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## Intracoronary Injection of Mononuclear Bone Marrow Cells in Acute Myocardial Infarction

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### ABSTRACT

#### BACKGROUND

Previous studies have shown improvement in left ventricular function after intracoronary injection of autologous cells derived from bone marrow (BMC) in the acute phase of myocardial infarction. We designed a randomized, controlled trial to further investigate the effects of this treatment.

#### METHODS

Patients with acute ST-elevation myocardial infarction of the anterior wall treated with percutaneous coronary intervention were randomly assigned to the group that underwent intracoronary injection of autologous mononuclear BMC or to the control group, in which neither aspiration nor sham injection was performed. Left ventricular function was assessed with the use of electrocardiogram-gated single-photon-emission computed tomography (SPECT) and echocardiography at baseline and magnetic resonance imaging (MRI) 2 to 3 weeks after the infarction. These procedures were repeated 6 months after the infarction. End points were changes in the left ventricular ejection fraction (LVEF), end-diastolic volume, and infarct size.

#### RESULTS

Of the 50 patients assigned to treatment with mononuclear BMC, 47 underwent intracoronary injection of the cells at a median of 6 days after myocardial infarction. There were 50 patients in the control group. The mean ( $\pm$ SD) change in LVEF, measured with the use of SPECT, between baseline and 6 months after infarction for all patients was  $7.6\pm 10.4$  percentage points. The effect of BMC treatment on the change in LVEF was an increase of 0.6 percentage point (95% confidence interval [CI],  $-3.4$  to  $4.6$ ;  $P=0.77$ ) on SPECT, an increase of 0.6 percentage point (95% CI,  $-2.6$  to  $3.8$ ;  $P=0.70$ ) on echocardiography, and a decrease of 3.0 percentage points (95% CI,  $0.1$  to  $-6.1$ ;  $P=0.054$ ) on MRI. The two groups did not differ significantly in changes in left ventricular end-diastolic volume or infarct size and had similar rates of adverse events.

#### CONCLUSIONS

With the methods used, we found no effects of intracoronary injection of autologous mononuclear BMC on global left ventricular function. (ClinicalTrials.gov number, NCT00199823.)

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\*Members of the Steering Committee and the Data and Safety Monitoring Board of the Autologous Stem-Cell Transplantation in Acute Myocardial Infarction (ASTAMI) study are listed in the Appendix.

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CONTRARY TO PREVIOUS BELIEF, THERE is evidence of regeneration of the myocardium throughout life, and the rate of this regeneration is increased after large myocardial infarctions.<sup>1</sup> However, regeneration seems to be limited to the viable myocardium and its border zone,<sup>1</sup> and the net loss of cardiomyocytes during myocardial infarction is a key factor in the resulting remodeling and in the impairment of cardiac-pump function.<sup>2,3</sup>

The bone marrow harbors stem cells and progenitor cells that may be capable of solid-organ repair.<sup>4</sup> In experimental models of myocardial infarction, intramyocardial or intravenous injections of cells derived from bone marrow (BMC) have resulted in improved left ventricular function through angiogenesis or reduced apoptosis and remodeling.<sup>5,6</sup> The transdifferentiation of transplanted BMC to cardiomyocytes has also been reported.<sup>7,8</sup> Phase 1 studies have confirmed the feasibility of intracoronary injections of autologous mononuclear BMC a few days after myocardial infarction, and the results have indicated improvement of left ventricular function.<sup>9-12</sup> In two randomized trials of intracoronary injections of BMC in the acute phase of myocardial infarction, the effects on global left ventricular function were discrepant.<sup>13,14</sup>

We conducted a randomized, controlled trial — the Autologous Stem-Cell Transplantation in Acute Myocardial Infarction (ASTAMI) study — designed to investigate the effects on left ventricular function of intracoronary injections of autologous mononuclear BMC 4 to 8 days after myocardial infarction treated with acute percutaneous coronary intervention (PCI). In order to study the patients who were best suited for an evaluation of left ventricular function by imaging,<sup>15</sup> we included patients with anterior-wall infarction only. In addition, this type of infarct has the greatest effect on left ventricular function.<sup>16</sup> The primary aim of our study was to examine whether intracoronary injection of autologous mononuclear BMC results in a clinically important improvement in left ventricular function as measured by the left ventricular ejection fraction (LVEF) after acute myocardial infarction. Additional objectives were to examine whether this treatment reduces end-diastolic volume and infarct size.

