Original Article

# Stem Cell Transplantation in Patients with Acute Myocardial Infarction: a Single Center Registry

Mojtaba Salarifar, MD[1*], Kamran Ali Moghaddam, MD[2], Seyed Ebrahim Kassaian, MD[1], Mohammad Alidoosti, MD[1], Ali Mohammad Haji Zeinali, MD[1], Hakimeh Sadeghian, MD[1] Jalil Majd Ardakani, MD[1], Elham Hakki Kazazi, MD[1], Ardeshir Ghavamzadeh, MD[2]

[1]*Tehran Heart Center, Medical Sciences/University of Tehran, Tehran, Iran.*
[2]*Shariati Hospital, Medical Sciences/University of Tehran, Tehran, Iran.*

Received 12 April 2007; Accepted 28 July 2007

## Abstract

***Background:*** *Early clinical investigations indicate that an infusion of autologous bone-marrow cells into the infarct-related coronary artery is feasible after acute myocardial infarction. There is increasing evidence that cell transplantation may improve the perfusion and contractile function of the ischemic myocardium. The present study reports primarily the safety of intracoronary bone marrow mononuclear cell (BMMNC) injections and secondarily the hypothesis that intracoronary injections of autologous BMMNC in patients with acute myocardial infarction may have a favorable impact on tissue perfusion and contractile performance.*

***Methods:*** *Twelve patients with acute ST-elevation myocardial infarction of the anterior wall treated with percutaneous coronary intervention were enrolled in this prospective, nonrandomized, open-label study. Left ventricular function and number of nonviable segments were assessed with the use of echocardiography and Technetium-sestamibi single photon emission tomography respectively at baseline and after a 4-month follow-up.*

***Results:*** *At 4 months' follow-up, global left ventricular ejection fraction in echocardiography increased from a mean of 31.78±7.56% at baseline to 38.89±6.97% (p=0.018). Mean wall motion score in rest echocardiography was 29.5±6.67 in basal and 26.75±5.44 at 4 months' follow-up (p=0.05). Nuclear perfusion imaging studies in the patients for the mean number of nonviable segments were 6.5 at baseline and 6 in 4 months' follow-up (p=0.17). Three patients were lost to follow-up and did not undergo the 4-month evaluations.*

***Conclusion:*** *This study is small and very preliminary. Data from large, randomized, controlled trials are needed to clarify the effect of stem-cell injection in myocardial function.*

*J Teh Univ Heart Ctr 4 (2007) 201-206*

*Keywords: Stem cell transplantation • Myocardial infarction • Ventricular function*

## Introduction

Myocardial infarction (MI) leads to the loss of tissue and impairment of cardiac performance. The irreversible loss of cardiomyocytes after MI begets left ventricular remodeling, eventually resulting in ischemic heart failure. Remodeling of

[*]**Corresponding author:** ***Mojtaba Salarifar,*** *Assistant Professor of Interventional Cardiology, Cardiology Department, Tehran Heart Center, North Kargar Street, Tehran, Iran. 1411713138. Tel: +98 21 88029257. Fax: +98 21 88029256. E-mail: salarifar@tehranheartcenter.org.*

the left ventricle after MI represents a major cause of infarct-related heart failure and death.[1,2] Despite the application of pharmacotherapeutics and mechanical interventions, the cardiomyocytes lost during MI cannot be regenerated. The recent finding that a small population of cardiac muscle cells is able to replicate itself is encouraging but is still consistent with the concept that such regeneration is restricted to viable myocardium.[3] One approach to reverse myocardial remodeling is to repair myocardial tissue by using bone marrow-derived cells. In recent years, stem cell transplantation has emerged as a potential modality for the treatment of cardiovascular disease on the basis of its possible ability to induce neovascularization and tissue replacement. Several recent experimental studies have confirmed the potential of pluripotential cells in differentiating into cardiomyocytes and endothelial cells.[4-6] Early clinical investigations indicate that the infusion of autologous bone-marrow cells into the infarct-related coronary artery is feasible after acute MI[7-8] and there is increasing evidence that cell transplantation may improve the perfusion and contractile function of the ischemic myocardium.[8-12] In this way, the present study reports primarily the safety of intracoronary bone marrow mononuclear cell (BMMNC) injections and secondarily the hypothesis that intracoronary injections of autologous BMMNC in patients with acute MI may have a favorable impact on tissue perfusion and contractile performance of the injured heart.

