# Cardiac bone marrow cell injection for chronic ischemic heart disease

Beeres, S.L.M.A.

# Chapter 9

Saskia L.M.A. Beeres[1]
Jeroen J. Bax[1]
Petra Dibbets-Schneider[2]
Marcel P.M. Stokkel[2]
Willem E. Fibbe[3]
Ernst E. van der Wall[1]
Martin J. Schalij[1]
Douwe E. Atsma[1]

*[1]Department of Cardiology, [2]Department of Nuclear Medicine and the [3]Department of Hematology, Leiden University Medical Center, Leiden, The Netherlands*

# Sustained Effect of Autologous Bone Marrow Mononuclear Cell Injection in Patients with Refractory Angina Pectoris and Chronic Myocardial Ischemia: 12-month Follow-up Results

*American Heart Journal 2006;152:684.e11-684.e16*

## Abstract

**Background:** Cell therapy has recently been introduced to treat patients with refractory angina. Because most studies have only included short-term follow-up, the effects of cell therapy over a longer period are unknown.

**Methods:** In 25 patients (64±10 years, 21 male) with refractory angina, a total of 84±29x$10^6$ bone marrow-derived mononuclear cells was injected intramyocardially in regions with ischemia on technetium-99m tetrofosmin single-photon emission computed tomography. Anginal symptoms and quality-of-life were evaluated at baseline and at 3, 6 and 12 months. Gated single-photon emission computed tomography was performed at baseline and at 3 and at 12 months to assess myocardial perfusion and left ventricular function.

**Results:** Bone marrow cell injection was performed without any complication. At 7 months, 1 patient died of intracranial hemorrhage. Canadian Cardiovascular Society class improved from 3.4±0.5 to 2.3±0.6 at 3 months, 2.4±0.6 at 6 months and 2.7±0.8 at 12 months (P<0.01). Quality-of-life improved from 53±10% to 71±11% at 3 months, 72±14% at 6 months, and 68±14% at 12 months (P<0.01). The number of segments with ischemia per patient decreased from 4.7±3.3 to 2.1±2.6 at 3 months and 1.6±2.5 at 12 months (P<0.01). Left ventricular ejection fraction increased from 47±13% to 53±17% at 3 months and 51±17% at 12 months (P<0.01). Regional wall motion improved from 5.9±1.7 mm to 6.6±2.2 mm at 3 months and 6.4±2.0 mm at 12 months (P=0.01).

**Conclusions:** Autologous bone marrow cell injection in patients with ischemia is safe and resulted in a sustained beneficial effect on anginal symptoms, myocardial perfusion and left ventricular function.

# Introduction

Intramyocardial injection of autologous bone marrow mononuclear cells has been introduced as a new therapeutic option for patients with refractory angina pectoris and chronic myocardial ischemia who are ineligible for conventional revascularization.[1] The short-term effects of this therapy are encouraging; different studies have demonstrated a reduction in anginal symptoms, with an improvement in myocardial perfusion and left ventricular function.[2-5]

The long-term safety and efficacy of bone marrow cell injection in patients with refractory angina pectoris and chronic myocardial ischemia remain to be investigated. Data obtained by Fuchs et al. suggest that the favourable effect on anginal symptoms is maintained up to 12 months after bone marrow cell injection.[3] Only one pilot study (primarily designed to assess safety and feasibility) reported a sustained favourable effect on myocardial perfusion in 4 of 8 patients with refractory angina pectoris.[4]

We recently reported on the initial safety and efficacy of autologous bone marrow mononuclear cell injection into the ischemic myocardium of patients with refractory angina pectoris. Short-term follow-up showed a reduction in anginal symptoms accompanied by an improvement in myocardial perfusion and left ventricular function.[5]

The present study investigated whether the short-term safety and clinical benefit of bone marrow cell injection in patients with refractory angina pectoris and chronic myocardial ischemia are sustained over a longer period of follow-up (i.e. 12 months). In particular, we report on the effects of bone marrow cell injection on anginal symptoms, myocardial perfusion and left ventricular function.

