$ cells			
Absolute number (×10^6)	4.0 (2.1–6.5)	0.3 (0.2–0.4)	—
Percentage	1.5 (0.8–2.1)	0.10 (0.06–0.14)	—
CD14$^+$ cells			
Absolute number (×10^6)	20.7 (14.2–31.7)	50.5 (41.6–81.6)	—
Percentage	8.6 (5.2–11.0)	22.0 (17.0–26.0)	—
Medication at discharge[b] (%)			
Aspirin	65 (96)	62 (94)	65 (100)
Clopidogrel	68 (100)	66 (100)	65 (100)
Coumarin derivate	6 (9)	15 (23)	11 (17)
Beta-blockers	64 (94)	63 (95)	62 (95)
ACE-inhibitor or AT II-receptor blocker	63 (93)	58 (88)	65 (100)
Statins	68 (100)	65 (98)	65 (100)

Continued

Table 1 *Continued*

Characteristic	Bone marrow group ($n = 69$)	Peripheral blood group ($n = 66$)	Control group ($n = 65$)
Medication at 4-month follow-up[c] (%)			
Aspirin	65 (96)	53 (82)	61 (94)
Clopidogrel	58 (85)	52 (80)	62 (95)
Coumarin derivate	7 (10)	19 (29)	10 (15)
Beta-blockers	63 (93)	60 (92)	60 (92)
ACE-inhibitor or AT II-receptor blocker	66 (97)	54 (83)	63 (97)
Statins	67 (99)	63 (97)	63 (97)

Data are number (%), mean ± SD or median (25–75th percentile) unless otherwise indicated. TIMI, thrombolysis in myocardial infarction; PCI, percutaneous coronary intervention; MB, myocardial band; ULN, upper limit of normal; ACE, angiotensin-converting-enzyme; AT, angiotensin.
[a]This analysis included only patients in whom cell infusion was performed: 66 patients in the bone marrow group and 65 in the peripheral blood group. There was no difference between the total number of injected cells between the bone marrow and peripheral blood group: $P = 0.79$ by non-parametric testing.
[b]The analysis included 68 patients in the bone marrow group, 66 in the peripheral blood group, and 65 in the control group.
[c]The analysis included 68 patients in the bone marrow group, 65 in the peripheral blood group, and 65 in the control group.

patients treated at Day 5 or 6 ($n = 28$), and 1.8 ± 7.3% in patients treated ≥7 days ($n = 19$) ($P = 0.34$) and for the peripheral blood group the increase was 5.8 ± 6.6% ($n = 25$), 3.0 ± 6.0% ($n = 24$), and 3.4 ± 6.0% ($n = 12$), respectively ($P = 0.22$). In the BM and peripheral blood group, the different stem cell laboratory used for cell isolation did not show any relation with the primary endpoint or the change in the LVEF (BM: $P = 0.59$ and $P = 0.16$; peripheral blood: $P = 0.74$ and $P = 0.83$, respectively).

In an additional, *post hoc* exploratory analysis, we excluded patients that did not receive intracoronary infusion in the two treatment arms and excluded all patients with a clinical event up to 4-month follow-up (death, recurrent myocardial infarction, or revascularization). The differences between either of the treatment groups and control did not substantially change with a treatment effect for the primary endpoint of −3.1 (−11.4 to 5.2), $P = 0.46$ for BM ($n = 63$) vs. control ($n = 56$) and −3.5 (−10.9 to 3.9), $P = 0.35$ for peripheral blood ($n = 53$) vs. control ($n = 56$). The treatment effect for LVEF was 0.5 (−1.9 to 2.9), $P = 0.69$ and 0.3 (−1.9 to 2.6), $P = 0.78$, respectively.