## Methods

### Patient selection

This is a prospective, nonrandomized, open-label study on 12 patients with ST- elevation MI. The following inclusion criteria were required for patient enrollment: 1) age between 18 and 75 years; 2) first acute ST- elevation MI and in left anterior descending coronary artery (LAD) territory; 3) left ventricular ejection fraction (LVEF)<45% in echocardiography; 4) at least 2 nonviable segments in dipyridamole single photon emission computed tomography (SPECT); 5) akinetic and nonviable scar, as demonstrated by a lack of response to low-dose dobutamine echocardiography; 6) successful revascularization procedure with stent implanting; and 7) signed, informed consent. Patients were not enrolled in the study if any the following exclusion criteria was met: 1) presence of cardiogenic shock (defined as systolic blood pressure<80 mm Hg requiring intravenous pressors or intra-aortic balloon counterpulsation); 2) major bleeding requiring blood transfusion after reperfusion treatment; 3) a history of leucopenia, thrombocytopenia, or hepatic or renal dysfunction; 4) evidence for malignant disease, and 5) unwillingness to participate. The ethics committee of Tehran Heart Center, affiliated with Tehran University of Medical Sciences approved the study protocol, and a written informed consent was obtained from each patient.

### Study protocol

All 12 patients had suffered transmural infarction according to World Health Organization criteria with the involvement of the LAD. We assessed patients with left-heart catheterization and coronary angiography, transthoracic dobutamine echocardiography, and $^{99m}$Tc-mibi SPECT perfusion scan. After coronary angiography, mechanical treatment was initiated by balloon angioplasty and stent implantation in infarct-related artery and other arteries. According to the inclusion criteria, if the patient was eligible for stem cell implantation, he/she was prepared for this procedure.

### TC-mibi SPECT

Baseline Technetium-Sestamibi single photon emission tomography ($^{99m}$Tc-mibi SPECT) was performed after angioplasty and stenting for the assessment of viable myocardium. All the patients underwent rest-redistribution $^{99m}$Tc-mibi SPECT imaging according to a standardized clinical protocol. Several minutes after the injection of $^{99m}$Tc-mibi, rest imaging was performed. Then, 3 to 4 h later the patient returned for redistribution imaging.

### Stress echocardiography

Baseline dobutamine stress echocardiography was carried out to assess viable myocardium. Dobutamine was infused with doses of 5, 10, and 15 μg/kg per minute in 3-minute stages. Regional wall motion analysis was performed as described by the Committee on Standards of the American Society of Echocardiography[13] dividing the left ventricle into 16 segments and scoring wall motion of 1=normal, 2=hypokinesia, 3=akinesia, and 4=dyskinesia for each segment. Contractile reserve was defined as an improvement of ≥1 in the wall motion score between the baseline images and the dobutamine low- dose stage (15μg/kg per minute). The wall motion score index (WMSI) was calculated as the sum of the scores of the segments divided by the number of the segments evaluated. Echocardiograms were read by investigators unaware of the clinical, angiographic, and SPECT imaging findings in each patient.