## METHODS

We included patients with myocardial infarction who were admitted to Rikshospitalet University Hospital or Ullevål University Hospital — both in Oslo — between September 11, 2003, and May 4, 2005. Inclusion criteria were an age of 40 to 75 years, the presence of ST-elevation myocardial infarction of the anterior wall and treatment with PCI 2 to 12 hours after the onset of symptoms, successful PCI with stent implantation performed on the culprit lesion in the left anterior descending coronary artery proximal to the second diagonal branch, three or more hypokinetic left-ventricle segments observed on echocardiography, and a creatine kinase MB level more than three times the upper reference value. Exclusion criteria were previous Q-wave myocardial infarction, cardiogenic shock, and severe coexisting conditions that interfered with the ability of the patient to comply with the protocol. All patients received medication according to current guidelines,<sup>17</sup> followed standard rehabilitation programs for myocardial infarction, and were given general advice on diet, smoking, and lifestyle changes.

The study protocol conformed to the Declaration of Helsinki and was approved by the regional ethics committee. All patients gave written informed consent. An independent data and safety monitoring board was informed of adverse events as they occurred.

### STUDY DESIGN AND TREATMENT RANDOMIZATION

The study design is shown in Figure 1. The day of acute PCI was defined as day 0. On days 3 to 5, patients were randomly assigned in a 1:1 ratio to either the mononuclear BMC group or the control group with the use of permuted-block randomization stratified according to center. Consecutively numbered, sealed envelopes were provided by the Center for Clinical Research at Ullevål University Hospital. Single-photon-emission computed tomography (SPECT) and echocardiography were performed before treatment with mononuclear BMC in the treatment group, on days 4 to 7 (hereafter referred to as baseline). No aspiration or sham injection was performed in the control group. Magnetic resonance imaging (MRI) was performed 2 to 3 weeks after myocardial infarction to prevent overestimation of the infarct

size owing to tissue edema. Six months after myocardial infarction, SPECT, echocardiography, MRI, and coronary angiography were repeated. Clinical evaluations (assessments of functional status and adverse events) and biochemical analysis of blood samples were performed 2 to 3 weeks and 3 and 6 months after myocardial infarction.

#### CELL PREPARATION AND TRANSFER

Bone marrow (50 ml in 10,000 IU of heparin) was obtained from the iliac crest 4 to 7 days after PCI. The aspirate was centrifuged on a Ficoll density gradient to isolate the mononuclear cells, which were washed and resuspended in heparin-treated plasma. Before intracoronary injection, the mononuclear cells were filtered and subjected to quality-control procedures. During intracoronary BMC injection, a PCI technique with intermittent balloon inflation was used to ensure the absence of flow distal to the culprit-lesion stent in the left anterior descending coronary artery.

