Percutaneous coronary intervention in acute myocardial infarction: from procedural considerations to long term outcomes
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Part IV Intracoronary bone marrow cell therapy
Chapter 11

Long term outcome after mononuclear bone marrow or peripheral blood cells infusion after myocardial infarction


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ABSTRACT

Objectives This study reports the long-term follow-up of the randomised controlled HEBE trial. The HEBE study is a multicentre trial that randomised 200 patients with large first acute myocardial infarction (AMI) treated with primary percutaneous coronary intervention to either intracoronary infusion of bone marrow mononuclear cells (BMMCs) (n=69), peripheral blood mononuclear cells (PBMCs) (n=66) or standard therapy (n=65).

Methods In addition to 3–5 days, and 4 months after AMI, all patients underwent cardiac MRI after 2 years. A follow-up for 5 years after AMI was performed to assess clinical adverse events, including death, myocardial re-infarction and hospitalisation for heart failure.

Results Of the 200 patients enrolled, 9 patients died and 12 patients were lost to follow-up at 5 years after AMI. BMMC group showed less increase in LV end-diastolic volume (LVEDV) (3.5±16.9 mL/m²) compared with (11.2±19.8 mL/m², p=0.03) in the control group, with no difference between the PBMC group (9.2±20.9 mL/m²) and controls (p=0.69). Moreover, the BMMC group showed a trend for decrease in LV end systolic volume (−1.8±15.0 mL/m²) as compared with controls (3.0±16.3 mL/m², p=0.07), with again no difference between PBMC (3.3±18.8 mL/m²) and controls (p=0.66). The combined endpoint of death and hospitalisation for heart failure was non-significantly less frequent in the BMMC group compared with the control group (n=4 vs n=1, p=0.20), with no difference between PBMC and controls (n=6 vs n=4, p=0.74). The composite endpoint of death or recurrent myocardial infarction was significantly higher in the PBMC group compared with controls (14 patients vs 3 patients, p=0.008), with no difference between the BMMC group and controls (2 vs 3 patients, p=0.67).

Conclusions Long-term follow-up of the HEBE trial showed that increase in LVEDV was lower in the BMMC group. This study supports the long-term safety of intracoronary BMMC therapy. However, major clinical cardiovascular adverse events were significantly more frequent in the PBMC group.

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

The effect of intracoronary infusion of mononuclear bone marrow mononuclear cells (BMMCs) after acute myocardial infarction (AMI) in patients has been analysed in the past, with a moderate positive effect of BMMC treatment on LV function at short-term follow-up.

Data regarding LV function at the long term are scarce and contradicting. Some studies demonstrated a sustained positive or neutral effect at long term; another study had a transient positive effect and one study even had a neutral effect on short term and positive effect on the long term. Long-term clinical outcomes are essential for further evaluation of the efficacy of BMMC therapy after AMI, as currently, intracoronary BMMC therapy is used in research setting only and a large randomised controlled trial with a primary clinical endpoint is awaited.

The HEBE trial was a multicentre, randomised, open trial with blinded evaluation of endpoints of which the details of the design and main results have been published previously. Briefly, 200 patients with first ST-segment elevation myocardial infarction treated with primary percutaneous coronary intervention (PCI) were enrolled in this study. After cardiac MRI (CMR), on day 2–7, patients were randomly assigned in a 1:1:1 ratio to either intracoronary infusion of autologous mononuclear BMMCs (n=69), intracoronary infusion of peripheral blood mononuclear cells (PBMCs) (n=66) or standard therapy (without placebo infusion) (n=65). There were no significant differences between the two treatment groups and the control group. The HEBE trial included 3 treatment groups. Because the paracrine function of the injected cells is considered as an important mechanism 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 progenitor cell subpopulation. These considerations constituted the rationale for the third randomisation arm (PBMC) in our trial. Here, we evaluated the 5-year long-term clinical events follow-up and a 2-year CMR follow-up to assess effects on regional and global LV functions.

METHODS

As described previously, we estimated the 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 SD of 10%. It was assumed that up to 10% of patients would not have paired CMR studies and, therefore, a total of 200 patients were required. Cell harvesting in the BMMC and PBMC groups was performed within 8 days after primary PCI. Bone
marrow (60 mL) was aspirated from the iliac crest under local anaesthesia. In the PBMC group, 150 mL of venous blood was collected. BMMCs or PBMCs were isolated by density gradient centrifugation, and 15 mL of cell suspension was used for intracoronary infusion.