### Bone marrow aspiration and isolation of mononuclear cells

Within the first week after angioplasty, bone marrow (≈50

ml) was aspirated under local anesthesia from the posterior iliac crest. Bone marrow mononuclear cells (BMMNC) were isolated by Ficol density separation on Lymphocyte Separation Medium 199 before the erythrocytes were lysed with $H_2O$. Mononuclear cells were exhaustively washed with heparinized saline containing 1% human serum albumin. The cells were finally resuspended in saline with 1% human serum albumin for injection. A small fraction of the cell suspension was used for cell counting and viability testing by trypan blue exclusion. Post-hoc characterization of leukocyte differentiation markers by flow cytometry and functional assay were performed on another fraction of the cells.

### *Intracoronary transplantation of Bone marrow cells (BMC)*

A day after bone marrow harvest, the final preparation of bone marrow cells was infused into the infarct-related artery via the central lumen of an over-the- wire balloon catheter. To allow bone marrow cells maximum contact time with the microcirculation of the infarct-related artery, the balloon was inflated inside the stent to transiently interrupt antegrade blood flow during infusions. The entire bone marrow cell preparation was infused during four to five coronary occlusions, each lasting 3 minutes. Between occlusions, the coronary artery was reperfused for 3 minutes.

### *Assessment of outcomes*

Follow-up visits were performed by physicians 1 and 4 months post transplantation. Specific attention was paid to any potential signs or symptoms of arrhythmia during the follow-up. Four months after stem cell injection, stress echocardiography and $^{99m}$Tc-mibi SPECT were repeated to measure LVEF, WMSI, and number of nonviable segments. The primary end points were feasibility and safety. Feasibility was defined as the ability of the expansion procedure to yield the target numbers of cells within two to three weeks. Safety referred to any procedural complication, including: ventricular arrhythmia, visible thrombus formation, distal embolization, injury of the coronary artery associated with the cell-infusion catheterization procedure or any adverse events related to cell injection within the follow-up period. The secondary end point was efficacy, which was primarily assessed by two-dimensional echocardiographic analysis of LV function at four months' follow-up.

### *Statistics*

Results are reported as mean±SD. A comparison of the preoperative and postoperative data was done using the paired t test, with $p<0.05$ as the limit of significance.

## *Results*

Between July 2003 and August 2004, 12 patients were informed and enrolled in this prospective study. Table 1 shows the clinical and interventional details of the 12 patients in this series. Although 12 patients were enrolled in this study, 3 patients were not included in the analysis. One patient was lost to follow-up and did not undergo the 4-month evaluations, the second patient had additional non-ST elevation MI 2 weeks after cell therapy. A Third patient had pulmonary edema 2 hours after the procedure; Cardio pulmonary resuscitation (CPR) was done successfully and in repeated angiogram the related artery was patent. The patient was subsequently transferred to CCU ward. However, the patient's situation was complicated with fever, pneumonia, acute renal failure, and acute respiratory distress syndrome (ARDS). After 8 days, the patient died in CCU.

In all the patients, aspirin, plavix, statin, β-blocker, and angiotensin converting enzyme (ACE) inhibitor therapy were initiated during the hospitalization for acute MI and continued until the 4-month follow-up examination. After the exclusion of above-mentioned patients, there were no deaths, and none of the patients had any malignant arrhythmias during the follow-up. At 4 months' follow-up examination, none had any clinical findings suggestive of heart failure.

On average, 43±5 ml of bone marrow was aspirated from the posterior iliac crest during a brief general analgesia with morphine. No bleeding complications at the harvest site were noted. During the preparation of bone marrow cells, the sedimentation process reduced the volume of bone marrow cells to a mean of 7.5±3.5 ml. The final preparation of bone marrow cells contained $15.5\times10^6\pm6.4\times10^6$ nucleated cells (mean viability 90%), $1.55\times10^6$ CD34+ cells, and $7.75\times10^6$ CD45+cells.