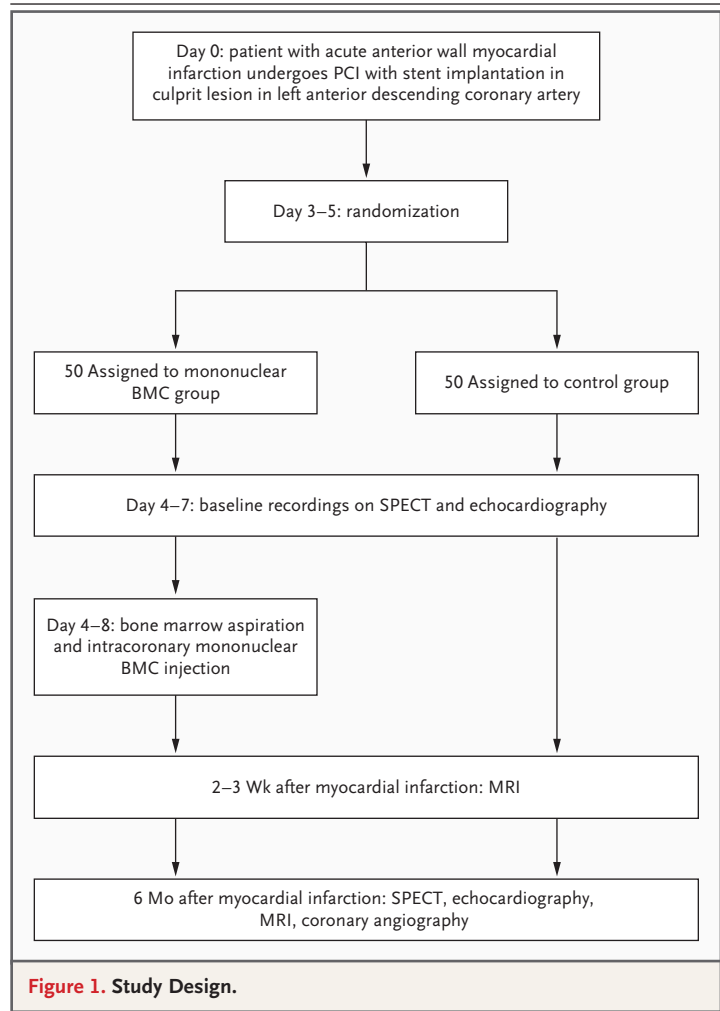
#### CARDIAC IMAGING

Perfusion imaging was performed according to electrocardiogram-gated SPECT, and an Exeleris processing station (GE Medical Systems) with 4D-MSPECT software was used to calculate left ventricular volumes and infarct size. Echocardiograms were obtained with a Vivid 7 scanner (GE Vingmed Ultrasound), and left ventricular volumes were calculated according to the modified Simpson's rule.<sup>18,19</sup> MRI was performed with a 1.5-tesla scanner (Siemens), and left ventricular volumes were calculated according to the area-length method.<sup>20,21</sup> Infarct size on MRI was determined after the administration of gadolinium contrast medium. Infarct size is presented as the total late-enhancement volume (in milliliters) and as a proportion (total late enhancement volume divided by total volume of the left ventricular wall).

A detailed description of cardiac imaging and the characterization and transfer of mononuclear BMC is provided in the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org).

#### STATISTICAL ANALYSIS

The study was designed with 80% power to detect a significant difference in LVEF between baseline and 6 months after infarction at a significance



level of 5%. A clinically important difference was defined as a difference in LVEF between baseline and 6 months of 5 percentage points between the two treatment groups. With an estimated standard deviation of the effect measure of 8.3%,<sup>22</sup> we calculated that 45 patients would need to be enrolled in each group. To allow for some dropouts, we decided to enroll 100 patients in total. All analyses were performed according to the intention-to-treat principle.<sup>23</sup> Prespecified end points were assessed by analysis of covariance, with the baseline values used as a covariate.<sup>24</sup> All variables for end-point analysis approximated a normal distribution, and Bartlett's test confirmed homogeneity of the variance between the two groups.

Values for continuous variables that approximated a normal distribution are presented as

means  $\pm$ SD, and two-sample t-tests were performed for comparisons between groups. Values for variables that were not normally distributed are presented as medians with interquartile ranges, and Mann–Whitney tests were performed for between-group comparisons. Categorical variables were analyzed with the chi-square test or Fisher's exact test, as appropriate. Regression analyses were performed to assess correlations between baseline variables and outcomes. All tests were two-sided, and P values of less than 0.05 were considered to indicate statistical significance. Analyses were performed with Epi Info software, version 3.3.2, and SPSS software, version 12.0.1.

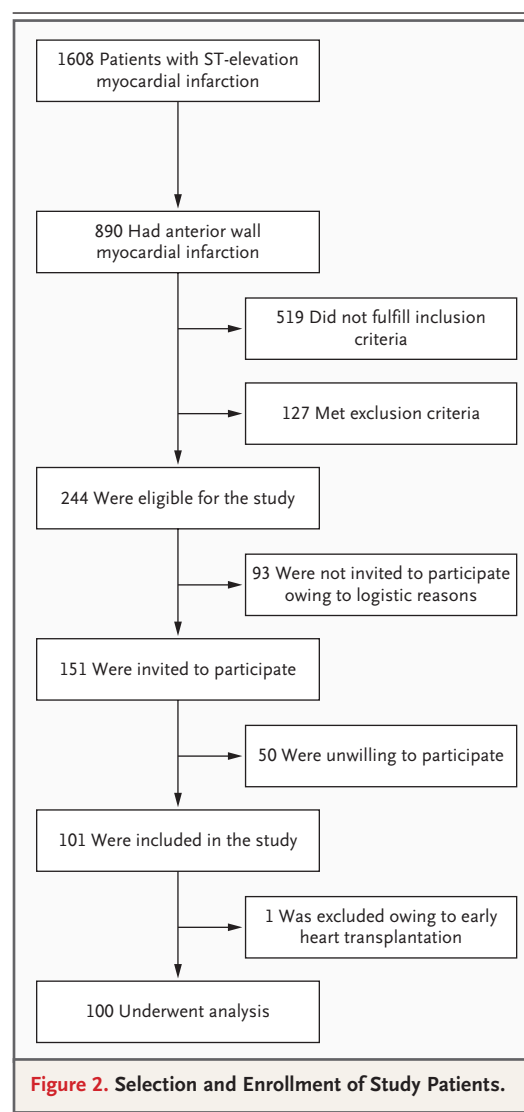
## RESULTS

### PATIENTS AND IMAGING

During the enrollment period, 1608 patients with ST-elevation myocardial infarction were admitted to the study centers for acute PCI (Fig. 2). A total of 101 patients were enrolled in the study, after consideration of inclusion and exclusion criteria, logistics, and patient consent. Of these, 50 were assigned to the mononuclear BMC group, and 51 to the control group. One patient in the control group was later excluded owing to reinfarction and cardiogenic shock on day 11, followed by heart transplantation on day 30. Intracoronary BMC injection was not performed in three patients assigned to the BMC group (because of acute stent thrombosis in two patients and low cell viability [89%] in one).

