 Coronary artery disease is a major cause of morbidity and mortality in the western world. Despite optimal medical management and advanced revascularization techniques, an increasing number of patients end up with no-option end-stage coronary artery disease. These patients may have intracoronal anginal complaints because of stress-inducible ischemia that is no longer amenable for additional conventional treatment modalities.1

Cardiac cell therapy has been proposed as a treatment option for these patients. On the basis of preclinical studies, it is hypothesized that bone marrow cell administration stimulates angiogenesis by the release of growth factors or by direct incorporation of cells into new capillaries, resulting in improved perfusion.2–3 We previously found in a randomized controlled trial that intramyocardial mononuclear bone marrow cell injection in patients with chronic myocardial ischemia improves myocardial perfusion, cardiac function, and angina-related quality of life.4 Similar results were obtained in other clinical trials.5,6

However, despite improvements in myocardial perfusion and anginal complaints, some patients may have areas of remaining myocardial ischemia causing complaints. In
WHAT IS KNOWN

- Bone marrow cell injection is associated with improvements in myocardial perfusion, left ventricular function, and symptoms ≤12 months after injection.
- In a substantial number of patients with refractory angina treated with intramyocardial bone marrow cell injection, recurrence of anginal complaints occurs after the 12-month follow-up time point.

WHAT THE STUDY ADDS

- Repeated bone marrow cell injection in previously responding patients with refractory angina is safe.
- Repeated bone marrow cell injection in previously responding patients with refractory angina is associated with improvements in myocardial perfusion and decreased angina complaints ≤12 months after the second injection.
- The extent of improvement after repeated bone marrow cell injection in previously responding patients with refractory angina is comparable with the extent of improvement after the first injection.

addition, because of progression of atherosclerosis, new myocardial ischemic territories may develop resulting in recurrent anginal complaints.7 Therefore, we hypothesized that repeated bone marrow cell injection may be effective to stimulate neovascularization, thereby improving myocardial perfusion and anginal complaints.

Previously, it was found that in patients with heart failure and recent acute myocardial infarction, repeated injection of bone marrow cells and peripheral blood stem cells is safe and can result in increased cardiac function.8,9 However, the effect of repeated bone marrow cell treatment in patients with refractory angina is unknown.

Therefore, this study investigates the effect of repeated intramyocardial mononuclear bone marrow cell injection on myocardial perfusion and anginal complaints in patients with residual or recurrent stress-inducible myocardial ischemia who previously had been successfully treated using cardiac cell therapy, with a follow-up of 12 months after repeated injection.

Methods

Patients

The patient population of this pilot study consisted of 23 no-option refractory angina patients who received intramyocardial bone marrow mononuclear cell injection >1 year ago. These patients had received their first intramyocardial bone marrow mononuclear cell injections during 1 of 3 previous subsequent study protocols: a safety and feasibility study, a randomized, double-blind, placebo-controlled trial, and an ongoing nonrandomized registry that was initiated after the randomized trial. In all 3 studies, patients were treated according to the same protocol that has been published previously.3,10,11 Patients were eligible for this study if the first bone marrow cell injection had resulted in improved myocardial stress perfusion and remaining or recurrent complaints of angina pectoris were present in combination with stress-inducible myocardial ischemia on Tc-99m tetrofosmin single-photon emission computed tomographic (SPECT) myocardial perfusion imaging (MPI). In addition, the same inclusion criteria were applied as in the abovementioned studies: patients received maximal tolerated medical therapy and were ineligible for percutaneous or surgical revascularization. This was reassessed before the repeated injection procedure. Also, the same exclusion criteria were applied: left ventricular (LV) ejection fraction of ≤35%, acute myocardial infarction within 6 months of enrolment, history of malignancy, renal dysfunction (glomerular filtration rate, <30 mL/min per 1.73 m²), and unexplained hematologic or biochemical abnormalities.

The protocol was approved by the institutional ethics committee and registered at the Dutch trial registry (http://www.trialregister.nl; number, NTR2664). Written informed consent was obtained from all patients.

