

Intramyocardial Bone Marrow Cell Injection for Chronic Myocardial Ischemia

A Randomized Controlled Trial

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BONE MARROW CELL THERAPY IS currently being investigated as a new therapeutic option for patients with ischemic heart disease. The goal of this treatment is to improve myocardial perfusion and contractile performance through administration of therapeutic cells into ischemically damaged myocardium. The majority of clinical studies conducted so far investigated whether intracoronary bone marrow cell infusion could enhance functional recovery after acute myocardial infarction.¹⁻³

Animal model studies, however, suggested that bone marrow cell therapy may also improve myocardial perfusion and increase left ventricular (LV) function in chronic ischemia.^{4,5} A number of nonrandomized clinical studies indicated the safety and feasibility of intramyocardial bone marrow cell injection.⁶⁻⁹ Moreover, a beneficial effect on myocardial perfusion and LV function was presumed. Until now, only 2 small-sized randomized controlled studies assessed the effect of bone marrow cell

Context Previous studies have suggested that bone marrow cell injection may improve myocardial perfusion and left ventricular (LV) function in patients with chronic myocardial ischemia.

Objective To investigate the effect of intramyocardial bone marrow cell injection on myocardial perfusion and LV function in patients with chronic myocardial ischemia.

Design, Setting, and Patients Randomized, double-blind, placebo-controlled trial at a Netherlands university hospital, May 1, 2005–March 3, 2008 (6-month follow-up ended September 2008) of 50 patients with chronic myocardial ischemia (mean age [SD], 64 [8] years; 43 men). Inclusion criteria: severe angina pectoris despite optimal medical therapy and myocardial ischemia. All patients were ineligible for conventional revascularization.

Interventions Intramyocardial injection of 100×10^6 autologous bone marrow-derived mononuclear cells or placebo solution.

Main Outcome Measures Primarily, the summed stress score, a 17-segment score for stress myocardial perfusion assessed by Tc-99m tetrofosmin single-photon emission computed tomography (SPECT). Secondary included LV ejection fraction (LVEF), Canadian Cardiovascular Society (CCS) class, and Seattle Angina Questionnaire quality-of-life score (mean difference $>5\%$ considered clinically significant).

Results After 3-month follow-up, the summed stress score (mean [SD]) improved from 23.5 (4.7) to 20.1 (4.6) ($P < .001$) in the bone marrow cell group, compared with a decrease from 24.8 (5.5) to 23.7 (5.4) ($P = .004$) in the placebo group. In the bone marrow cell-treated patients who underwent magnetic resonance imaging (MRI), a 3% absolute increase in LVEF was observed at 3 months (95% CI, 0.5% to 4.7%; $n = 18$), but the placebo group showed no improvement. CCS angina score improved significantly in the bone marrow cell group (6-month absolute difference, -0.79 ; 95% CI, -1.10 to -0.48 ; $P < .001$) compared with no significant improvement in the placebo group. Quality-of-life score increased from 56% (9%) to 64% (12%) at 3 months and 69% (12%) at 6 months in bone marrow cell-treated patients, compared with a smaller increase in the placebo group from 57% (11%) to 61% (14%) to 64% (17%). The improvements in CCS class and quality of life score were significantly greater in bone marrow cell-treated patients than in placebo-treated patients ($P = .03$ and $P = .04$, respectively).

Conclusions In this short-term study of patients with chronic myocardial ischemia refractory to medical treatment, intramyocardial bone marrow cell injection resulted in a statistically significant but modest improvement in myocardial perfusion compared with placebo. Further studies are required to assess long-term results and efficacy for mortality and morbidity.

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injection in patients with chronic myocardial ischemia.^{10,11} Since the results of these 2 studies were discrepant, the beneficial effect of this treatment mo-

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dality on myocardial perfusion and LV function remains unclear.

The goal of the current randomized, double-blind, placebo-controlled trial was to assess the effect of intramyocardial bone marrow cell injection on myocardial perfusion and LV function in patients with chronic ischemia who are ineligible for conventional treatment.

METHODS

Patients

The study population consisted of patients with severe angina (Canadian Cardiovascular Society [CCS] class III-IV) despite optimal medical therapy, and myocardial ischemia in at least 1 myocardial segment on Tc-99m tetrofosmin single-photon emission computed tomography (SPECT). All patients were ineligible for both surgical and percutaneous revascularization as determined by an independent expert panel that reviewed the coronary angiograms.