Six months after infarction, all patients were alive, and none had been lost to follow-up. SPECT and echocardiography were performed in all patients at baseline and at 6 months. SPECT gating values were not obtained at baseline in two patients owing to irregular heart rhythm. MRI was not performed in 11 patients at 2 to 3 weeks and in 7 at 6 months owing to contraindications or logistics, and data from MRI were incomplete for one patient at baseline and one at 6 months.

The characteristics of the patients did not differ significantly between the two groups (Table 1). Among all patients, the mean age was  $57.4 \pm 9.1$  years, the median time from the onset of symptoms to PCI was 210 minutes (interquartile range, 180 to 330), and the median value for maximum



creatinine kinase MB was  $369 \mu\text{g}$  per liter (interquartile range, 220 to 444).

Intracoronary cell injection was performed a median of 6 days (interquartile range, 5 to 6) after the acute PCI. Bone marrow was aspirated the day before injection in 43 patients and on the same day in 4 patients. The median number of mononuclear cells injected was  $68 \times 10^6$  (interquartile range,  $54 \times 10^6$  to  $130 \times 10^6$ ), the median percentage of viable cells was 95% (interquartile range, 94 to 97), and the median number of CD34+ cells was  $0.7 \times 10^6$  (interquartile range,  $0.4 \times 10^6$  to  $1.6 \times 10^6$ ). Bone marrow aspiration and preparation were repeated in one patient owing to low cell viability and another patient owing to

contamination. Of the 47 patients who received intracoronary cell injections, 34 had mild chest pain and 36 had transient ischemic ST deviation during balloon inflation. No patients had reinfarction related to the procedure.

Baseline recordings were obtained for SPECT at  $4.0 \pm 1.4$  days, for echocardiography at  $4.5 \pm 1.1$  days, and for MRI at  $18.8 \pm 4.3$  days after myocardial infarction. LVEF, end-diastolic volume, and infarct size did not differ significantly between the two groups at baseline (Table 2). On SPECT, the mean baseline value among all patients was  $41.9 \pm 11.0\%$  for LVEF ( $P=0.57$  for the comparison of the two groups),  $155.3 \pm 53.4$  ml for end-diastolic volume ( $P=0.19$ ), and  $41.1 \pm 19.4\%$  for infarct size ( $P=0.16$ ). At 6 months, LVEF had increased in both groups (mean change,  $7.6 \pm 10.4$  percentage points). There were no significant differences between groups in the increase in LVEF, end-diastolic volume, or infarct size. For the mononuclear BMC group, there was no significant correlation between the increase in LVEF and the number of mononuclear cells injected ( $r=0.03$ ,  $P=0.82$ ), the time from PCI to BMC injection ( $r=-0.01$ ,  $P=0.97$ ), or the patient's age ( $r=0.02$ ,  $P=0.88$ ). As measured by echocardiography, the mean baseline value among all patients was  $46.3 \pm 9.5\%$  for LVEF ( $P=0.51$  for the comparison of the two groups) and  $134.0 \pm 32.5$  ml for end-diastolic volume ( $P=0.53$ ). The changes in these values of LVEF and end-diastolic volume at 6 months did not differ significantly between groups.

On MRI at 2 to 3 weeks, the mean value among all patients was  $54.2 \pm 12.6\%$  for LVEF ( $P=0.64$  for the comparison between the two groups),  $163.5 \pm 46.2$  ml for end-diastolic volume ( $P=0.72$ ), and  $22.1 \pm 13.3\%$  for infarct size ( $P=0.95$ ). There were no significant differences between the two groups in changes in LVEF, end-diastolic volume, or infarct size (Table 3).

#### ADVERSE EVENTS

Contamination of the cell suspension with coagulase-negative staphylococci was discovered after treatment in one patient with a serum creatinine level of  $80 \mu\text{mol}$  per liter ( $0.9 \text{ mg}$  per deciliter), who was given intravenous vancomycin ( $500 \text{ mg}$ , four times daily) for 3 days without evidence of infection on the basis of clinical evaluation and laboratory tests. Two patients in the mononuclear

BMC group had stent thrombosis in the acute phase, and a new PCI was performed instead of mononuclear BMC injection. Reinfarction was confirmed in one of these patients, who was also treated with PCI in the right coronary artery on day 27 because of unstable angina. During the 6-month follow-up, PCI was performed for culprit-lesion restenosis in one patient in the control group, and coronary-artery bypass grafting was performed in one patient in each group. The numbers of elective PCI procedures performed on the circumflex and right coronary arteries during the first 7 weeks after myocardial infarction are given in Table 1.