# Methods

### Patients

Between February 2004 and March 2005, 25 patients with stress-induced ischemia on gated single-photon emission computed tomography (SPECT) were recruited into the study. All patients had severe angina pectoris (Canadian Cardiovascular Society (CCS) class III or IV) despite maximal tolerated medical therapy and were ineligible for conventional (percutaneous or surgical) revascularization. Exclusion criteria were: acute myocardial infarction within 6 months of enrollment, history of malignancy, renal dysfunction (serum creatinine >200 µmol/L), or unexplained hematological/biochemical laboratory abnormalities. The local ethics committee approved the protocol and all patients provided written informed consent. The detailed study protocol and short-term follow-up data have been reported previously.[5]

**Study Protocol**

In brief, the study protocol was as follows. Before bone marrow cell injection the clinical status was assessed according to the CCS classification and patients were asked to keep record of their daily angina pectoris frequency and sublingual nitrate use. The Seattle Angina Questionnaire was used to assess patients' quality-of-life. Technetium-99m tetrofosmin gated SPECT (stress-rest) was performed to assess myocardial perfusion and left ventricular function.

Bone marrow was aspirated from the iliac crest on the day of the injection procedure. Mononuclear cells were isolated by Ficoll density gradient. Autologous bone marrow-derived mononuclear cell injections were targeted at myocardial regions with stress-induced ischemia (on gated SPECT) and injected with the NOGA system (Biosense-Webster, Waterloo, Belgium).[5]



The clinical status (CCS class and quality-of-life) was re-assessed at the outpatient clinic at 3, 6 and 12 months follow-up. In the 2 weeks before each visit, patients kept record of their daily angina pectoris frequency and sublingual nitrate use. At 3 and 6 months follow-up, 24-hour Holter electrocardiogram recordings were obtained to monitor occurrence of ventricular tachycardia. Technetium-99m tetrofosmin gated SPECT (stress-rest) was repeated at 3 and 12 months follow-up to evaluate myocardial perfusion and left ventricular function.

**Gated SPECT Imaging**

Technetium-99m tetrofosmin stress and resting SPECT scans were performed as previously described.[5] Briefly, the stress protocol included a symptom-limited bicycle exercise test. Whenever possible, patients discontinued their β-blockers and calcium channel antagonists for 24 hours before undergoing SPECT. Technetium-99m tetrofosmin (500 MBq) was injected intravenously at peak exercise, which was continued for another 1 to 2 minutes after tracer injection. In patients unable to perform a bicycle stress test, pharmacological stress (adenosine, n=12 or dobutamine, n=2) was used. Resting imaging was performed on a separate day using 500 MBq technetium-99m tetrofosmin. The resting studies were acquired in gated mode, to assess left ventricular volumes and ejection fraction and regional wall motion. SPECT imaging at 12 months was performed under identical conditions (type of stressor and discontinuation of the same medications) as at baseline and 3 months. Two experienced observers who were blinded to all clinical data (including the time-point of the study) reviewed the SPECT images.

**Gated SPECT Data Acquisition and Data Analysis**

Both rest and stress imaging were performed with a triple-head SPECT camera system (GCA 9300/HG, Toshiba Corp., Tokyo, Japan) equipped with low energy general-purpose collimators. Reconstruction yielded standard short- and long-axis projections perpendicular to the heart-axis. The short-axis slices were displayed in polar map format,

adjusted for peak myocardial activity (100%). The myocardium was divided into 17 segments.[6] Stress and rest perfusion was analyzed quantitatively (quantitative gated SPECT (QGS)-software, Cedars-Sinai Medical Center, Los Angeles, California, USA) using segmental tracer activity on a 4-point scale: 1= normal tracer activity ≥75%; 2= tracer activity 50-75%; 3= tracer activity 25-50%; 4= tracer activity <25%. Perfusion defects on stress images were considered to be present when tracer activity was <75% of maximum. When significant fill-in (>10%) of perfusion defects was observed on the resting images, segments were classified as ischemic.