Exclusion criteria were LV ejection fraction (LVEF) of less than 35%, acute myocardial infarction within 6 months before enrollment, history of malignancy, renal dysfunction (glomerular filtration rate <30 mL/min/1.73 m²), or unexplained hematological abnormalities.

The protocol was approved by the institutional ethics committee and written informed consent was obtained from each patient.

Study Protocol

At baseline, the clinical status of the patients was assessed according to the CCS classification, ranging from class I (mild) to IV (severe).¹² Quality of life was assessed using the disease-specific Seattle Angina Questionnaire ranging from 0% to 100% (higher scores indicating better health status; mean difference $>5\%$ considered clinically significant).^{13,14} A bicycle exercise test (to evaluate exercise capacity), SPECT (to assess myocardial perfusion), and magnetic resonance imaging (MRI [to assess LV function and volumes]) were performed at baseline.

On the day of the injection procedure, 80 mL of bone marrow was har-

vested from each patient. The bone marrow was aspirated from the iliac crest by an experienced hematologist under local anesthesia and placed in a heparinized Hanks balanced salt solution. The mononuclear cells were isolated using Ficoll density gradient centrifugation according to good manufacturing practice regulations, washed in phosphate-buffered saline with 0.5% human serum albumin, and resuspended in phosphate-buffered saline with 0.5% human serum albumin.¹⁵ The final suspension of bone marrow mononuclear cells contained 40×10^6 /mL. The filtered bone marrow was checked for the presence of clots, and the bone marrow cell population was analyzed by fluorescence-activated cell sorting using anti-CD34 and anti-CD45 antibodies (Becton Dickinson, Palo Alto, California).

After cell isolation, patients were randomly assigned in a 1:1 ratio to the bone marrow cell group or the placebo group, using sequentially numbered sealed envelopes provided by the Department of Medical Statistics and Bioinformatics. A block size of 4 was used without further stratification. Following randomization, a blinded syringe with either bone marrow cell suspension or placebo solution (NaCl 0.9% with 0.5% human serum albumin) was brought to the catheterization laboratory. The patients, study coordinators, and investigators responsible for patient assessment were unaware of group assignment.

During cell isolation and randomization, a 3-dimensional electromechanical map of the LV was obtained¹⁶ using the NOGA system (Biologics Delivery Systems Group, Cordis; Bridgewater, New Jersey). The ischemic regions on SPECT were visually matched with the 3-dimensional electromechanical map based on anatomical landmarks including LV long axis, position of apex, mitral valve area, aortic valve location, and basal inferoseptal point. Cross-referencing was also performed using fluoroscopic identification of anterior, septal, lateral, and inferior orientations. Subsequently, 8 to 10 intramyocardial injections of 0.2

0.3 mL each were delivered (see video at <http://www.jama.com/cgi/content/full/301/19/1997/DC1>).

At 3 and 6 months, CCS class, quality-of-life score, and exercise capacity were reassessed and 24-hour Holter electrocardiogram recordings were obtained to monitor the occurrence of arrhythmias. At 3 months, SPECT and MRI were repeated to reassess myocardial perfusion and LV function and volumes.

SPECT Imaging

SPECT imaging was performed¹⁶ using adenosine stress (0.14 mg/kg/min for 6 minutes) and intravenously injecting 500 MBq of Tc-99m tetrofosmin after 3.5 minutes of adenosine infusion. Resting images were obtained after a second injection of 500 MBq of Tc-99m tetrofosmin. Reconstruction yielded long- and short-axis projections perpendicular to the heart axis. The short-axis slices were displayed in polar map format and adjusted for peak myocardial activity (100%).

The myocardium was divided into 17 segments according to the American Heart Association/American College of Cardiology recommendations.¹⁷ Myocardial perfusion was analyzed using Quantitative Gated SPECT software (Cedars-Sinai Medical Center, Los Angeles, California) and segmental tracer activity was categorized on a 4-point scale: 1 denoting tracer activity greater than 75%; 2 denoting tracer activity of 50% to 75%; 3 denoting tracer activity of 25% to 49%; and 4 denoting tracer activity of less than 25%. Perfusion defects on stress images were considered present when tracer activity was less than 75% of maximum. When significant fill-in ($>10\%$) of perfusion defects was observed on the resting images, segments were classified as ischemic. Summation of the patients' segmental scores at stress yielded the summed stress score, and summation of the patients' segmental rest scores yielded the summed rest score.