In the bone marrow group, 3 patients did not have intracoronary infusion; in the peripheral blood group, 1 patient refused intracoronary infusion. The 3 groups were comparable with respect to baseline characteristics (table 1). Overall, the mean age was 56±9 years and 85% of the patients were men. At discharge, 95% were treated with β-blockers, 99% with statins and 93% with angiotensin-converting-enzyme or angiotensin inhibitors. There was no significant difference between the two treatment groups and the control group regarding the primary end point, namely the change in regional myocardial function in dysfunctional segments at 4 months relative to baseline, based on segmental analysis as measured by CMR. Also, no significant differences in the secondary endpoints of change in LVEF, volumes, mass and infarct size were observed. Furthermore, the 3 groups had similar rates of clinical events at 4 months follow-up. After 4 months, patients were contacted for additional informed consent for CMR at 2 years of follow-up and clinical follow-up at 5 years.

<table>
<thead>
<tr>
<th>Table 1. Baseline characteristics of the HEBE trial</th>
</tr>
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<tbody>
<tr>
<td><strong>Characteristic</strong></td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Male gender</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Known hypertension</td>
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<tr>
<td>Family history of coronary heart disease</td>
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<td>Hypercholesterolemia</td>
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<td>Current cigarette smoking</td>
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<tr>
<td>Infarct Related Artery</td>
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<tr>
<td>Left anterior descending artery</td>
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<tr>
<td>Left circumflex artery</td>
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<tr>
<td>Right coronary artery</td>
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<tr>
<td>Multivessel disease</td>
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</table>

BMMC=mononuclear bone marrow cells; PBMC=peripheral blood mononuclear cells

Data previously published in *Eur Heart J* 2011;32:1736-47.

**Cardiac MRI**

Patients were studied using a clinical magnetic resonance scanner (Siemens, Erlangen, Germany; Philips, Best, The Netherlands; GE Healthcare, Buckinghamshire, UK). The
CMR protocol at 24 months was similar to the CMR protocol at baseline and 4 months, with the exception that no contrast medium was administrated. In short, contiguous short-axis slices were acquired every 10 mm covering the whole LV using a cine retrospectively ECG-gated segmented steady state free precession pulse sequence, with image parameters identical to the baseline and follow-up scan at 4 months. LV volumes and mass were measured on the cine images and indexed for body-surface area. LVEF was calculated.

For analysis of regional myocardial function, each short-axis slice was divided into 12 equi-angular segments to calculate wall thickening (in mm) of each segment by subtracting end-diastolic from end-systolic wall thickness. Myocardial segments were considered dysfunctional if segmental wall thickening was <3 mm. Improved wall thickening of a segment at follow-up was defined as >1.5 mm improvement in segmental wall thickening as compared with baseline and complete recovery was defined as segmental wall thickening ≥3.0 mm at follow-up.

All CMR analyses were performed in a core laboratory blinded to treatment allocation using a standardised protocol. Both baseline and 4-month follow-up scans were used to match all 3 studies for slice position using anatomic landmarks, such as papillary muscles and RV insertion sites.

Clinical events

To assess clinical status and adverse events, patients were seen at the outpatient clinic at 1, 4, 12, 24 and 60 months after randomisation. At 36 and 48 months, clinical follow-up was performed via telephone contact. All potential outcome events were recorded. If a patient could not be contacted, information was obtained from the general practitioner, treating cardiologist or hospital records. Data were censored at 5 years (1825 days) of follow-up or at the date of last contact. All new events occurring after previous reported data were adjudicated by a clinical event committee blinded to treatment allocation.

Statistical analysis

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 ± SD. The analysis consisted of separate comparisons of the endpoints between the two active treatment groups and control. For the comparison of changes in CMR variables between groups, analysis of covariance was used including treatment group as the main factor and each baseline variable as a covariate. Safety of BMC administration was analysed using pre-specified clinical endpoints and included major adverse cardiovascular events defined as death, re-hospitalisation for heart failure and recurrent myocardial infarction. Cumulative event rates were assessed by the Kaplan–Meier method and compared
using a log-rank test. Follow-up was censored at the date of last contact or at 5 years, whichever came first. If the patient was lost to follow-up, censoring was done at the date of last clinical follow-up.

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 V.19.0 for Windows).