After SPECT, echocardiography, and MRI were performed at 6 months, coronary angiography was carried out according to the standard protocol, resulting in PCI being performed for culprit-lesion restenosis in eight patients in each group and coronary-artery bypass grafting being performed in two patients in the mononuclear BMC group and one in the control group. One patient in each group was rehospitalized with progressive heart failure. In the mononuclear BMC group, one patient had sustained ventricular tachycardia before intracoronary injection of mononuclear BMC, and one had ventricular fibrillation at day 6, 24 hours after injection. Both patients recovered without sequelae after resuscitation, and they underwent implantation of cardiac defibrillators, with no therapies delivered during follow-up. In the control group, one patient had pulseless ventricular tachycardia, which was converted to sinus rhythm by means of a precordial thump on day 2. Lung cancer was diagnosed in one patient in the mononuclear BMC group. Retrospectively, the cancer was evident at the time of the primary admission. Four patients in the mononuclear BMC group and six in the control group were rehospitalized for other reasons.

#### DISCUSSION

In our randomized, controlled trial, patients treated with intracoronary injection of mononuclear BMC in the infarct-related coronary artery at a median of 6 days after myocardial infarction, treated with PCI in the acute phase, had neither improved LVEF (Fig. 3) nor reduced left ventricular end-diastolic volume or infarct size at 6 months, as compared with the control group. Results were

**Table 1. Characteristics of the Patients.\***

Characteristic	Mononuclear BMC Group (N = 50)	Control Group (N = 50)	P Value
Age — yr	58.1±8.5	56.7±9.6	0.42
Female sex — no. (%)	8 (16)	8 (16)	1.00
Body-mass index†	26.3±3.8	27.1±3.5	0.30
Hypertension — no. (%)	17 (34)	17 (34)	1.00
Diabetes mellitus — no. (%)	5 (10)	4 (8)	0.73
Previous angina — no. (%)	14 (28)	10 (20)	0.35
Current smokers — no. (%)	20 (40)	24 (48)	0.72
Blood pressure at admission — mm Hg			
Systolic	132±21	132±23	0.96
Diastolic	82±14	83±17	0.92
Heart rate at admission — beats/min	76±16	75±13	0.82
Time from symptom onset to PCI — min			0.54
Median	210	230	
Interquartile range	180–330	180–330	
Thrombolysis before PCI — no. (%)	15 (30)	14 (28)	0.83
TIMI flow grade before primary PCI — no.‡			1.00
Grade 0	31	32	
Grade 1	2	2	
Grade 2	9	8	
Grade 3	8	8	
TIMI flow grade after primary PCI — no.‡			1.00
Grade 2	3	4	
Grade 3	47	46	
Platelet glycoprotein IIb/IIIa inhibitor — no. (%)	24 (48)	25 (50)	0.84
Drug-eluting culprit-lesion stent — no. (%)	3 (6)	2 (4)	1.00
Concomitant coronary-vessel stenosis >50% — no.			0.56
Circumflex coronary artery	5	10	
Right coronary artery	5	6	
No. of diseased vessels — no.			0.29
1	42	36	
2	6	12	
3	2	2	

consistent for the three imaging methods. Our inclusion and exclusion criteria resulted in the enrollment of a relatively homogeneous patient population with definite and extensive anterior-wall infarction, which was well suited for the evaluation of a therapy aimed at improving left ventricular function and reducing infarct size. In 2003, when the study was designed, only a few trials using this treatment had been reported.

Thus, for ethical reasons, bone marrow aspiration and sham intracoronary injections were not performed in our control group. However, all imaging data were analyzed by investigators who were unaware of the treatment assignment. Potential bias related to patient and physician behavior would be expected to favor the mononuclear BMC group. Medical treatment was the same for the two groups.