Magnetic Resonance Imaging

MRI studies were performed¹⁵ using a 1.5-Tesla system (Philips Medical Sys-

tems; Best, the Netherlands) with a 5-segment synergy coil and vector electrocardiographic gating. Two experienced observers (J.v.R. and S.D.R.), blinded to all clinical data, analyzed the images. Previously validated software was used to determine parameters of global systolic function (QMass MR, Medis Medical Imaging Systems; Leiden, the Netherlands).¹⁸ The mean (SD) levels for intraobserver and interobserver variability were 1 (3) mL and 2 (4) mL for LV end-systolic volume, 1 (4) mL and 2 (6) mL for LV end-diastolic volume, and 0.2% (1.6%) and 0.5% (2.1%) for LVEF, respectively.¹⁵

Assessment of Exercise Capacity

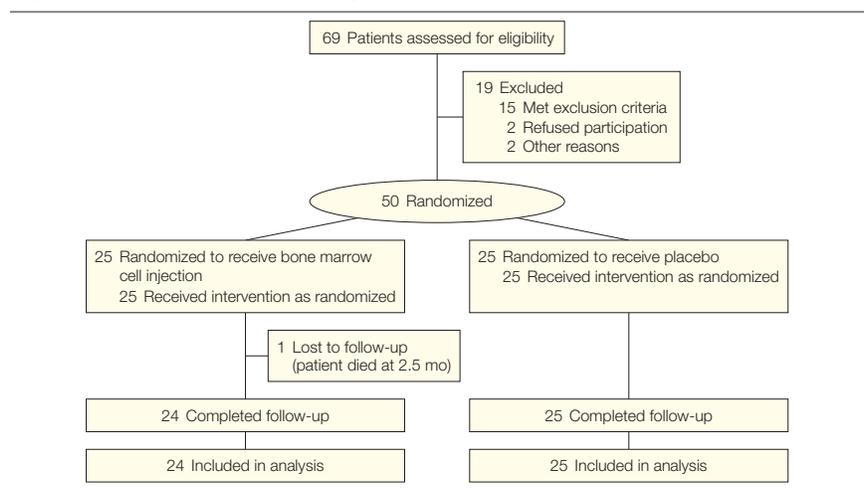
Patients performed a symptom-limited bicycle exercise test with a 20-W starting load and an increment of 10 W/min. Exercise tests were monitored by a study coordinator who was not aware of the patients' group assignment. Patients were encouraged to perform as much exercise as possible while their symptoms and 12-lead electrocardiogram were continuously assessed. Test end points were angina, physical exhaustion, dyspnea, or significant decrease in systolic blood pressure (>10 mm Hg).

Statistical Analysis

This study was designed to demonstrate a treatment difference of at least 3 points in summed stress score between bone marrow cell-treated patients and placebo-treated patients. Based on an earlier study from our group, the SD of this effect measure was estimated at 3.35 points.¹⁶ To obtain a power of at least 85% in a 2-sided test with a type I error of at most 5%, 23 patients needed to be enrolled in each group. Fifty patients were enrolled to account for dropouts.

Data are reported as mean (SD). Categorical variables were compared using the χ^2 test or Fisher exact test. We applied repeated measures analysis of variance and the Friedman test to study the relation between randomly allocated treatment and changes in continuous outcome data at baseline and follow-up

Figure 1. Flow of Participants Through the Trial



time points. For the analysis of summed stress score, summed rest score, and variables with significant differences in baseline values, an analysis of covariance was performed to assess differences between both groups at 3-month follow-up adjusted for baseline values. The analysis of covariance included the values at 3 months as dependent variables, and the associated baseline values and a factor for treatment as independent variables. Treatment effects were estimated by computing the differences between the adjusted means of the bone marrow cell-treated patients and placebo-treated patients and their corresponding 95% confidence intervals (CIs). A Mann-Whitney test was used to compare changes in semiquantitative data. A *P* value of less than .05 was considered statistically significant. All statistical analyses were performed with SPSS software version 16.0 (SPSS, Chicago, Illinois).