Several baseline features were examined for potential effects in subgroup analyses. With regard to the primary endpoint, no interactions were observed between any of these subgroups and intracoronary cell infusion (BM or peripheral blood; *Figure 3*). For example, microvascular obstruction was present in 59% of the patients: 55% in the BM group, 56% in the peripheral blood group, and 65% in the control group ($P = 0.49$) and no interaction was found between the presence of microvascular obstruction and treatment.

Figure 4 shows the observed changes in regional wall thickness and function in dysfunctional segments in relation to infarct transmurality and treatment group. As expected, segments with increasing transmural extent of infarction showed a larger decrease in end-diastolic and end-systolic wall thickness. Furthermore, the likelihood of complete recovery of dysfunctional segments was the lowest in segments with >75% hyperenhancement. However, these results did not differ between the three treatment groups. Therefore, there was no indication of enhanced recovery in any of the infarct zones due to the cell infusion.

Clinical outcome

During follow-up one patient assigned to the peripheral blood group died of ventricular fibrillation at 18 days after randomization (13 days after cell delivery). Autopsy revealed thrombus in the infarct-related artery. The patient was discharged with aspirin and clopidogrel, and platelet inhibition was not discontinued. Ventricular fibrillation occurred in another patient in the peripheral blood group 1 day after randomization (within a few hours after cell infusion) and in one patient in the control group 3 days after randomization. Both patients survived without sequelae after resuscitation and received an implantable cardioverter-defibrillator. *Table 2* summarizes all clinical events from randomization to 4-month follow-up. With regard to clinical symptoms, at 4 months 19% (13/68) of the patients in the BM group were in New York Heart Association class II or higher compared with 20% (13/65) and 18% (12/65) in the peripheral blood and control group.

Discussion

We evaluated the potential benefit of intracoronary infusion of mononuclear cells from BM or peripheral blood in the subacute phase after AMI in patients treated with primary PCI. There were no significant differences between the treatment groups and standard therapy in the efficacy endpoints that were evaluated, including the primary endpoint of percentage of dysfunctional segments at baseline with improved segmental wall thickening at 4 months and the secondary endpoints of change in the LVEF, volumes, mass, and infarct size.

To date, intracoronary injection of BM-derived cells as an adjunctive therapy in patients with AMI has been tested in several small- and medium-sized trials with various results. The results of the ASTAMI trial, REGENT trial, and the study by Janssens *et al.*[4–6] did not indicate an incremental improvement of LV function compared with the control group, whereas data from the BOOST and REPAIR-AMI trial, respectively, showed a

Table 2 Adverse events and clinical outcomes from randomization to 4-month follow-up

Event	Bone marrow group ($n = 69$)	Peripheral blood group ($n = 66$)	Control group ($n = 65$)
Catheterization for cell delivery			
Adverse events during cell delivery			
Coronary spasm	1	3	—
Transient bradycardia	1	0	—
Thrombus in infarct-related artery[a]	1	0	—
Occlusion of small side branch of infarct-related artery	0	1	—
Recurrent myocardial infarction[b]	0	3	—
Additional revascularization[c]			
Target lesion revascularization	3	3	—
Target vessel, non-target lesion revascularization	1	2	—
At 4-month follow-up (cumulative)			
Death	0	1	0
Recurrent myocardial infarction	0	4	1
Related to cell infusion procedure	0	3	—
Spontaneous	0	1	1
Revascularization	4	6	6
Target lesion revascularization	3	3	4
Target vessel, non-target lesion revascularization	1	3	0
Non-target vessel revascularization	0	0	3
Documented ventricular arrhythmia treated by ICD	0	1	1
Hospitalization for heart failure	0	1	1
Stroke	0	0	0
Cancer	0	1	0
Composite of death, recurrent myocardial infarction or target lesion revascularization	3	6	4
Composite of death, recurrent myocardial infarction or any revascularization	4	9	6
Composite of death, recurrent myocardial infarction or hospitalization for heart failure	0	5	2