**Table 1. (Continued.)**

Characteristic	Mononuclear BMC Group (N=50)	Control Group (N=50)	P Value
PCI at time of culprit-lesion PCI — no.			1.00
Circumflex coronary artery	2	1	1.00
Right coronary artery	1	0	
Elective PCI within 7 wk after PCI for MI — no. (%)			
Circumflex coronary artery	0	3	0.24
Right coronary artery	1	3	0.62
Killip class — no.‡			0.17
I	29	36	
II	16	13	
III	4	0	
IV	1	1	
Maximum creatine kinase MB — $\mu\text{g/liter}$			0.50
Median	400	357	
Interquartile range	223–444	220–400	
Q-wave on electrocardiography at primary discharge — no. (%)	42 (84)	38 (76)	0.32
Medication at primary discharge — no. (%)			
Aspirin	50 (100)	50 (100)	1.00
Clopidogrel	50 (100)	50 (100)	1.00
Warfarin	5 (10)	6 (12)	0.75
Beta-blockers	49 (98)	50 (100)	1.00
ACE inhibitors or angiotensin-receptor blockers	50 (100)	50 (100)	1.00
Statins	50 (100)	50 (100)	1.00
Diuretics	21 (42)	16 (32)	0.30
Medication at 6-mo follow-up — no. (%)			
Aspirin	50 (100)	50 (100)	1.00
Clopidogrel	41 (82)	42 (84)	1.00
Warfarin	7 (14)	8 (16)	0.78
Beta-blockers	50 (100)	50 (100)	1.00
ACE inhibitors or angiotensin-receptor blockers	50 (100)	50 (100)	1.00
Statins	50 (100)	48 (96)	0.49
Diuretics	20 (40)	13 (26)	0.14

\* Plus-minus values are means  $\pm$ SD. MI denotes myocardial infarction, and ACE angiotensin-converting enzyme.

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡ The Thrombolysis in Myocardial Infarction (TIMI) trial grades<sup>25</sup> are defined as follows: grade 0, no perfusion; grade 1, penetration without perfusion; grade 2, partial perfusion; and grade 3, complete perfusion.

§ Killip classes<sup>26</sup> are defined as follows: grade I, no heart failure; grade II, heart failure; grade III, severe heart failure; and grade IV, cardiogenic shock. Patients were graded according to the worst score during primary hospital admission for acute myocardial infarction.

The similar increases in LVEF in the two randomly assigned groups were greater as measured with SPECT than with echocardiography or MRI. The reason for this difference may be related to timing. Baseline recordings for SPECT were ob-

tained at 4 days, for echocardiography at about 4.5 days, and for MRI at about 19 days after the infarction. Thus, the potential for observing improvement in left ventricular function due to recovery after stunning<sup>27</sup> and reduction of infarct

**Table 2. LVEF, End-Diastolic Volume, and Infarct Size on SPECT and Echocardiography at Baseline and 6 Months after Myocardial Infarction.\***

Analysis	Baseline		6 Mo		Change between Baseline and 6 Mo		Treatment Effect Change (95% CI)	P Value
	Mononuclear BMC Group (N = 50)	Control Group (N = 50)†	Mononuclear BMC Group (N = 50)	Control Group (N = 50)	Mononuclear BMC Group (N = 50)	Control Group (N = 50)		
<b>SPECT</b>								
LVEF (%)	41.3±10.4	42.6±11.7	49.3±13.2	49.3±11.0	8.1±11.2	7.0±9.6	0.6 (-3.4 to 4.6)	0.77
End-diastolic volume (ml)	162.3±59.1	148.0±46.3	151.1±52.9	146.0±50.0	-11.2±36.0	-1.8±17.6	-7.0 (-18.0 to 4.0)	0.21
Infarct size (%)	43.8±17.4	38.3±21.1	32.8±20.4	30.5±20.9	-11.0±12.7	-7.8±8.7	-2.8 (-7.1 to 1.6)	0.21
<b>Echocardiography</b>								
LVEF (%)	45.7±9.4	46.9±9.6	48.8±10.7	49.0±9.5	3.1±7.9	2.1±9.2	0.6 (-2.6 to 3.8)	0.70
End-diastolic volume (ml)	136.1±30.5	132.0±34.6	145.0±42.0	142.7±45.2	8.9±28.5	10.8±29.1	-1.9 (-13.4 to 9.6)	0.74