RESULTS

Between May 1, 2005, and March 3, 2008, 50 patients (64 [8] years; 43 men) were enrolled in the study. Of these, 25 patients were assigned to the bone marrow cell group and 25 to the placebo group (FIGURE 1). All analyses were performed in line with the intention-to-treat principle. For all paired tests, complete case analysis was performed. There were no differences in

baseline characteristics between the groups (TABLE 1). During the study period, the type and dose of cardiovascular medications remained unchanged in all patients.

Procedural Data

Mean procedural time for bone marrow aspiration was 20 (8) minutes in the bone marrow cell group and 23 (9) minutes in the placebo group (*P* = .22). Procedural time for mapping and injection was 62 (18) minutes in the bone marrow cell group and 59 (16) minutes in the placebo group (*P* = .42). Bone marrow cell-treated patients received 8.5 (1.3) injections, whereas placebo-treated patients received 8.3 (1.0) injections (*P* = .24). The cell suspension contained $98 (6) \times 10^6$ bone marrow cells, with a cell viability of 98% (1%) and a CD34-positive cell fraction of 2.4% (0.9%).

Myocardial Perfusion and Ischemia

Paired SPECT studies were available for 24 bone marrow cell-treated patients and 25 placebo-treated patients. In the bone marrow cell group, the summed stress score improved from 23.5 (4.7) at baseline to 20.1 (4.6) at 3 months (*P* < .001). In the placebo group, the summed stress score also improved from 24.8 (5.5) at baseline to 23.7 (5.4) at 3 months (*P* = .004). However, when the 2 groups were compared, the improvement in summed stress score was significantly

greater in bone marrow cell–treated patients as compared with placebo-treated patients (treatment effect, -2.44 ; 95% CI, -3.58 to -1.30 ; $P < .001$). The summed rest score improved from 18.9 (3.9) at baseline to 18.3 (3.9) at 3 months in the bone marrow cell group ($P = .002$).

In the placebo group, the summed rest score remained unchanged at 21.0 (5.4) at baseline vs 20.7 (4.8) at 3 months ($P = .10$). The change in summed rest score was not significantly different between both groups (treatment effect, -0.32 ; 95% CI, -0.87 to 0.23 ; $P = .25$).

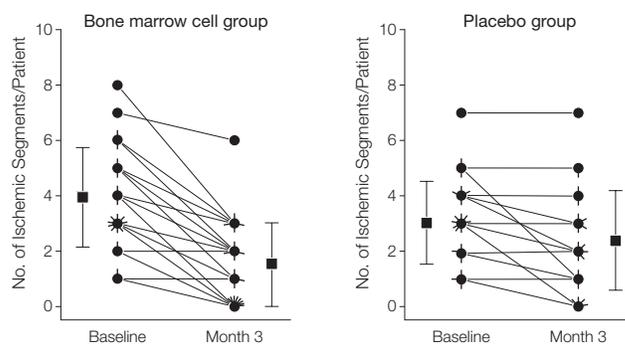
The number of ischemic myocardial segments per patient at baseline was not significantly different between the groups ($P = .10$). In the bone marrow cell group, the mean number of ischemic myocardial segments per patient decreased from 3.9 (1.8) at baseline to 1.5 (1.5) at 3 months ($P < .001$). In the placebo group, the number of ischemic myocardial segments also decreased from 3.1 (1.5) at baseline to 2.4 (1.8) at 3 months ($P = .003$). FIGURE 2 displays that the absolute decrease in the number of ischemic myocardial segments per patient was significantly greater in the bone marrow cell group (-2.4 ; 95% CI, -2.9 to -1.9 vs -0.8 ; 95% CI, -1.2 to -0.3 ; $P < .001$).

Table 1. Baseline Characteristics of the Study Population

	No. (%)		P Value
	Bone Marrow Cell Group (n = 25)	Placebo Group (n = 25)	
Age, mean (SD), y	64 (8)	62 (9)	.55
Men	23 (92)	20 (80)	.41
Cardiovascular risk factors			
Smoking	10 (40)	12 (48)	.77
Hypertension	12 (48)	11 (44)	>.99
Diabetes	13 (52)	8 (32)	.25
Dyslipidemia	12 (48)	15 (60)	.57
Family history of coronary artery disease	16 (64)	13 (52)	.56
Body mass index, mean (SD), kg ^a	29 (3)	28 (4)	.40
Current medication			
Nitrates	21 (84)	21 (84)	>.99
β -Blockers	24 (96)	24 (96)	>.99
Calcium channel blockers	18 (72)	18 (72)	>.99
Statins	25 (100)	25 (100)	>.99
Angiotensin-converting enzyme inhibitors	19 (76)	14 (56)	.23
Clopidogrel	13 (52)	8 (32)	.25
Aspirin	20 (80)	21 (84)	>.99
Oral anticoagulants	5 (20)	4 (16)	.68
Medical history			
Prior myocardial infarction	14 (56)	18 (72)	.37
Prior coronary artery bypass grafting	24 (96)	19 (76)	.10
Prior percutaneous coronary intervention	16 (64)	13 (52)	.57

^aBody mass index was calculated as weight in kilograms divided by height in meters squared.