Data are number of patients. ICD, implantable cardioverter-defibrillator.
[a]The occlusion was treated with a glycoprotein IIb/IIIa inhibitor, thrombosuction and balloon inflation resulting in TIMI grade 3 flow. This event did not result in a procedural-related myocardial infarction.
[b]Causes of myocardial infarctions related to cell delivery were an occlusion of a small side branch in one patient, coronary spasm in another, and in one patient no cause was identified.
[c]This included an additional PCI in a patient in the bone marrow group who did not undergo cell delivery due to a total occlusion of the infarct-related artery. The attempt to reopen the vessel failed. In the peripheral blood group, one patient was treated by stent implantation for a local dissection of the infarct-related artery caused by an intracoronary flow wire, and one patient was treated by balloon inflation for a thrombus in the infarct-related artery during cell delivery as described above. All other patients were treated before cell infusion without complications.

significant 6.0 and 2.5% absolute increase in the LVEF compared with control treatment.[7,8] However, long-term follow-up of the BOOST trial showed no sustained improvement of LV systolic function.[22] In relation to the earlier studies, we combined the following important aspects in the HEBE trial: MRI was used for assessment of regional myocardial function, patients with relatively large first myocardial infarctions and short total ischaemic time were included, cell infusion was performed at the same day of cell harvesting, and a second treatment group with infusion of mononuclear peripheral blood cells was included.

We have chosen the change in regional systolic myocardial function measured by MRI as our primary endpoint, based on the assumption that regional function is more sensitive than global LV function for the evaluation of cell therapy.[23] Several mechanisms of action by which cell therapy may enhance functional cardiac recovery have been suggested including cardiac and vascular regeneration. Alternatively, paracrine activities of the transplanted mononuclear cells may be responsible for the functional recovery.[13,15] Restoration of microvascular function determined by intracoronary flow measurements in patients in the REPAIR-AMI trial provided first clinical proof of concept of vascular repair by intracoronary cell therapy.[24] However, these measurements were secondary endpoints and in part *post hoc* analyses.

Our study is the largest study so far that used a highly accurate and quantitative imaging technique for assessment of regional systolic function in a multicentre setting. In the recently published REGENT trial, also 200 patients were included and MRI was used for assessment of the change in the LVEF. However, a major limitation of this study is the low number of patients with paired MRI images (59%).[6] Another important issue is the timing of

Table 3 Quantitative measures of regional and global left ventricular function, volumes, mass, and infarct size by magnetic resonance imaging