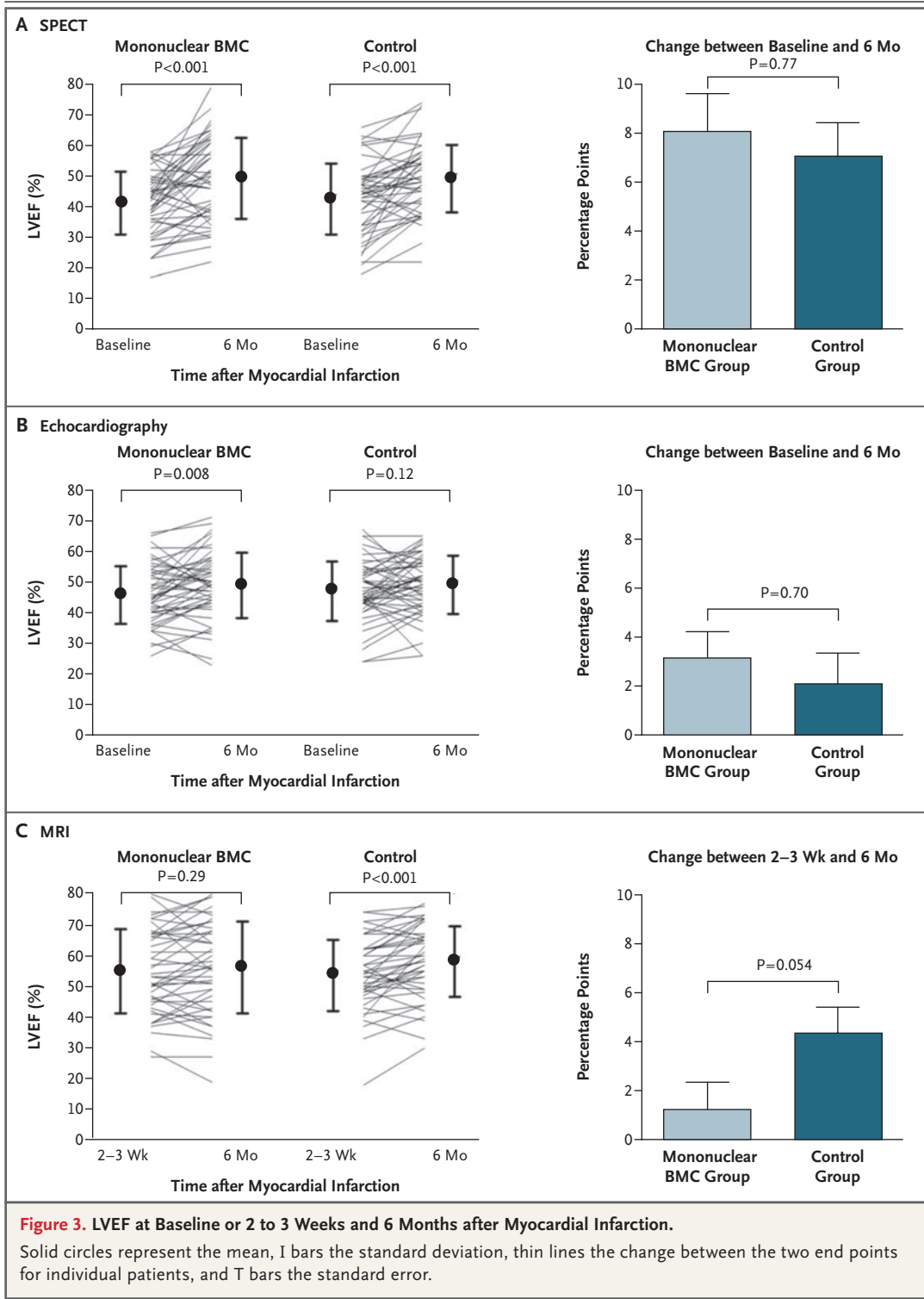
\* Plus-minus values are means ±SD. The change between baseline and 6 months, as well as the treatment effect, was calculated for patients for whom data from both time points were available. Treatment-effect data and P values were obtained from analysis of covariance.

† SPECT readings for LVEF and end-diastolic volume could not be obtained for two patients in the control group at baseline owing to irregular heart rhythm.

**Table 3. LVEF, End-Diastolic Volume, and Infarct Size on MRI 2 to 3 Weeks and 6 Months after Myocardial Infarction.\***

Measure	2-3 Wk		6 Mo		Change between 2-3 Wk and 6 Mo		Treatment Effect Change (95% CI)	P Value
	Mononuclear BMC Group	Control Group	Mononuclear BMC Group	Control Group	Mononuclear BMC Group	Control Group		
LVEF								
No. of patients	45	44	46	47	44	43	87	0.054
Mean ±SD (%)	54.8±13.6	53.6±11.6	56.2±14.9	58.1±11.4	1.2±7.5	4.3±7.1	-3.0 (-6.1 to 0.05)	
End-diastolic volume								
No. of patients	45	44	46	47	44	43	87	0.49
Mean ±SD (ml)	161.7±46.3	165.3±46.7	154.1±54.1	162.5±45.3	-6.9±34.3	-2.8±20.0	-4.2 (-16.1 to 7.7)	
Infarct size								
No. of patients	45	43	45	47	43	43	86	0.11
Mean ±SD (ml)	41.4±27.6	39.0±29.5	38.7±26.9	33.6±23.7	-2.3±11.2	-5.9±13.6	3.9 (-0.9 to 8.8)	
Infarct size								
No. of patients	45	43	45	47	43	43	86	0.07
Mean ±SD (%)	22.0±12.8	22.2±14.0	20.9±11.5	19.6±12.5	-0.7±5.1	-2.6±5.2	1.8 (-0.2 to 3.9)	

\* The change between 2 to 3 weeks and 6 months, as well as the treatment effect, was calculated for patients for whom data from both time points were available. Treatment-effect data and P values were obtained from analysis of covariance.



size due to regression of tissue edema<sup>28</sup> was greatest when SPECT was used and least when MRI was used.