Figure 2. Improvements in Segments With Inducible Myocardial Ischemia as Assessed by SPECT



Each additional spoke on the data markers denotes another patient with the same number of ischemic segments. In the bone marrow cell group, there is a larger decrease in segments with inducible ischemia than in the placebo group ($P < .001$). Square data markers with error bars are the mean (SD) number of ischemic myocardial segments per patient for each group.

Myocardial Perfusion in Injected and Noninjected Segments

In the 25 bone marrow cell–treated patients, a total of 213 injections were targeted at 92 ischemic myocardial segments (3.7 [0.6] injected segments/patient). In the 25 placebo-treated patients, a total of 207 injections targeting 97 ischemic myocardial segments were performed (3.9 [0.5] injected segments/patient; $P = .38$).

In the bone marrow cell group, 52 of 92 injected segments (57%) increased at least 1 point in rest or stress perfusion. In these patients, 15 of 333 noninjected segments (5%) showed an improved perfusion ($P < .001$). In the placebo group, an improved perfusion was observed in 19 of 97 injected segments (20%) and in 19 of 328 noninjected segments (6%), P was less than .001. The percentage of injected segments with an improved perfusion was significantly higher in the bone marrow cell group than in the placebo group (57% vs 20%; $P < .001$). The percentage of noninjected segments with an improved perfusion was similar in both groups (5% vs 6%; $P = .48$).

LV Function and Volumes

Paired MRI was performed in 22 bone marrow cell–treated patients and in 18 placebo-treated patients. In this subset of patients, no differences in baseline characteristics existed between the

groups. At baseline, LV volumes and LVEF were not significantly different between the 2 groups (TABLE 2). In the bone marrow cell group, LVEF increased from 56% (12%) at baseline to 59% (11%) at 3 months ($P = .02$; FIGURE 3). In the placebo group, LVEF was 54% (10%) at baseline and 53 (10%) at 3 months ($P = .55$). When the 2 groups were compared, the absolute increase in LVEF was significantly larger in bone marrow cell-treated patients (change, 3%; 95% CI, 0.5% to 4.7% vs -1%; 95% CI, -2.1 to 1.1; $P = .03$). In both treatment groups, no significant changes in LV end-diastolic volume and LV end-systolic volume were noted (Table 2).

Clinical Outcome

Complete clinical follow-up data were available in 24 patients in the bone marrow cell group and 25 patients in the placebo group. Assessment of the clinical status according to the CCS class revealed a significant improvement in the bone marrow cell group from 3.0 (0.6) to 2.3 (0.7) at 3 months, and to 2.2 (0.6) at 6 months (absolute difference at 6 months, -0.79; 95% CI, -1.10 to -0.48; $P < .001$; FIGURE 4A). In the placebo group, no significant difference in CCS class was observed (2.9 [0.7] to 2.6 [0.8] at 3 months and to 2.5 [0.9] at 6 months), absolute difference at 6 months -0.39 (95% CI, -0.9 to 0.12; $P = .058$). In line with this observation, the quality of life score increased from 56% (9%) to 64% (12%) at 3 months, and to 69% (12%) at 6 months (absolute improvement at 6 months, 13.0%; 95% CI, 8.2% to 17.9%) in the bone marrow cell-treated patients ($P < .001$; Figure 4B). In the placebo group, a modest but significant improvement in the quality-of-life score was noted from 57% (11%) to 61% (14%) at 3 months and to 64% (17%) at 6 months (absolute improvement at 6 months, 6.3%; 95% CI, 0.5% to 13.0%; $P = .04$). The improvements in CCS class and quality-of-life score were significantly greater in the bone marrow cell group ($P = .03$ and $P = .04$, respectively).