As is shown in Figure 1, at 4 months' follow-up, global LVEF in echocardiography increased from a mean of 31.78±7.56% at baseline to 38.89±6.97% at 4 months ($p=0.018$). Mean WMS in rest echocardiography was 29.5±6.67 in basal and 26.75±5.44 at 4 months' follow-up ($p=0.05$). Figure 2 illustrates WMSI at resting and 15 µg dobutamine stress echocardiography at baseline before stem cell therapy as well as the WMSI at resting echocardiography at 4 months' follow-up. Nuclear perfusion imaging studies in the patients for the mean number of nonviable segments were 6.6 at baseline and 6.1 at 4 months' follow-up ($p=0.17$). Two patients showed improvement of nonviable segment in SPECT at 4 months' follow-up.

Table 1. Patients' Characteristics

| Patient No | Age (y) | Sex | LVEF (%) (pre inj) | LVEF (%) (post inj) | No of nonviable segments (pre inj) | No of nonviable segments (post inj) | WMS (pre inj) | WMS (post inj) |
|---|---|---|---|---|---|---|---|---|
| 1 | 39 | Male | 25 | 30 | 6 | 6 | 27 | 28 |
| 2 | 46 | Female | 35 | 30 | 8 | 8 | 28 | 28 |
| 3 | 62 | Male | 25 | 40 | 6 | 6 | 26 | 26 |
| 4 | 52 | Male | 25 | 35 | 9 | 9 | 42 | 38 |
| 5 | 36 | Female | 40 | 45 | 5 | 5 | 22 | 22 |
| 6 | 62 | Male | 30 | 45 | 7 | 4 | 34 | 26 |
| 7 | 42 | Male | 36 | 40 | 7 | 5 | 34 | 28 |
| 8 | 53 | Male | 45 | 50 | 6 | 6 | 25 | 20 |
| 9 | 65 | Female | 30 | 35 | 6 | 6 | 24 | 24 |
| 10 | 75 | Male | 30 | - | - | - | - | - |
| 11 | 50 | Female | 40 | - | - | - | - | - |
| 12 | 69 | Male | 35 | - | - | - | - | - |

LVEF, Left ventricalar ejection fraction: Inj, Injection; WMS, Wall motion score

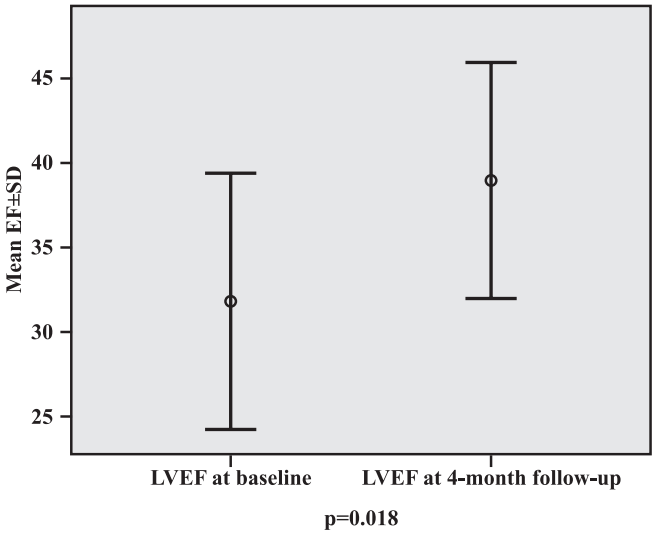


Figure 1. Mean left ventricular ejection fraction (LVEF) of patients at baseline and 4-month follow-up

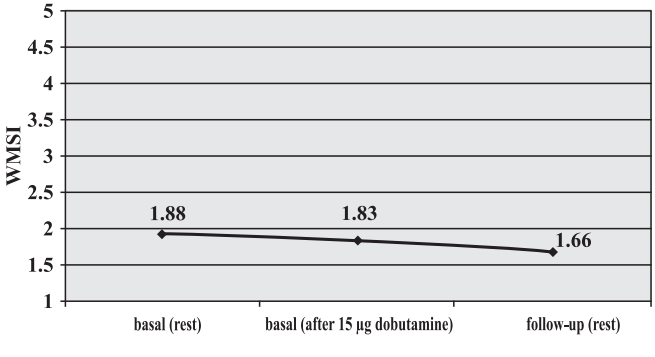


Figure 2. Wall motion score index (WMSI) before injection of bone marrow stem cell (rest and after dobutamine injection) and at 4-month follow-up

Follow-up visits were continued after 4 months. Mean follow-up visit duration was 24±4.5 months. There were no deaths, and none of the patients had any malignant arrhythmias during follow-up visit duration.