	Bone marrow group ($n = 67$)	Peripheral blood group ($n = 62$)	Control group ($n = 60$)	Bone marrow vs. Control		Peripheral blood vs. Control	
				Treatment effect[a] Estimate (95%CI)	*P*-value	Treatment effect[a] Estimate (95%CI)	*P*-value
Primary endpoint (%)							
Dysfunctional segments at baseline	53.3 ± 19.6	57.5 ± 19.6	56.2 ± 18.4				
Dysfunctional segments that improved during follow-up	38.6 ± 24.7	36.8 ± 20.9	42.4 ± 18.7	−3.9 (−11.7 to 4.0)	0.33	−5.3 (−12.3 to 1.7)	0.14
Segmental wall thickening in dysfunctional segments (mm)							
Baseline	1.19 ± 0.55	1.18 ± 0.49	1.14 ± 0.52				
Follow-up	2.31 ± 1.32	2.21 ± 1.21	2.31 ± 0.97				
Change	1.12 ± 1.20	1.03 ± 0.99	1.18 ± 0.80	−0.06 (−0.43 to 0.30)	0.73	−0.15 (−0.48 to 0.17)	0.35
P-value (baseline vs. 4 months)	<0.0001	<0.0001	<0.0001				
LV ejection fraction (%)							
Baseline	43.7 ± 9.0	41.7 ± 9.1	42.4 ± 8.3				
Follow-up	47.5 ± 9.9	46.0 ± 9.3	46.4 ± 9.2				
Change	3.8 ± 7.4	4.2 ± 6.2	4.0 ± 5.8	0.1 (−2.2 to 2.4)	0.94	0.1 (−2.0 to 2.2)	0.90
P-value (baseline vs. 4 months)	<0.0001	<0.0001	<0.0001				
LV end-diastolic volume (mL/m^2)							
Baseline	97.3 ± 14.0	98.0 ± 15.4	100.0 ± 16.9				
Follow-up	102.6 ± 19.1	103.4 ± 22.6	108.2 ± 24.6				
Change	5.4 ± 13.4	5.3 ± 16.3	8.2 ± 13.5	−2.5 (−7.2 to 2.2)	0.29	−2.6 (−8.0 to 2.7)	0.33
P-value (baseline vs. 4 months)	0.002	0.01	<0.0001				
LV end-systolic volume (mL/m^2)							
Baseline	55.4 ± 14.5	57.8 ± 15.9	58.1 ± 15.1				
Follow-up	54.9 ± 19.5	57.1 ± 21.6	59.3 ± 21.7				
Change	−0.5 ± 13.4	−0.7 ± 14.4	1.2 ± 11.7	−1.5 (−5.9 to 3.0)	0.52	−1.9 (−6.6 to 2.8)	0.43
P-value (baseline vs. 4 months)	0.75	0.71	0.42				
LV mass (g/m^2)							
Baseline	59.8 ± 12.2	59.6 ± 11.4	59.1 ± 11.9				
Follow-up	51.7 ± 10.5	51.3 ± 10.2	51.4 ± 10.6				
Change	−8.0 ± 9.6	−8.3 ± 7.9	−7.8 ± 7.6	−0.03 (−2.6 to 2.6)	0.98	−0.4 (−2.8 to 2.0)	0.74
P-value (baseline vs. 4 months)	<0.0001	<0.0001	<0.0001				
Infarct size (g)[b]							
Baseline	22.9 ± 12.6	21.1 ± 11.2	23.6 ± 13.8				
Follow-up	15.2 ± 8.2	13.2 ± 7.3	14.2 ± 8.9				
Change	−7.7 ± 8.5	−7.9 ± 6.5	−9.4 ± 7.1	1.3 (−0.5 to 3.2)	0.16	0.4 (−1.1 to 1.9)	0.62
P-value (baseline vs. 4 months)	<0.0001	<0.0001	<0.0001				

Plus-minus values are means ± SD. *P*-values for the change between baseline and follow-up within each study group were calculated with paired Student's *t*-test. LV, left ventricular.

[a]Treatment effect and *P*-values were determined by analysis of covariance.

[b]The analysis included 58 patients in the bone marrow group, 57 in the peripheral blood group, and 52 in the control group.