RESULTS

Of the 200 patients originally enrolled in the HEBE study, at 24 months one had withdrawn the informed consent, three had died, three patients did not consent to additional long-term follow-up and three patients were lost to follow-up. At 24 months, 19 patients had clinical follow-up but did not undergo CMR because of implantable cardioverter-defibrillator implantation (n=8), pacemaker implantation (n=1) or because they refused (n=10). In 12 patients the CMR scan was of poor quality due to breathing or triggering artefacts, or it was not possible to match with the baseline and 4-month studies, and therefore the patients were excluded from the analysis by the core lab (see figure 1). During long-term follow-up, 12 patients were lost to follow-up at 60 months.

LV function, volumes and infarct size

Paired cine CMR images for functional analyses were available for 59 patients in the BMMC group, 48 in the PBMC group and 52 in the control group. At 24 months, 45.0±26.3% of the dysfunctional segments showed improved segmental wall thickening in patients treated with BMMCs, compared with 52.3±22.6% in the control group (p=0.14). Patients treated with PBMCs showed less improvement of dysfunctional segments at 24 months of follow-up compared with control (42.5 ±20.7%, p=0.03; table 2).

Improvement of LVEF was 4.2±8.6% in the BMMC group, 3.0±8.3% in the PBMC group as compared with 4.0±8.6% in the control group (p=0.37 and p=0.17, respectively). Patients treated with intracoronary BMMC therapy had less increase in LV end diastolic volume (LVEDV) (3.5±16.9 mL/m²) as compared with controls (11.2±19.8 mL/m², p=0.03). Moreover, the BMMC group showed a trend for less increase in LV end systolic volume (LVESV) (−1.8±15.0 mL/m²) as compared with controls (3.0±16.3 mL/m², p=0.07). There were no other significant differences in the changes in LV volumes between the BMMC, PBMC and control group (table 2).
Clinical outcome

At 5 years of follow-up, 88% were treated with \(\beta\)-blockers, 96% with statins and 89% with angiotensin-converting-enzyme or angiotensin inhibitors. There was no difference in medicine use in the treatment arms. Six patients in the BMMC group were classified as New York Heart Association (NYHA) Functional Classification class II and one patient as class III, compared with three patients in the control group with NYHA class II. All other patients were classified as NYHA class 1. In the PBMC group, 5 patients were classified as NYHA class II and 1 patient as class III. Two patients in the BMMC and two patients in the control group suffered from angina symptoms at 5 years of follow-up; one patient Canadian Cardiovascular Society (CCS) class 1 and one patient CCS class 2 in both treatment arms. One patient in the PBMC group suffered from angina symptoms.
Table 2. Quantitative measures of regional and global left ventricular function, volumes, mass and infarct size by CMR.

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Dysfunctional segments</th>
<th>Improvement at 4 months</th>
<th>Baseline</th>
<th>4 months</th>
<th>Baseline</th>
<th>4 months</th>
<th>Improvement at 24 months</th>
<th>Baseline</th>
<th>24 months</th>
<th>Baseline</th>
<th>24 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis at 4 months</td>
<td>Dysfunctional segments</td>
<td>Improvement at 4 months</td>
<td>Baseline</td>
<td>4 months</td>
<td>Baseline</td>
<td>4 months</td>
<td>Improvement at 24 months</td>
<td>Baseline</td>
<td>24 months</td>
<td>Baseline</td>
<td>24 months</td>
</tr>
<tr>
<td>BMMC group (n=67)</td>
<td>43.7±9.0</td>
<td>38.6±24.7</td>
<td>42.5±20.7</td>
<td>36.8±20.9</td>
<td>42.4±18.7</td>
<td>0.33</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group (n=60)</td>
<td>49.2±8.1</td>
<td>45.0±26.3</td>
<td>42.5±20.7</td>
<td>42.4±18.7</td>
<td>0.14</td>
<td>0.03</td>
<td></td>
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</tr>
<tr>
<td>PBMC group (n=62)</td>
<td>51.8±14.5</td>
<td>47.5±9.9</td>
<td>46.0±9.1</td>
<td>46.0±9.1</td>
<td>0.52</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis at 24 months</td>
<td>Dysfunctional segments</td>
<td>Improvement at 24 months</td>
<td>Baseline</td>
<td>24 months</td>
<td>Baseline</td>
<td>24 months</td>
<td>Improvement at 24 months</td>
<td>Baseline</td>
<td>24 months</td>
<td>Baseline</td>
<td>24 months</td>
</tr>
<tr>
<td>BMMC group (n=59)</td>
<td>47.5±9.9</td>
<td>43.7±9.0</td>
<td>42.5±20.7</td>
<td>36.8±20.9</td>
<td>42.4±18.7</td>
<td>0.33</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group (n=52)</td>
<td>51.8±14.5</td>
<td>47.5±9.9</td>
<td>46.0±9.1</td>
<td>46.0±9.1</td>
<td>0.52</td>
<td>0.80</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>PBMC group (n=59)</td>
<td>61.3±26.4</td>
<td>54.9±19.5</td>
<td>57.1±21.6</td>
<td>57.1±21.6</td>
<td>0.31</td>
<td>0.93</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

BMMC=mononuclear bone marrow cells; PBMC=peripheral blood mononuclear cells

Baseline and 4 month LV function previously published in *Eur Heart J* 2011; 32:1736-47.
Long term outcome of the HEBE trial

(CC class 1). These differences in NYHA classifications and CCS classifications were all non-significant.