Quantitative assessment of left ventricular volumes, left ventricular ejection fraction and regional wall motion was performed using previously validated and automated software (QGS-software).[7,8] Regional wall motion was determined as the distance (in millimeters) between a given endocardial point at end-diastole and end-systole, perpendicular to the average midmyocardial surface between end-diastole and end-systole.

### Statistical Analysis

Categorical data, presented as frequencies, were compared using chi-square test (with Yates' correction when appropriate). A mixed-model analysis of variance using SPSS 12.0 (SPSS Inc., Chicago, Illinois, USA) was used for comparisons of continuous data acquired at >2 time points (e.g. baseline vs. 3 months vs. 6 months vs. 12 months). All tests are 2 sided and a P-value <0.05 was considered statistically significant.

## Results

The baseline characteristics of the 25 patients are summarized in **Table 1**. Anti-anginal therapy included high-dosages of long-acting nitrates (100%), calcium channel

**Table 1.** Clinical characteristics of the study population (n=25)

| Characteristics | | |
|---|---|---|
| Age (yrs) | 64±10 | |
| Gender (Male) | 21 | ( 84 %) |
| Systemic hypertension | 12 | ( 48 %) |
| Diabetes mellitus | 13 | ( 52 %) |
| Insulin dependent | 7 | ( 28 %) |
| Non-insulin dependent | 6 | ( 24 %) |
| Hyperlipidemia | 22 | ( 88 %) |
| Smoking | 2 | ( 8 %) |
| Coronary artery disease in family | 18 | ( 72 %) |
| Body mass index (kg/m$^2$) | 28±4 | |
| Prior myocardial infarction | 18 | ( 72 %) |
| Prior percutaneous coronary intervention | 19 | ( 76 %) |
| Prior coronary artery bypass grafting | 22 | ( 88 %) |

antagonists (72%) and ß-blockers (92%). In all patients, the type and dosages of anti-anginal medication remained unchanged during the 12-month follow-up period.

**Procedural Data**

The mean procedural time for mapping and cell injection was 58±17 minutes. Patients received 8-13 injections of approximately 0.2 ml each targeted at a total of 87 segments with stress-inducible ischemia. The injected suspension contained 84±29x$10^6$ mononuclear cells in total.

**Safety Parameters**



Intramyocardial bone marrow cell injection was performed without major periprocedural complication in all patients. Continuous electrocardiographic monitoring did not reveal sustained ventricular tachycardia during left ventricular mapping, cell injection or hospitalization. No increases in Troponin T or Creatine Kinase levels occurred after cell injection. Two-dimensional echocardiography excluded post-procedural pericardial effusion. Patients were discharged 3±2 days after bone marrow cell injection.

One patient died of intracranial haemorrhage 7 months after bone marrow cell injection. Another patient was hospitalized for decompensated heart failure at 7 months follow-up, while 1 patient developed a non ST-segment elevation acute myocardial infarction in a non-injected territory at 8 months follow-up. Repeat coronary angiography in the latter patient excluded the presence of new significant lesions. During the study period none of the patients underwent a coronary revascularization procedure. In addition, syncope or sustained ventricular tachycardia was not observed.

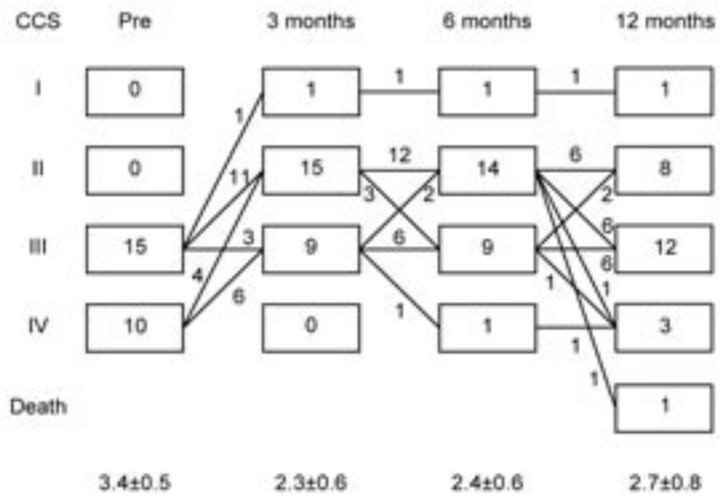


**Figure 1**

Individual CCS class at baseline (pre), and at 3, 6 and 12 months after autologous bone marrow cell injection. Mean CCS class improved from 3.4±0.5 at baseline to 2.3±0.6 at 3 months, 2.4±0.6 at 6 months and 2.7±0.8 at 12 months.