Study Protocol

Baseline assessment was similar to the protocols of the first intramyocardial bone marrow mononuclear cell injection. Briefly, myocardial perfusion was evaluated using Tc-99m tetrofosmin–gated SPECT-MPI, whereas LV function and volumes were evaluated using magnetic resonance imaging (MRI). A bicycle exercise test was used to assess maximal exercise capacity. Severity of angina was graded according to the Canadian Cardiovascular Society score, ranging from class I (mild) to IV (severe), and quality of life was quantified on a range from 0% to 100% with the use of the disease-specific Seattle Angina Questionnaire. Furthermore, the 5 domains of the Seattle Angina Questionnaire, (1) physical limitation scale, (2) angina stability scale, (3) angina frequency scale, (4) treatment satisfaction, and (5) disease perception scale, were analyzed separately.

On the day of the injection procedure, bone marrow was aspirated from the iliac crest under local anesthesia and mononuclear cells were isolated by Ficoll density gradient separation.7 During cell isolation, a 3-dimensional electromechanical map of the LV was obtained using the NOGA system (Biologics Delivery Systems Group; Cordis, Bridgewater, NJ). Intramyocardial cell injections were targeted at myocardial regions with ischemia on SPECT-MPI and 8 to 12 injections of 0.2 to 0.3 mL each were delivered with the NOGA system.4

After the procedure, heart rhythm monitoring was continued during 2 days and laboratory markers of myocardial necrosis and systemic infection were measured. Echocardiography was performed 1 day after the injection procedure to exclude postprocedural pericardial effusion. At 6 weeks and 6 months of follow-up, 24-hour Holter recording was obtained to monitor the occurrence of ventricular arrhythmias.

To evaluate the efficacy of bone marrow mononuclear cell treatment, myocardial perfusion was reassessed at 3 and 12 months using Tc-99m tetrofosmin–gated SPECT-MPI and LV function and volumes were measured at 3 months, by MRI. Exercise testing was repeated at 3 and 6 months of follow-up, and Canadian Cardiovascular Society score and quality of life score were reevaluated at 3, 6, and 12 months of follow-up.

SPECT Myocardial Perfusion Imaging

A 2-day stress–rest protocol was used for SPECT-MPI examination.10 Imaging was performed with a dual-head SPECT camera system (GCA 7200-P; Toshiba Corp, Tokyo, Japan). For stress imaging, adenosine (0.14 mg/kg per minute) was injected intravenously for 6 minutes and 500 MBq of Tc-99m tetrofosmin was injected after 3.5 minutes of adenosine injection. At the second day, rest images were obtained after injection of 500 MBq of Tc-99m tetrofosmin. Myocardial perfusion was analyzed by reconstruction of a standard short- and long-axis projection, perpendicular to the heart axis (adjusted for peak myocardial activity [100%]).

The myocardium was divided into 17 segments according to the American Cardiology/American College of Cardiology recommendations. Segmental tracer activity was categorized on a 4-point scale: 1=tracer activity >75%; 2=tracer activity 50% to 75%; 3=tracer activity 25% to 49%; and 4=tracer activity ≤25%. The summed stress score was calculated by summation of the segmental scores at stress. Similarly, patients’ summed rest score was calculated by summation of the segmental scores at rest. The summed differences score was
calculated by summation of the differences in stress and rest segmental scores and reflected the extent of stress-inducible ischemia.

Magnetic Resonance Imaging
MRI studies were performed using a 1.5-Tesla MRI scanner (Philips Medical Systems, Best, the Netherlands) with 5-element cardiac synergy coil and vector electrocardiographic gating as previously described. To determine LV end-systolic volumes and LV end-diastolic volumes and to calculate LV ejection fractions, previously validated software (QMass MR; Medis Medical Imaging Systems, Leiden, the Netherlands) was used. Scans were analyzed by an observer blinded to all clinical data.

Exercise Capacity Assessment
Patients performed a symptom-limited bicycle exercise test, starting with 20-W load and 10-W increments per minute. During the test, patients were encouraged to perform as much exercise as possible and their symptoms and 12-lead ECG were continuously assessed. Test end points were angina pectoris, physical exhaustion, dyspnea, and significant decrease in systolic blood pressure (>10 mm Hg). All antianginal medication was continued.

Statistical Analysis
In the study by Yao et al., repeated intracoronary bone marrow cell administration in patients with acute myocardial infarction led to an additive treatment effect of 50% on top of the effect of a single-dose bone marrow cell administration. On the basis of these data, this study was designed to detect a treatment effect of 50% of the initial treatment effect after repeated intramyocardial bone marrow cell injection. The initial treatment effect as found in the randomized double-blinded placebo-controlled trial was an improvement of 3.4±2.3 points in summed stress score. Thus, to obtain a power of 90% with an α of 0.05 for detecting 50% of this value, power analysis demonstrated that 20 patients needed to be enrolled. To allow a 15% drop out, 23 patients were included.