During 5 years of follow-up, six patients assigned to the PBMC group died, compared with two patients in the control group (p=0.27). Ten patients treated with PBMC therapy had a recurrent myocardial infarction, compared with one in the control group (p=0.009). Three recurrent myocardial infarctions occurred during cell infusion. Of the remaining seven other recurrent myocardial infarctions, six were in the same vessel as the infarct related artery.

Eight patients in the BMMC group had a target lesion revascularisation, seven in the PBMC group and six in the control group (respectively, p=0.78 and 1.00). Table 3 summarises all clinical events from randomisation to 60-month follow-up. The combined endpoint of death and hospitalisation for heart failure was non-significantly more frequent in the control group compared with the BMMC group (n=4 vs n=1, p=0.20). Figure 2 shows the Kaplan–Meier survival curves for the composite of death or recurrent myocardial infarction of all three groups. The composite endpoint of death, recurrent myocardial infarction was significantly higher in the PBMC group compared with controls (14 patients vs 3 patients, p=0.008).

**Table 3. Adverse events and clinical outcomes from randomization to 5 years of follow up**

<table>
<thead>
<tr>
<th>Event</th>
<th>BMMC (n=65)</th>
<th>PBMC (n=63)</th>
<th>Control Group (n=60)</th>
<th>BMMC vs. control p value</th>
<th>PBMC vs. control p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 60 months follow-up</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>0.61</td>
<td>0.27</td>
</tr>
<tr>
<td>Recurrent myocardial infarction</td>
<td>1</td>
<td>10</td>
<td>1</td>
<td>1.00</td>
<td>0.009</td>
</tr>
<tr>
<td>Related to cell infusion procedure</td>
<td>0</td>
<td>3</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revascularization</td>
<td>17</td>
<td>15</td>
<td>13</td>
<td>0.68</td>
<td>0.83</td>
</tr>
<tr>
<td>Target lesion revascularization</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>0.78</td>
<td>1.00</td>
</tr>
<tr>
<td>Target vessel, nontarget lesion revascularization</td>
<td>12</td>
<td>9</td>
<td>8</td>
<td>0.47</td>
<td>1.00</td>
</tr>
<tr>
<td>Nontarget vessel revascularization</td>
<td>5</td>
<td>8</td>
<td>6</td>
<td>0.76</td>
<td>0.78</td>
</tr>
<tr>
<td>Documented ventricular arrhythmia treated by ICD</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0.49</td>
<td>1.00</td>
</tr>
<tr>
<td>Hospitalization for heart failure</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0.11</td>
<td>0.68</td>
</tr>
<tr>
<td>Hospitalization for chest pain</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>0.78</td>
<td>1.00</td>
</tr>
<tr>
<td>Stroke</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1.00</td>
<td>0.50</td>
</tr>
<tr>
<td>Composite of death or recurrent myocardial infarction</td>
<td>2</td>
<td>14</td>
<td>3</td>
<td>0.67</td>
<td>0.008</td>
</tr>
<tr>
<td>Composite of death or hospitalization for heart failure</td>
<td>1</td>
<td>6</td>
<td>4</td>
<td>0.20</td>
<td>0.74</td>
</tr>
<tr>
<td>Composite of death, recurrent myocardial infarction or hospitalization for heart failure</td>
<td>2</td>
<td>15</td>
<td>5</td>
<td>0.26</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are number of patients; in composite endpoints multiple events in one patient are possible . BMMC=mononuclear bone marrow cells; PBMC=peripheral blood mononuclear cells ICD=implantable cardioverter-defibrillator.
DISCUSSION

The present analysis confirms and extends our previous reports as we could not demonstrate a beneficial effect of intracoronary delivery of mononuclear cells from bone marrow or peripheral blood on regional and global systolic myocardial function at 2 years of follow-up in patients treated with primary PCI. Nevertheless, we observed that the increase in LVEDV was lower in the BMMC group. No late effects were observed in the BMMC group compared with controls, with comparable increments in the outcomes death and recurrent myocardial infarction up to 5 years of follow-up. The composite endpoint of death, recurrent myocardial infarction was significantly higher in the PBMC group compared with controls.