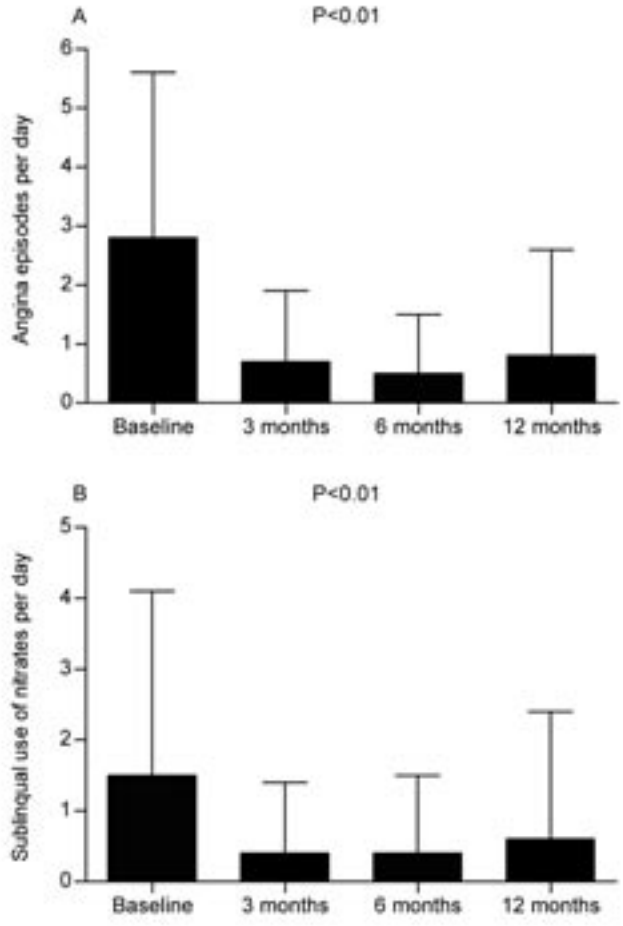


**Figure 2**
Daily angina episodes (A) and use of sublingual nitrates (B) at baseline, and at 3, 6 and 12 months follow-up.

## Clinical Outcome

Assessment of the patients' clinical status according to the CCS classification at 3 and 6 months follow-up revealed significant improvement as compared to baseline (**Figure 1**). The effect was sustained at 12 months of follow-up (P<0.01). As shown in **Figure 2**, the frequency of angina pectoris episodes and the use of sublingual nitrates significantly decreased after bone marrow cell injection. The daily number of angina episodes decreased from 2.8±2.8 at baseline to 0.7±1.2 at 3 months, 0.5±1.0 at 6 months and 0.8±1.8 at 12 months of follow-up (P<0.01). The frequency of sublingual use of nitrates per day decreased from 1.5±2.6 at baseline to 0.4±1.0 at 3 months, 0.4±1.1 at 6 months and 0.6±1.8 at 12 months of follow-up (P<0.01). Mean quality-of-life score improved from 53±10% at baseline to 71±11% at 3 months, 72±14% at 6 months, and 68±14% at 12 months follow-up (P<0.01).

## Myocardial Perfusion

Since 1 patient died at 7 months of follow-up, stress-rest technetium-99m tetrofosmin gated SPECT at 12 months was available in 24 of 25 patients. The mean number of segments with stress-inducible ischemia per patient decreased from 4.7±3.3 at baseline to 2.1±2.6 at 3 months and 1.6±2.5 at 12 months (P<0.01). A typical example of a series of SPECT polar maps at baseline and at 3 and at 12 months follow-up is shown in **Figure 3**.