Exercise Capacity

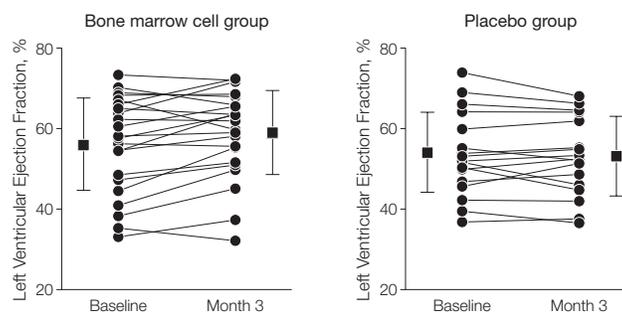
Bicycle exercise testing at all time points was available in 24 bone marrow cell-treated patients and 25 placebo-treated patients. In the bone marrow cell group, the maximal achieved workload increased from 107 (29) W to 114 (34) W at 3 months and to 116 (32) W at 6 months ($P = .01$). In the placebo group, no significant change in maximal workload was detected with a level of 101 (35) W vs 99 (34) W at 3 months

vs 103 (41) W at 6 months, $P = .49$). The time to significant ST-segment depression increased from 7.0 (1.7) minutes to 8.3 (1.7) minutes at 3 months and 8.1 (2.0) minutes at 6 months in bone marrow cell-treated patients ($P = .001$). No significant change in time to significant ST-segment depression was observed in placebo-treated patients: 8.5 (3.0) minutes vs 8.6 (3.2) minutes vs 8.1 (2.6) minutes ($P = .25$). The change in time to significant ST-segment de-

Table 2. Left Ventricular Function and Volumes as Assessed by Magnetic Resonance Imaging

Characteristics	Mean (SD)		P Value
	Bone Marrow Cell Group (n = 22)	Placebo Group (n = 18)	
Left ventricular ejection fraction, %			
Baseline	56 (12)	54 (10)	.54
3 mo	59 (11)	53 (10)	.11
Change from baseline	3 (5)	-1 (3)	.03
P value	.02	.55	
Left ventricular stroke volume, mL			
Baseline	98 (47)	94 (16)	.51
3 mo	103 (20)	92 (17)	.06
Change from baseline	5 (10)	-2 (11)	.048
P value	.03	.45	
Left ventricular end-systolic volume, mL			
Baseline	83 (47)	87 (41)	.77
3 mo	79 (45)	86 (40)	.61
Change from baseline	-4 (15)	-1 (10)	.45
P value	.09	.64	
Left ventricular end-diastolic volume, mL			
Baseline	181 (54)	181 (50)	>.99
3 mo	182 (54)	178 (49)	.80
Change from baseline	1 (15)	-3 (19)	.43
P value	.76	.43	

Figure 3. Individual Changes in Left Ventricular Ejection Fraction



In the bone marrow cell group, left ventricular ejection fraction (LVEF) increased significantly ($P = .02$), whereas LVEF remained unchanged in the placebo group ($P = .55$). Improvement in LVEF was significantly greater in the bone marrow cell group ($P = .03$). Square data markers with error bars are the mean (SD) percent LVEF for each group.

pression improved more in the bone marrow cell group than in the placebo group (treatment effect, 1.3 minutes; 95% CI, 0.6 to 2.0; $P = .001$).

Safety Data

During the 6-month follow-up period, no arrhythmias were observed in any of the patients. In addition, electrocardiograms at rest on 24-hour Holter recordings and during exercise testing revealed no arrhythmias in both study groups.

In the bone marrow cell group, 1 patient died 2.5 months after the injection procedure because of myocardial ischemia leading to acute heart failure. At 3.5 months, another bone marrow cell–treated patient developed a rel-

evant stenosis in an arterial bypass graft that was located outside the injected myocardial territory. This patient underwent a percutaneous coronary intervention and completed the study.

In the placebo group, a greater than 0.5 cm pericardial effusion was detected on 2-dimensional echocardiography in an asymptomatic patient 2 days after the injection procedure and pericardiocentesis was subsequently performed. One cerebrovascular accident was observed in another placebo-treated patient with a history of transient ischemic attacks. Four months after the injection procedure, 1 placebo-treated patient was diagnosed with spondylodiscitis caused by a *Staphylococcus aureus* infection. Furthermore,

1 patient in the placebo group was diagnosed with breast cancer at 5.5 months follow-up.