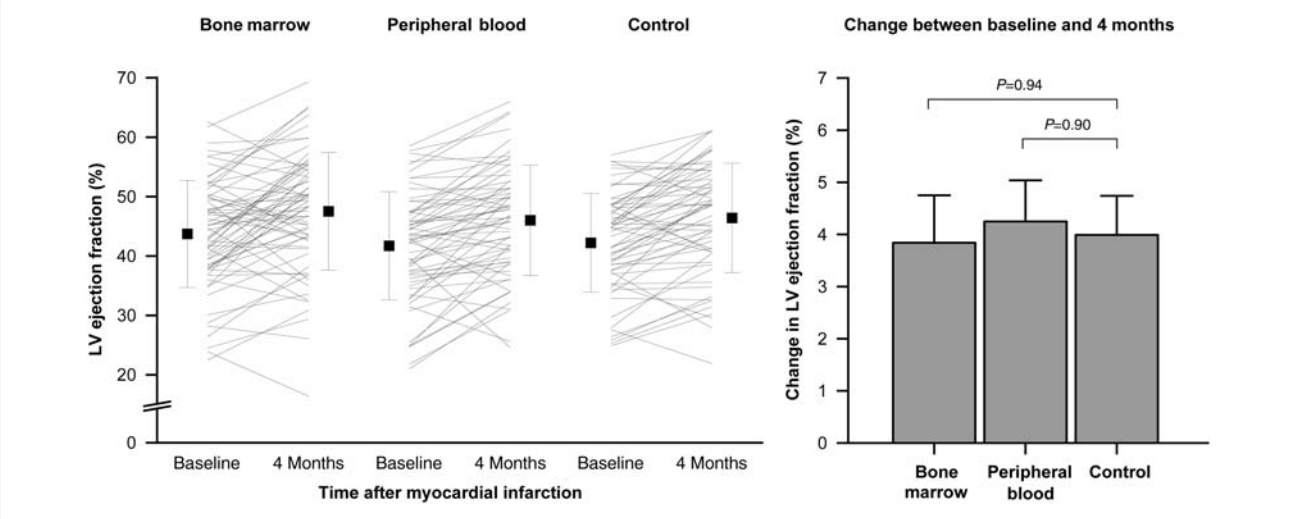

Figure 2 Estimation of the effect of intracoronary injection of mononuclear cells from bone marrow or peripheral blood on left ventricular ejection fraction. In the left panel, the lines represent the change observed in individual patients and the squares represent the mean with the standard deviation. In the right panel, the mean change between baseline and follow-up at 4 months is presented with the standard error. LV, left ventricular.