Intramyocardial bone marrow cell injection improved stress perfusion, whereas resting perfusion remained unchanged. The proportion of segments with ≥75% tracer uptake (normal perfusion) during stress increased from 242/408 (59%) at baseline to 308/408 (75%) at 3 months and 318/408 (78%) at 12 months follow-up (P<0.01, **Table 2**). In contrast, the proportion of segments with normal perfusion at rest remained unchanged (343/408 (84%) at baseline vs. 353/408 (87%) at 3 months vs. 363/408 (89%) at 12 months; P=0.27, **Table 2**). As compared to baseline, myocardial stress perfusion at 12 months in particular improved in injected segments, whereas only limited improvement was seen in non-injected segments. Of the 87 injected segments, 58 (67%) increased at least 1 point in stress perfusion, whereas 42/321 (13%) of the non-injected segments increased at least 1 point in stress perfusion (P<0.01).



## Left Ventricular Function

Repeat gated SPECT at 12 months was available in 24 of 25 patients. Left ventricular ejection fraction demonstrated a modest increase from 47±13% at baseline to 53±17% at 3 months and 51±17% at 12 months (P<0.01, **Figure 4**). The increase in left ventricular ejection fraction was mainly based on a reduction in end-systolic volume (from 84±68 ml at baseline to 77±70 ml at 3 months and 80±72 ml at 12 months; P=0.04), whereas end-diastolic volume remained unchanged (141±80 ml at baseline to 142±83 ml at 3 months

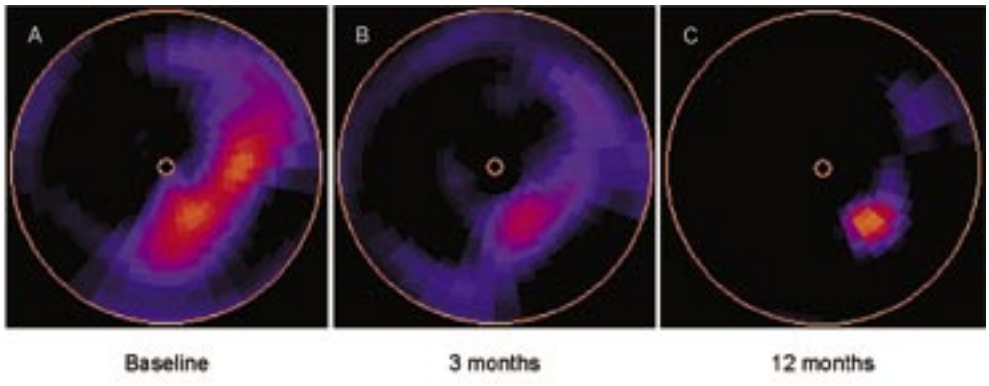


**Figure 3**

Tc-99m tetrofosmin SPECT polar map of a patient with stress-induced ischemia in the inferolateral myocardium at baseline (A). Three months after intramyocardial injection of autologous bone marrow-derived mononuclear cells there is a reduction in the extent of stress-induced ischemia (B). The effect is sustained at 12 months follow-up (C).

**Table 2.** Number of segments according to tracer activity

| | Baseline | 3 months | 12 months | P-value |
|---|---|---|---|---|
| Myocardial stress perfusion | | | | |
| Tracer activity ≥75% | 242 | 308 | 318 | |
| Tracer activity 50-75% | 109 | 65 | 55 | <0.01 |
| Tracer activity 25-50% | 30 | 9 | 9 | |
| Tracer activity <25% | 27 | 26 | 26 | |
| Myocardial rest perfusion | | | | |
| Tracer activity ≥75% | 343 | 353 | 363 | |
| Tracer activity 50-75% | 31 | 24 | 14 | =0.27 |
| Tracer activity 25-50% | 10 | 9 | 7 | |
| Tracer activity <25% | 24 | 22 | 24 | |