We previously demonstrated that of the 22 randomised controlled trials assessing intracoronary bone marrow cell therapy after AMI, only 7 included long-term follow-up, defined as longer than 1 year after AMI. The REPAIR AMI trial and 2 other studies, conducted by Cao et al and Plewka et al, demonstrated more improvement
in LV function after intracoronary BMMC therapy compared with controls at the
time of primary endpoint assessment, between 3 and 6 months. In these three studies
this effect remained significant in favour of intracoronary cell therapy at 24,5 484 and
60 months6 after AMI. The BOOST trial demonstrated a benefit for patients treated
with intracoronary BMMC therapy compared with controls at 6 months of follow-up.15
However, this positive effect was no longer significant after 60 months.7 This has been
explained by a catch up phenomenon in the control group, suggesting that bone marrow
cell therapy may, in fact, accelerate the post-AMI functional recovery, but that this effect
is transient.

The study conducted by Penicka et al was prematurely ended due to futility and serious
adverse events in the BMMC group.16 However, the authors demonstrated that BMMC
infusion in patients with AMI was associated with a significant improvement of global
myocardial function during 24 months of follow-up compared with standard therapy.8
In the ASTAMI trial17 and the study conducted by Wohrle et al,18 intracoronary infusion
of mononuclear cells did not improve global LV function at the primary endpoint, as
well as after 3 years of observation.9,19 These data are in line with our observations in the
HEBE trial.

We did find that the increase in LVEDV was lower in the BMMC group, with a trend
for less increase in LVESV suggesting a long-term beneficial effect on adverse LV
remodelling after AMI. The acute loss of myocardium results in an abrupt increase in
loading conditions that induces a remodelling involving the infarcted border zone and
remote non-infarcted myocardium. It has been postulated that intracoronary cell therapy,
might specifically benefit the remote non-infarcted myocardium. Although this finding
is interesting, in all CMR endpoints there were no differences compared with controls.
Therefore we cannot exclude that this finding is due to chance and multiple testing.

Finally, several postulated safety issues about intracoronary cell therapy have been raised.
We have found low rates of adverse events, with no differences between BMMC group
and controls. This is reassuring in terms of safety. Although the study is not powered
to detect differences in clinical endpoints, long-term data from this study and others
establish safety of intracoronary BMMC therapy. The combined endpoint of death and
hospitalisation for heart failure was in the REPAIR-AMI trial, (non-significantly) more
frequent in the placebo compared with the BMMC group (n=19 vs n=10 events, p=0.10)
during the 5 years of follow-up.5 Unfortunately, we could not confirm these beneficial
effects in the HEBE trial.

We did demonstrate that long-term clinical follow-up is worse in patients treated with
PBMC. Although these findings could be a matter of chance, it cannot be fully excluded
that PBMC infusion in the acute phase has aggravated underlying atherosclerotic disease leading to an increase in coronary ischaemic events. The events occurred during long-term follow-up, suggesting it is rather the progression of atherosclerosis than the acute effect of intracoronary infusion of PBMCs. Some small studies using granulocyte-colony stimulating factor or granulocyte-macrophage colony-stimulating factor suggested that an increase in the number of circulating leucocytes is related to plaque destabilisation.\textsuperscript{20,21} Our data do not support further research assessing the effect of local PBMC infusion.

Limitations of this long-term study follow-up include a more than 10\% loss of CMR follow-up at 24 months. However, overall loss of clinical follow-up at 60 months was low. Second, the original protocol did not include a pre-specified statistical plan for the 5 years of analysis, as the 2-year and 5-year follow-up were later added to the study protocol. Lastly, the HEBE study was, due to the ethical reasons, a non-blinded study with no true placebo control. Although there was a blinded evaluation of all endpoints, we cannot exclude the occurrence of potential biases.

In conclusion, we did not show a beneficial effect on clinical outcomes of intracoronary delivery BMMC compared with controls, with comparable increments in the outcomes death and recurrent myocardial infarction up to 5 years of follow-up. There was a significantly worse clinical outcome in patients treated with PBMC during 5 years of follow-up.

**Acknowledgments**

We thank all the investigators and coordinators of the HEBE trial and all the medical and nursing staff who made the trial possible. The complete list of investigators has been published previously.\textsuperscript{10}
REFERENCES