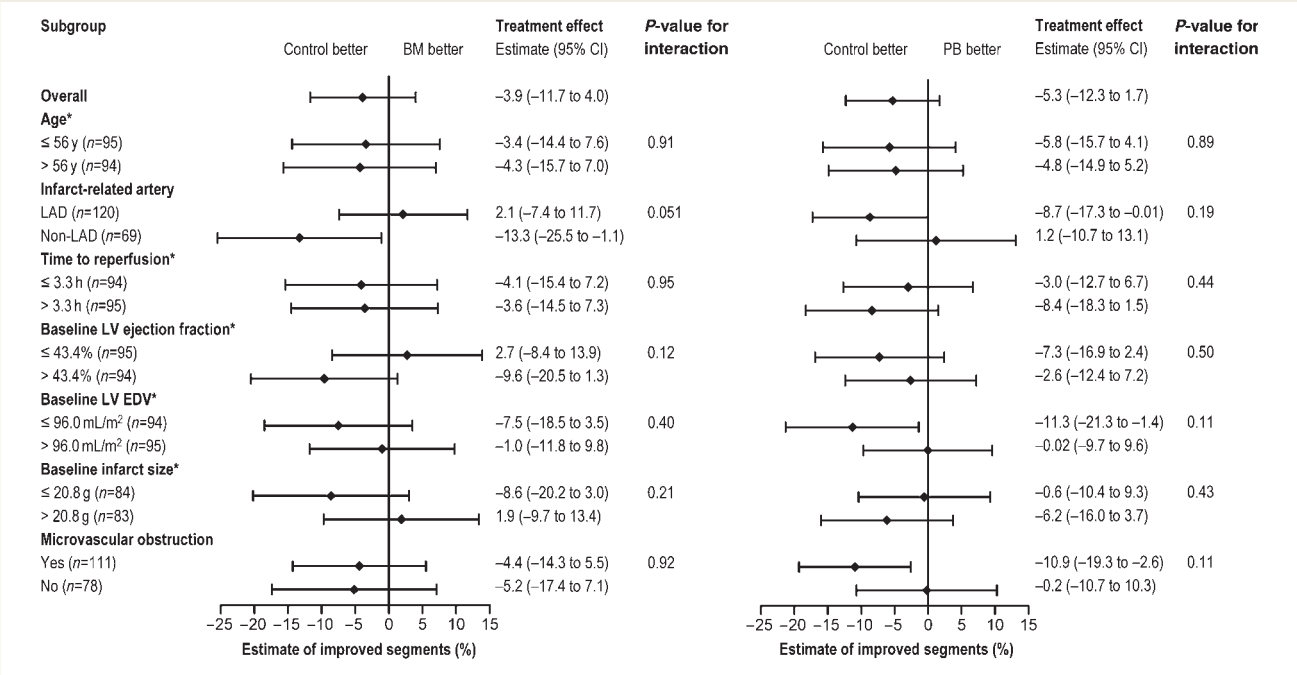

Figure 3 Estimated treatment effect of intracoronary injection of mononuclear cells from bone marrow or peripheral blood in different subgroups on the primary endpoint of percentage of dysfunctional segments at baseline with improved segmental wall thickening at 4 months. BM, bone marrow, CI, confidence interval, EDV, end-diastolic volume, EF, ejection fraction, LAD, left anterior descending, LV, left ventricular, PB, peripheral blood. *Median values of the whole study population were used to create subgroups of equal size. Treatment effects and *P*-values for interaction were estimated by analysis of covariance.

the baseline MRI. In the REGENT trial, MRI was performed after cell infusion, up to 2 weeks after myocardial infarction and in the ASTAMI trial baseline MRI was performed even later at 2–3 weeks after PCI.[5,6] In contrast, we performed MRI in all patients before randomization and within 1 week after primary PCI.

Compared with other cell therapy studies after AMI we included relatively large infarcts. This resulted in a population with a