Values are expressed as n

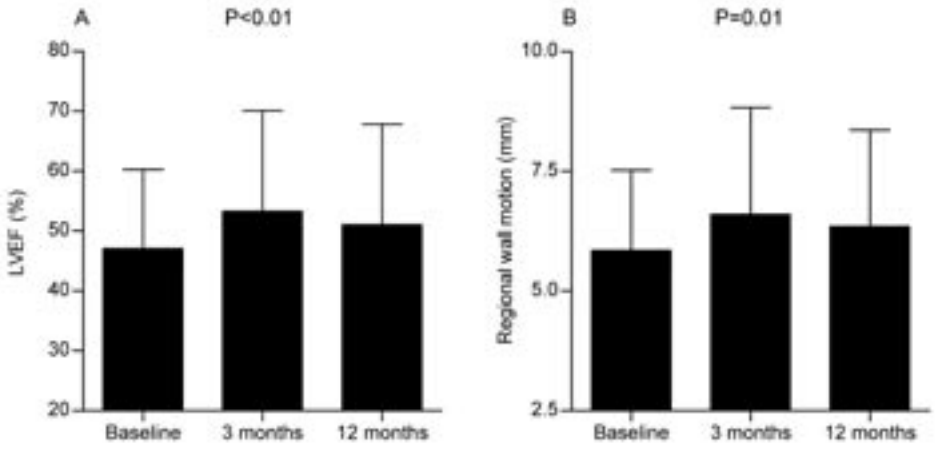


**Figure 4**
Left ventricular ejection fraction (LVEF, A) and mean segmental regional wall motion (B) at baseline and at 3 and 12 months follow-up as assessed by gated SPECT.

and 143±86 ml at 12 months follow-up; P=0.86). Regional left ventricular function increased significantly after bone marrow cell injection (**Figure 4**). Mean segmental regional wall motion per patient improved from 5.9±1.7 mm at baseline to 6.6±2.2 mm at 3 months and remained unchanged at 12 months of follow-up (6.4±2.0 mm; P=0.01).

## Discussion

Various studies have evaluated the feasibility of bone marrow cell injection in patients with refractory angina and chronic myocardial ischemia.[2-5] These studies demonstrated a beneficial effect on symptoms with a reduction in ischemia and an improvement in left

ventricular function. For example, Tse et al. reported on a reduction in anginal symptoms with an improvement in perfusion and an increase in regional left ventricular function at 3 months follow-up in 8 patients who underwent cell therapy.[2] Similarly, we recently demonstrated in 25 patients with refractory angina pectoris and chronic myocardial ischemia that cell injection improved myocardial perfusion with an increase in left ventricular function at 3 months follow-up.[5]

However, the available studies have mainly assessed the short-term effects of cell therapy; thus, the long-term effects of cell therapy are unknown. Preliminary data by Fuchs et al. demonstrated that the beneficial effect on anginal symptoms was sustained at 12 months follow-up.[3] In 27 patients with refractory angina pectoris and chronic myocardial ischemia, the mean CCS score improved from 3.2±0.3 at baseline to 2.2±1.0 (P<0.01) at 12 months after cell therapy. However, it should be noted that 7 of 27 (26%) patients underwent an additional (surgical or percutaneous) revascularization procedure during the study period, which may have influenced the results.



Only one study in acute myocardial infarction patients and a pilot study in patients with chronic ischemia evaluated the effects of cell therapy over a longer period of follow-up. In the BOOST trial, a single dose of intracoronary bone marrow cell infusion did not provide a sustained beneficial effect on left ventricular systolic function after acute myocardial infarction as compared with a randomized control subjects.[9] On the other hand, Briguori et al. reported a sustained reduction in severity and extent of ischemia in 4 of 8 patients with refractory angina 12 months after intramyocardial bone marrow cell injection.[4] In addition, a modest (but non-significant) improvement in left ventricular ejection fraction was observed (from 53±10% at baseline to 57±16% at 12 months follow-up; P=NS).