## *Discussion*

A reduced LVEF during the acute phase of myocardial infarction is the most important independent predictor of a poor outcome, even in the era of optimal reperfusion therapy with the stenting of the infarct-related artery.[14] Thus, enhanced recovery of contractile function may be beneficial, especially in patients with large infarcts and depressed left ventricular function.[15]

Our findings indicate that a catheter-based intramyocardial implantation of bone marrow cells is relatively safe and feasible for the treatment of patients with acute MI. The present report describes the effect of intracoronary, autologous, mononuclear bone marrow cell transplantation on improving heart function and myocardial perfusion in patients after acute MI. After 4 months, the global LVEF was significantly higher compared with basal LVEF. However, the number of patients is not sufficient to conclude that an intracoronary infusion of bone marrow stem cell (BMS) is associated with persistent improvements in global left ventricular function and improved functional status among patients who have had MI at least 4 months prior to the transplantation. The enhanced recovery of global left ventricular contractile function after the intracoronary administration of BMS was due to a significant reduction in the extent and magnitude of regional left ventricular dysfunction within the territory of the infarct. This finding is in agreement with other studies where unfractionated BMC was utilized.[7,16-18] Our 4-month follow-up showed that the mean number of nonviable segments and WMSI were not significantly higher compared with the basal evaluation.

Some previous studies have shown no improvement in left ventricular function after treatment with BMC.[10,19] Technical differences in the characteristics or handling of the infused BMC might explain the different outcome. Also, differences in cell preparation and cell population may be important.

Although stem cell therapy has been shown to be safe, feasible, and effective in both animal and human studies,

the mechanism by which this therapy improves healing after ischemic injury remains unexplored. Other unanswered questions include: Is stem cell therapy safe and effective in the long term? Which stem cell (unfractionated bone marrow, bone marrow-derived mononuclear, or any of the multiple subpopulations of the latter) will produce optimal therapeutic effect in damaged myocardium? What is the optimal delivery approach (intravenous, intracoronary, intramyocardial, or transepicardial)? What is the optimal dosage and timing of the administration of cell therapy? Answers to these questions may require additional animal and human studies.

The major limitations of this study are the small number of the patients enrolled; the study design, which limits any conclusions about efficacy; and the lack of a randomized control group, which did not receive intracoronary infusion of BMC.

Additionally, as assessment of whether intracoronary infusion of BMCS can reduce the risk of complications and death among patients with acute MI was beyond the scope of the present study.

In summary, after acute MI, the intracoronary administration of BMC enhances left ventricular contractile recovery. Given the safety profile of this treatment and the beneficial effects in patients with the most severely impaired left ventricular function, large scale studies are warranted to examine the potential effects of this novel approach on the risk of death and complications in patients with large acute MI and depressed left ventricular contractile function. We emphasize the fact that all reported attempts of clinical cell transplantation for myocardial regeneration, including our study; have been done in association with surgical or interventional revascularization, so that the effectiveness of cell transplantation alone cannot clarify the role of cell transplantation in myocardial regeneration.

## *Conclusions*

This study is small and very preliminary. Data from large, randomized controlled trials are needed to clarify the effect of stem cell injection in myocardial function.

## *Acknowledgments*

This study has been approved by Tehran Heart Center's Review Board and Ethics Committee; and has been supported by Tehran Heart Center, Tehran University of Medical Sciences.