Continuous variables are reported as mean±SD and categorical data as numbers and percentages. Differences in baseline characteristics and procedural data, between the first and the repeated injection, were analyzed using McNemar test for categorical data and the Wilcoxon signed-rank test for continuous data. A linear mixed model, taking into account the within-patient repeated measure nature of the data (with random intercepts for patients), was used to analyze changes from baseline to follow-up time points after the first and repeated injection procedures (fixed factor) with time as a fixed continuous variable (time with a slope parameter, main analyses) and an interaction term between injection procedure and time. After repeated injection, an additional 12 months of follow-up, SPECT scan to assess myocardial perfusion was performed. Therefore, change in myocardial perfusion at 12 months after repeated injection was compared with baseline in a separate model, adding time as factor. All statistical analyses were performed with SPSS software version 20 (SPSS Inc, Chicago, IL). All tests were 2-sided, with a P value of <0.05 considered statistically significant.

Results
Between March 2011 and February 2013, 23 patients (69±9 years; 17 men) were enrolled in the study. Repeated intramyocardial bone marrow cell injection was performed 4.6±2.5 years (limits, 1.2–8.1 years) after the first injection. Apart from a higher baseline age, baseline patient characteristics were not significantly different. Changes in medical history and medication use between the first and repeated injection did not result in significant differences at baseline between the first injection and repeated injection (Table).

Procedural Data
Mean procedural time for mapping and injection was 49.8±11.4 minutes during repeated injection and 57.1±16.9 minutes during the first injection (P=0.091). Patients received a cell suspension containing 98.7±6.3×10^6 mononuclear cells, with a CD34-positive cell fraction of 1.8±1.2% during repeated injection. This is comparable with the number of injected cells and the CD34-positive cell fraction administered during the first treatment (93.5±20.1×10^6; P=0.269 and 2.0±1.4%; P=0.475, respectively). During the repeat injection procedure, 10.6±1.2 intramyocardial injections were performed, which is modestly higher number when compared with the first injection procedure in which patients received 9.2±1.4 injections (P=0.002).

Safety Data
The injection procedures were performed without major peri-procedural complications in all patients. Within 12 months after the first injection procedure, 1 patient underwent a percutaneous coronary intervention as was reported previously. During 12 months of follow-up after repeated injection, 1 patient developed, after a period of clinical improvement, renewed anginal complaints. The patient underwent coil embolization of a nonligated left internal mammary artery side branch, 11 months after repeated injection. No other clinical events occurred. Twenty-four–hour Holter ECG recording at 6 weeks and 6 months revealed no sustained ventricular tachycardia or ventricular fibrillation. Similarly, during exercise testing at 3 and 6 months, no sustained ventricular tachycardia or ventricular fibrillation was observed.

Myocardial Perfusion and Ischemia
SPECT-MPI studies before and 3 months after the first injection and at baseline of repeated injection were available for all 23 patients. One patient refused further follow-up after repeated injection; in 1 patient, the 3-month follow-up scan and in another patient, the 12-month follow-up scan were missing because of logistic reasons. The summed stress score and the summed rest score were higher before repeated injection when compared with baseline before the first injection (27.3±5.8 versus 24.9±6.7; P=0.019 and 22.6±5.0 versus 20.0±4.8; P=0.005, respectively), indicating a decrease in perfusion over time. However, the summed difference score was not significantly different at both baseline moments (4.9±4.4 before the first injection versus 4.7±2.9 before repeated injection; P=0.787), reflecting a similar amount of myocardial ischemic burden.

Three months after repeated injection, the summed stress score had improved from 27.3±5.8 to 24.5±4.4 (P=0.002), which is comparable with the improvement after the first injection (24.9±6.7 to 20.9±5.8; P<0.001, first versus repeated; P=0.379; Figure 1). Twelve months after repeated injection, the summed stress score was 25.4±4.9, demonstrating a sustained improvement (baseline versus 12 months; P=0.002).

The summed rest score after repeated injection showed a modest improvement from 22.6±5.0 at baseline to 21.3±4.2 after 3 months of follow-up (P=0.036). Although no significant improvement in summed rest score was demonstrated after the first injection (20.0±4.8 to 19.1±4.6; P=0.152), the
change in the summed rest score was not significant between the first and the repeated injection (P=0.602). Twelve months after repeated injection, the summed rest score was 21.8±4.6, showing still a modest but significant improvement when compared with baseline before repeated injection (baseline versus 12 months; P=0.034).