Previous, smaller trials have shown improve-

ment in left ventricular function after treatment with BMC. Why were we unable to confirm this finding? There are no data from experiments in animals or clinical studies indicating that a par-

ticular BMC population should be preferable in the setting of acute myocardial infarction. Consequently, we and others<sup>9,12,13,29</sup> have used unfractionated BMC. Nevertheless, differences in cell preparation and cell numbers may be important.

Although the cell population that may be responsible for a regenerative effect has not yet been identified, the level of circulating CD34+ endothelial progenitor cells is predictive of future cardiovascular events,<sup>30</sup> and bone marrow–derived CD34+ cells could be important for cardiovascular repair.<sup>31</sup> In most studies, mononuclear cells have been obtained on a Ficoll density gradient, and we used this technique as well. Cell quality was assessed according to prespecified criteria for viability and aggregation.

One of the previous, smaller studies demonstrating an effect of intracoronary injection of BMC on global LVEF in patients with acute myocardial infarction was that by Fernandez-Aviles et al.<sup>12</sup> The number of cells they injected was similar to ours, whereas in the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) study<sup>29</sup> and the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) study,<sup>13</sup> more cells were injected. In the BOOST study, all nucleated cells were isolated with the use of gelatin–polysuccinate density-gradient sedimentation. Thus, cell numbers from that study are not directly comparable with those in other studies. In both our study and the two studies with the highest cell numbers, there was no correlation between improvement in LVEF and total number of cells or CD34+ cells.<sup>11,13</sup> Of the groups that found no effect on global LVEF, Strauer et al.<sup>9</sup> injected fewer cells and Janssens et al.<sup>14</sup> injected more cells than we did. The two studies did suggest an effect on regional function and infarct size. However, since cell numbers have not been found to correlate with treatment efficacy, and an effect on LVEF has been demonstrated with cell numbers similar to those used in our study, the lack of effect of mononuclear BMC in our study probably cannot be explained by inadequate cell numbers.

The concept of intracoronary injection of unfractionated BMC for improvement of left ventricular function after myocardial infarction has several fundamental limitations. In a myocardial infarction involving 30% of the left ventricle, the number of cardiomyocytes is reduced by approximately  $1.7 \times 10^9$ .<sup>32</sup> CD34+ progenitor cells constitute only 1 to 2% of mononuclear cells in the bone marrow,<sup>33</sup> and true bone marrow stem cells are even more scarce.<sup>34</sup> After intracoronary injection of BMC, only a small proportion of the cells remain in the heart,<sup>35</sup> and a large proportion die after a few days.<sup>36</sup> In addition, the ability of bone marrow progenitor cells to differentiate into cardiomyocytes has been questioned,<sup>37,38</sup> and a mechanism for the improvement of cardiac function by means of mononuclear BMC treatment in humans has not been established.<sup>39</sup> If angiogenesis,<sup>5,6</sup> paracrine action,<sup>40</sup> or immune modulation<sup>41</sup> is involved, an observation period of 6 months may have been too short to demonstrate an effect. However, this argument is not supported by the long-term results of the BOOST trial, in which an effect of BMC treatment was reported at 6 months,<sup>13</sup> whereas at 18 months the difference between the groups had vanished.<sup>42</sup>

Our findings are confined to our methods used in a population with acute anterior-wall ST-elevation myocardial infarction. However, it is unlikely that a positive effect would have been found in patients with other types of infarcts. The study was powered to detect a 5% improvement in LVEF, which was considered to be a clinically important effect of this highly invasive intervention. Our results do not rule out a more modest effect of mononuclear BMC treatment, which may be addressed in future studies. Further research is needed before intracoronary injections of BMC can be recommended for patients with acute myocardial infarction.

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#### APPENDIX

The members of the Steering Committee and the Data and Safety Monitoring Board for the Autologous Stem-Cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial were as follows: **Steering Committee** — K. Forfang (chair), S. Aakhus, H. Arnesen, T. Egeland, K. Endresen, A. Ilebakk, A. Mangschau; **Data and Safety Monitoring Board** — University Hospital of North Norway, Tromsø, Norway: K. Rasmussen; Uppsala Clinical Research Centre, University Hospital, Uppsala, Sweden: L. Wallentin; University Hospital of Trondheim, Trondheim, Norway: R. Wiseth.

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