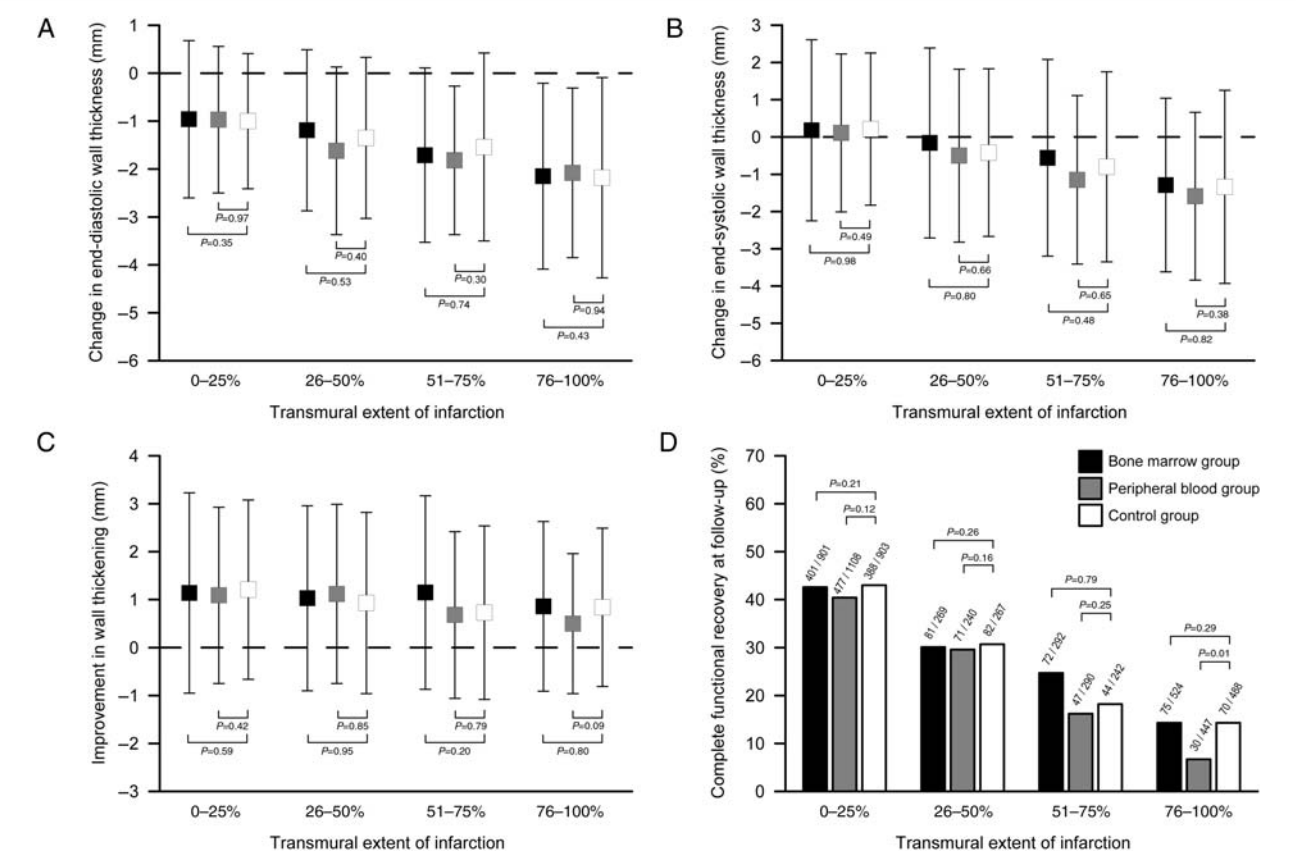

Figure 4 Changes in regional wall thickness and function in dysfunctional segments according to the transmural extent of infarction and treatment. Change of end-diastolic (*A*) and end-systolic regional wall thickness (*B*) and change of wall thickening (*C*) in dysfunctional segments between baseline and follow-up. Complete recovery at follow-up of dysfunctional segments (*D*). Outcomes were stratified by the transmural extent of infarction and treatment (bone marrow group: black, peripheral blood group: grey, and control group: white).

markedly depressed LVEF (42.6 ± 8.8%) despite a relatively short symptom onset to PCI time and contemporary post-infarct treatment.[9] While subgroup analyses of the REPAIR-AMI trial demonstrated an interaction between the baseline LVEF and the improvement seen after BM cell therapy with cell therapy being most effective in patients with a lower LVEF (<49%), we observed no improvement in our study.[7] Although data from the REGENT trial also suggest an interaction between baseline LVEF and treatment, a better outcome was only observed in patients with severely reduced baseline LVEF of <37%.[6] In *post hoc* analyses with regard to the primary endpoint, we found no interaction between several subgroups including baseline LVEF and treatment.

We have observed a relatively large number of adverse events during catheterization for cell infusion. The number of adverse events in the peripheral blood group seems higher although this was non-significant. We do not have an explanation for this possible increased event rate. Compared with the BM group, the peripheral blood group received very low numbers of $CD34^+$ cells. Our method for cell infusion using the stop-flow technique did not differ from previous trials.[2,3,16] In all patients, CK-MB concentrations were carefully and routinely measured after cell infusion (every 6 h after infusion for 24 h). However, in an additional analysis we showed that our main results did not change if patients with an event were excluded.

Most clinical studies have used the stop-flow technique with an over-the-wire balloon catheter for cell infusion after AMI. However, isolation protocols and numbers of injected cells have differed substantially. As shown by Seeger *et al.*,[11] the isolation protocol and incubation period are important and can have a major impact on the number of isolated cells and the functional activity of these cells. To validate our own isolation protocol in comparison with the REPAIR-AMI and ASTAMI protocol, we have previously tested several quantitative and qualitative *in vitro* parameters of the isolated cells (see Supplementary material online, *Table S1*). It has been suggested that differences in cell isolation procedures, especially the use of Lymphoprep instead of Ficoll, are responsible for the contrasting outcomes between the REPAIR-AMI and ASTAMI trial.[5,7,11] We and other investigators have shown that the choice of density gradient solution (Lymphoprep or Ficoll) has no effect on cell recovery and function.[12,25] However, the composition of the washing medium and centrifugation speed influence cell recovery and functional activity of the isolated cells.[12] The explanation for the lower cell recovery and lower functional activity of the cells found in the ASTAMI cell