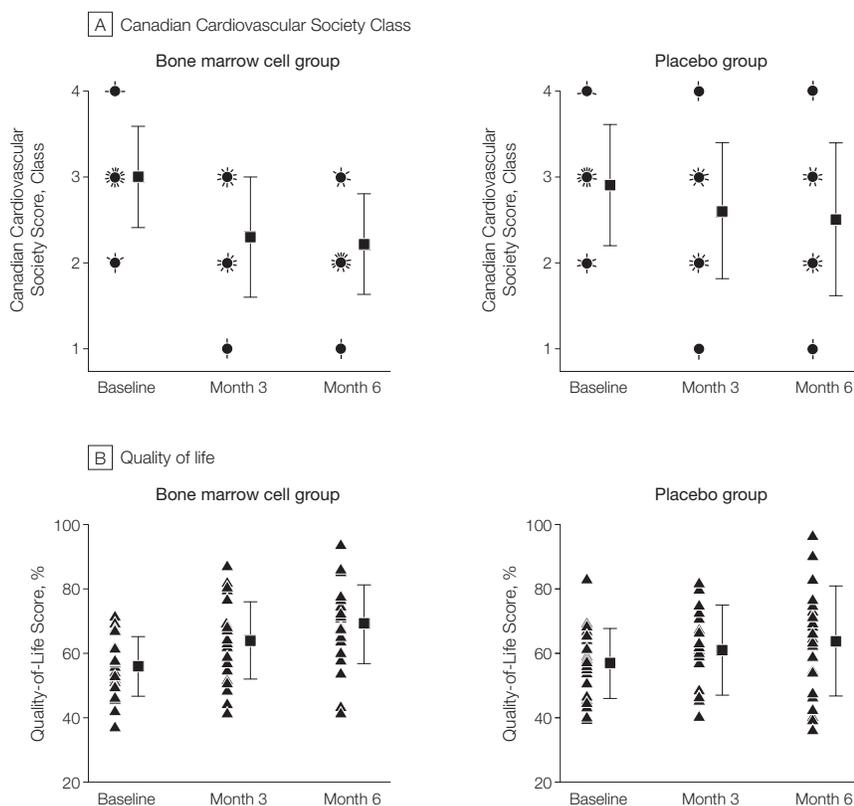
COMMENT

The current randomized, double-blind, placebo-controlled trial investigated the effect of intramyocardial bone marrow cell injection on myocardial perfusion and LV function in patients with chronic ischemia who are ineligible for conventional treatment. The main finding of the present trial is that bone marrow cell injection was associated with an improved myocardial perfusion and an increased LVEF. A more pronounced improvement in CCS class, quality-of-life score, and exercise capacity was observed in bone marrow cell–treated patients as compared with placebo-treated patients.

Animal model studies suggest that bone marrow cell therapy for ischemic heart disease may improve myocardial perfusion and contractile performance.¹⁸ These observations may be related to differentiation of bone marrow cells in endothelial cells, smooth muscle cells, or cardiac myocytes. In addition, the bone marrow cells may secrete paracrine factors that promote angiogenesis, exert cytoprotective effects, recruit resident cardiac stem cells, or alter mechanical properties of myocardial scar tissue. These preclinical findings provided the rationale for the initiation of various clinical studies investigating bone marrow cell therapy as a novel treatment for ischemic heart disease. In most clinical studies, bone marrow cells were infused in the infarct-related artery 3 to 8 days after percutaneous coronary intervention for acute myocardial infarction.¹⁻³

Only limited data are available in patients with severe angina and chronic ischemia who are ineligible for conventional revascularization. Initially, 4 nonrandomized pilot studies (64 patients) reported that intramyocardial bone marrow cell injection in chronic ischemic myocardium is safe and feasible.⁶⁻⁹ Also, bone marrow cell injection seemed to be associated with a beneficial effect on angina, myocardial

Figure 4. CCS Class and Quality-of-Life Score at Baseline and Follow-up



Data points with error bars are the mean (SD) scores for each group. A, For Canadian Cardiovascular Society (CCS) class, a spoke is added to the circle data marker for each additional patient with the same score. CCS class improved significantly in the bone marrow cell group ($P < .001$), whereas no significant improvement was observed in the placebo group ($P = .06$). Improvement in CCS class was significantly greater in the bone marrow cell group ($P = .03$). B, Quality of life improved significantly in both groups ($P < .001$ in the bone marrow cell group and $P = .04$ in the placebo group). Quality of life showed a larger improvement in the bone marrow cell group ($P = .04$).

perfusion, and LV function. A placebo effect, however, may have contributed to these beneficial effects since these studies did not comprise a control group.