## *Reference*

1. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. Circulation 1990;81:1161-1172.
2. Ertl G, Gaudron P, Hu K. Ventricular remodeling after myocardial infarction: experimental and clinical studies. Basic Res Cardiol 1993;88:125-137.
3. Quaini F, Urbanek K, Beltrami AP, Finato N, Beltrami CA, Nadal-Ginard B, Kajstura J, Leri A, Anversa P. Chimerism of the transplanted heart. N Engl J Med 2002;346:5-15.
4. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine DM, Leri A, Anversa P. Bone marrow cells regenerate infracted myocardium. Nature 2001;410:701-705.
5. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, Homma S, Edwards NM, Itescu S. Neovascularazation of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med 2001;7:430-436.
6. Menasché P, Hagège AA, Vilquin JT, Desnos M, Abergel E, Pouzet B, Bel A, Sarateanu S, Scorsin M, Schwartz K, Bruneval P, Benbunan M, Marolleau JP, Duboc D Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. J Am Coll Cardiol 2003;41:1078-1083.
7. Strauer BE, Brehm M, Zeus T, Köstering M, Hernandez A, Sorg RV, Kögler G, Wernet P. Repair of infracted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002;106:1913-18.
8. Assmus B, Schächinger V, Teupe C, Britten M, Lehmann R, Döbert N, Grünwald F, Aicher A, Urbich C, Martin H, Hoelzer D, Dimmeler S, Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002;106:3009-3017.
9. Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schümichen C, Nienaber CA, Freund M, Steinhoff G. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361:45-46.
10. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003;361:47-49.
11. Tomita S, Mickle DA, Weisel RD, Jia ZQ, Tumiati LC, Allidina Y, Liu P, Li RK. Improved hear function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation. J Thorac Cardiovasc Surg 2002;123:1132-1140.
12. Fuchs S, Baffour R, Zhou YF, Shou M, Pierre A, Tio FO, Weissman NJ, Leon MB, Epstein SE, Kornowski R. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. J Am Coll Cardiol 2001;37:1726-1732.
13. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography: American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr 1989;2:358-367.
14. Halkin A, Singh M, Nikolsky E, Grines CL, Tcheng JE, Garcia E, Cox DA, Turco M, Stuckey TD, Na Y, Lansky AJ, Gersh BJ, O'Neill WW, Mehran R, Stone GW. Prediction of mortality after primary percutaneous coronary intervention for acute myocardial infarction: the CADILLAC risk score. J Am Coll Cardiol 2005;45:1397-1405.
15. Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R,

Hölschermann H, Yu J, Corti R, Mathey DG, Hamm CW, Süselbeck T, Assmus B, Tonn T, Dimmeler S, Zeiher AM. Intra coronary bone marrow-derived progenitor cell in acute myocardial infarction. N Engl J Med 2006;355:1210-1221.

16. Fernández-Avilés F, San Román JA, García-Frade J, Fernández ME, Peñarrubia MJ, de la Fuente L, Gómez-Bueno M, Cantalapiedra A, Fernández J, Gutierrez O, Sánchez PL, Hernández C, Sanz R, García-Sancho J, Sánchez A. Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction. Circ Res 2004;95:742-748.

17. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, Fichtner S, Korte T, Hornig B, Messinger D, Arseniev L, Hertenstein B, Ganser A, Drexler H. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomized controlled clinical trial. Lancet 2004;364:141-148.

18. Schächinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, Abolmaali ND, Vogl TJ, Hofmann WK, Martin H, Dimmeler S, Zeiher AM. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. J Am Coll Cardiol 2004;44:1690-1699.

19. Lunde K, Solheim S, Aakhus S, Arnesen H, Abdelnoor M, Egeland T, Endresen K, Ilebekk A, Mangschau A, Fjeld JG, Smith HJ, Taraldsrud E, Grøgaard HK, Bjørnerheim R, Brekke M, Müller C, Hopp E, Ragnarsson A, Brinchmann JE, Forfang K. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. N Engl J Med 2006;355:1199-1209.