isolation protocol by Seegers *et al.*[11,12,25,26] is most likely the lower centrifugation speed during washing of the cells, the overnight storage of the cell suspension, and the storage medium. In this light, we demonstrated that the cell-processing protocol applied in the HEBE trial results in a cell fraction of which the quantity and quality (i.e. migratory capacity and expression of CXCR4, both important markers for the functional activity of the cells) are at least similar to a positive study like the REPAIR-AMI trial.[12] In fact, the number of isolated cells and $CD34^+$ cell fraction in the present study was similar to the REPAIR-AMI trial: $296 \pm 164 \times 10^6$ and $236 \pm 174 \times 10^6$ cells with 1.6 ± 0.9 and $1.5 \pm 0.7\%$ $CD34^+$ cells, respectively. Moreover, in this study cell infusion was performed at the day of harvesting thus avoiding overnight storage, a procedure that may have a negative impact on functional activity of isolated cells.[11] Considering these data, we believe that the lack of beneficial effect in our trial is not explained by the cell isolation protocol. However, we cannot exclude the possibility that differences in isolation protocols between studies have resulted in clinical important differences that we have not tested. For example, a recent *post hoc* analysis of the REPAIR-AMI trial indicates that contamination of the autologous cell product with red blood cells impairs functional improvement of the LVEF in patients after cell therapy.[27]

There are other possible factors that could account for the lack of benefit of cell therapy in the HEBE trial. First, patients with relatively short total ischaemic time were included (median time of 3.3 h). In a *post hoc* analysis of the REGENT trial, the investigators found that patients with longer than median delay from the pain onset to reperfusion (5 h) were more likely to have significant improvement.[6] Second, we performed follow-up MRI at 4 months after cell therapy. Owing to this relative short follow-up period, long-term effects on LV function and remodelling may have been missed. Third, previous studies have shown that after intracoronary cell injection only a small amount of the injected cells home to the myocardium. Future studies should focus on optimizing cell delivery. Fourth, the study was powered to detect a 6% improvement in the LVEF. A recent meta-analysis revealed that patients receiving BM cells had significant although small improvement of the LVEF of only 3%.[9] Our study may have been underpowered to detect a modest effect of cell therapy. Fifth, we performed cell infusion between 3 and 8 days after primary PCI. Results from the REPAIR-AMI trial suggest that the enhanced improvement of the LVEF was confined to patients who were treated >4 days after primary PCI.[7] Although we did not find a significant interaction (or trend), the timing of cell infusion may have not been optimal. Finally, although several quality measures were performed and there was no interaction between the hospital of inclusion or cell-processing laboratory and outcome, we cannot exclude the possibility that the use of multiple centres may have influenced the results.

Our trial has several limitations. First, for ethical reasons, the HEBE trial was not a double-blind placebo controlled study. Bone marrow aspiration and venous blood collection were not performed in all patients and the control group did not undergo sham infusion. However, there was a blind evaluation of endpoints using a core laboratory for MRI analysis. Second, baseline MRI was not performed on a fixed time point after myocardial infarction and this may influence the measured changes in LV parameters. However, in all patients MRI was performed before randomization and no differences between the three groups were observed. Third, the number of injected cells was not standardized and therefore there was considerable variability in injected cell numbers between patients. Finally, there was inhomogeneity between the number of included patients per participating centre, randomized among two treatment arms and a control group. Still, after exclusion of the centres with <10 included patients, the difference between either of the treatment groups and control did not significantly change the primary endpoint.

In conclusion, we did not show a beneficial effect of intracoronary delivery of mononuclear cells from BM or peripheral blood on regional and global systolic myocardial function at 4-month follow-up in patients with a first AMI treated with primary PCI.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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