In the current study the effects of cell therapy were evaluated at 12 months follow-up, showing the absence of acute or late complications. In particular, sustained ventricular tachycardia were not observed during cell injection or during the 12-month follow-up period. In addition, an improvement in symptoms was noted with a mean reduction in CCS class from 3.4±0.5 to 2.7±0.8 (P<0.01), in line with the results by Fuchs et al.[3] Moreover, a significant improvement in myocardial perfusion was demonstrated: the number of segments with stress-inducible perfusion defects decreased, whereas the number of segments with perfusion defects at rest remained unchanged, indicating a reduction in ischemia. Finally, a sustained improvement in regional and global contractile function was observed at 12 months after cell therapy.

The mechanism underlying the benefit of bone marrow cell injection is most probably related to promotion of angiogenesis, as proposed in animal model studies.[10,11] In a canine model of chronic ischemia, Silva et al. noted that 60 days after intramyocardial injection, bone marrow-derived mesenchymal stem cells had differentiated into vascular smooth muscle cells and endothelial cells.[10] This led to increased vascularity and improved left ventricular ejection fraction. Fuchs et al. described that NOGA-guided bone marrow cell injection in pigs with chronic ischemia enhanced collateral flow through secretion of

potent pro-angiogenic cytokines and proliferation of bone marrow-derived vascular and endothelial cells.[11] In addition, left ventricular contractility increased in bone marrow-treated pigs, whereas improvement was not observed in control animals. In short, these studies provided histological and functional evidence of bone marrow cell injection-induced angiogenesis in chronic ischemic myocardium.

The major limitation of the current study is the lack of a control group, and the observed clinical improvement could be considered a placebo effect. However, the improvement in symptoms was accompanied by an improvement in myocardial perfusion and left ventricular function, and these parameters were derived from gated SPECT imaging and quantified using automated software. Moreover, these effects could not be related to changes in cardiac medication, since medication remained unchanged during the study period. Still, the current findings need confirmation in larger, randomized placebo controlled trials.

## Conclusions

In summary, the observations in the present study demonstrate that intramyocardial injection of autologous bone marrow-derived mononuclear cells in patients with refractory angina pectoris and chronic myocardial ischemia is safe and results in a sustained beneficial effect on symptoms with an improvement in myocardial perfusion and left ventricular function.

# References


1. Wollert KC, Drexler H. Clinical applications of stem cells for the heart. Circ Res 2005;96:151-163.
2. Tse HF, Kwong YL, Chan JK, et. al. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet 2003;361:47-49.
3. Fuchs S, Kornowski R, Weisz G, et al. Safety and feasibility of transendocardial autologous bone marrow cell transplantation in patients with advanced heart disease. Am J Cardiol 2006;97:823-829.
4. Briguori C, Reimers B, Sarais C, et al. Direct intramyocardial percutaneous delivery of autologous bone marrow in patients with refractory myocardial angina. Am Heart J 2006;151:674-680.
5. Beeres SL, Bax JJ, Dibbets P, et al. Effect of intramyocardial injection of autologous bone marrow-derived mononuclear cells on perfusion, function, and viability in patients with drug-refractory chronic ischemia. J Nucl Med 2006;47:574-580.
6. Cerqueira MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: a statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation 2002;105:539-542.
7. Germano G, Kiat H, Kavanagh PB, et al. Automatic quantification of ejection fraction from gated myocardial perfusion SPECT. J Nucl Med 1995;36:2138-2147.
8. Sharir T, Berman DS, Waechter PB, et al. Quantitative analysis of regional motion and thickening by gated myocardial perfusion SPECT: normal heterogeneity and criteria for abnormality. J Nucl Med 2001;42:1630-1638.
9. Meyer GP, Wollert KC, Lotz J et al. Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. Circulation 2006;113:1287-1294.
10. Silva GV, Litovsky S, Assad JA, et al. Mesenchymal stem cells differentiate into an endothelial phenotype, enhance vascular density, and improve heart function in a canine chronic ischemia model. Circulation 2005;111:150-156.
11. Fuchs S, Baffour R, Zhou YF, et al. 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.