Recently, 2 small-sized randomized controlled trials were performed in patients with chronic ischemia refractory to medical therapy.^{10,11} Losordo and Schatz¹⁰ reported the feasibility and safety of intramyocardial injection of granulocyte-colony stimulating factor-mobilized CD34+ stem cells. There was no significant difference in angina frequency, exercise time, or CCS class, but this may have been due to underpowering for these outcomes. In the PROTECT-CAD trial,¹⁰ the effect of intramyocardial bone marrow cell injection on clinical outcome, myocardial perfusion, and LV function was assessed in 19 bone marrow cell-treated patients and 9 placebo-treated patients. Bone marrow cell injection was associated with a modest increase in exercise capacity and LVEF compared with placebo. However, no difference in myocardial perfusion between bone marrow cell-injected patients and placebo-treated patients was observed.

In this trial, bone marrow cell injection resulted in a significant improvement in anginal symptoms, quality of life, and exercise capacity, in line with the PROTECT-CAD trial. Also, the currently observed increase in LVEF in bone marrow cell-treated patients is consistent with the modest increase in LVEF in the PROTECT-CAD trial. The present trial, however, is the first randomized, double-blind, placebo-controlled trial to report that the improvement in anginal symptoms and LV function was accompanied by a significant improvement in myocardial perfusion. Particularly, the decrease in the number of ischemic myocardial segments per patient was significantly greater in bone marrow cell-treated patients as compared with placebo-treated patients, implicating a more pronounced improvement in myocardial perfusion as observed in the PROTECT-CAD trial. This may be related to the 2-fold higher number of bone marrow cells administered in the current trial ($98 [7] \times 10^6$ cells vs as many

as $42 [28] \times 10^6$ in PROTECT-CAD). Although this specific issue has not yet been addressed in large clinical studies, a recent trial in acute infarction patients has suggested the possibility of a dose-response relationship.¹⁹

In the past decades, a number of therapies have emerged for the management of refractory angina pectoris. For example, treatment with the metabolic agent ranolazine increased exercise time by 23.9 seconds compared with placebo treatment.²⁰ However, the time to onset of ST-segment depression during exercise testing did not change and the effect on myocardial perfusion was not investigated. Another adjunctive therapy, enhanced external counterpulsation (EECP), was associated with a decrease in angina pectoris frequency but no significant effect on exercise time was observed.²¹ Importantly, the effect of EECP on myocardial perfusion has not been investigated in a randomized trial. An invasive technique that has been investigated for the treatment of angina pectoris is transmyocardial laser revascularization. Although improvement in anginal complaints was reported in the initial studies,²² a large randomized, blinded trial did not show a beneficial effect of laser revascularization when compared with a sham procedure.²³ In particular, no improvement in myocardial perfusion was observed after ranolazine administration, EECP, or after transmyocardial laser revascularization in randomized studies.

In this trial, the beneficial effect of bone marrow cell injection on CCS class, quality-of-life score, and exercise capacity may be classified as modest. However, in contrast with the aforementioned techniques, the beneficial effect on clinical parameters was accompanied by objective improvements in myocardial perfusion and LV function.

In this trial, small but significant improvements in quality-of-life scores and myocardial perfusion were observed in the placebo group. These improvements may be related to improved adherence to use of medication and lifestyle changes (diet changes, smoking

cessation, physical activity). Finally, a beneficial effect of the intramyocardial placebo injections on myocardial perfusion, possibly by inflammation-mediated stimulation of angiogenesis, cannot be ruled out.

This trial had several limitations. The trial was not designed to assess whether the improvement in myocardial perfusion and global LV function is associated with reduced mortality and morbidity; moreover, whether the improvements noted after 3- and 6-month follow-up are sustained over time needs further study. Finally, the results of this study do not provide information on bone marrow cell homing, retention, and survival, and therefore do not explain the cellular mechanisms responsible for the observed improvement in myocardial perfusion.

In summary, the results of this randomized, double-blind, placebo-controlled trial demonstrate that intramyocardial bone marrow cell injection in patients with chronic ischemia is associated with significant improvements in anginal symptoms, myocardial perfusion, and LV function.

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