The summed difference score decreased from 4.7±2.9 to 3.2±2.3, 3 months after repeated injection, demonstrating a reduction in ischemia (P=0.031). This improvement was not significantly different from the amount of improvement after the first injection (from 4.9±4.4 to 1.8±2.5; P<0.001, first versus repeated; P=0.095). Twelve months after repeated injection, the summed difference score was 3.6±2.7 (baseline versus 12 months; P=0.050).

**LV Function and Volumes**

Paired MRI data were available in 19 patients after the first injection and after repeated injection. In 1 patient, only during the first injection, MRI was performed, and 2 patients did not undergo MRI at all because of the presence of a pacemaker or a transcutaneous electric nerve stimulator. Two follow-up scans after the first injections were not assessable, and 1 patient did not want to undergo follow-up after repeated injection.

Baseline LV ejection fraction was lower before repeated injection than that before the first injection (55.1±8.8% versus 56.1±8.4%; P=0.022). Differences in baseline end-systolic volumes (first 63.3±20.1 mL versus repeated 69.0±17.6 mL; P=0.194) and end-diastolic volumes (first 161.0±25.0 mL versus repeated 154.2±25.7 mL; P=0.488) were not significant.

Three months after repeated injection, no significant improvement in LV ejection fraction was observed (from 55.1±8.8% at baseline to 56.1±8.4% after 3 months of follow-up; P=0.582). Importantly, no substantial improvement in LV ejection fraction was observed after the first injection (60.8±8.4% to 61.1±8.2%; P=0.592). Thus, the effect on LV ejection fraction was not significantly different between the first and the repeated injection (P=0.986). In line with these findings, no significant changes in LV end systolic volume (from 69.0±17.6 to 67.0±19.4 mL; P=0.617) and LV end-diastolic volume (from 154.2±25.7 to 151.7±27.6; P=0.603) were observed after repeated injection or after the first injection (end-systolic volume: from 63.3±20.1 to 62.2±19.3; P=0.629 and end-diastolic volume: from 160.1±25.0 to 158.4±23.3; P=0.827).

**Exercise Capacity**

Baseline exercise capacity values at the first and repeated injections were similar (85±23 and 87±24 W, respectively; P=0.760). Mean exercise capacity did not significantly improve after repeated injection (from 87±24 to 88±25 W at 3 months and 83±24 W at 6 months of follow-up; P=0.550). In contrast, after the first injection procedure, mean exercise capacity demonstrated a significant improvement from 85±23 at baseline to 98±28 W at 3 months and 96±32 W at 6 months of follow-up (P<0.001, first versus repeated; P=0.008).
Clinical Outcome
Clinical status according to the Canadian Cardiovascular Society score was not significantly different before repeated injection when compared with before the first injection (3.0±0.7 versus 3.4±0.6; \( P = 0.110 \)). After repeated injection, Canadian Cardiovascular Society score improved from 3.0±0.7 at baseline to 2.3±0.8 at 3 months, 2.5±0.9 at 6 months, and 2.5±0.9 at 12 months (\( P_{\text{slope}} = 0.007 \)), which is comparable with the improvement after the first injection (from 3.4±0.6 at baseline to 2.5±0.8 at 3 months, 2.4±0.7 at 6 months, and 2.7±1.0 at 12 months; \( P_{\text{slope}} < 0.001 \), first versus repeated; \( P = 0.188 \)).

Similarly, baseline quality of life score did not significantly differ between the first and repeated injections (53±8% and 56±10%, respectively; \( P = 0.109 \)). After repeated injection, quality of life score improved from 56±10% to 63±14% at 3 months, 63±14% at 6 months, and 62±14% at 12 months (\( P_{\text{slope}} = 0.020 \)), comparable with the improvement observed after the first injection (from 53±8% to 66±13% at 3 months, 70±15% at 6 months, and 65±17% at 12 months; \( P_{\text{slope}} < 0.001 \), first versus repeated; \( P = 0.126 \)). After repeated injection, patients’ quality of life significantly improved on the angina frequency scale (\( P_{\text{slope}} = 0.024 \)) and on the disease perception scale (\( P_{\text{slope}} < 0.001 \)). Similarly, those domains had improved after the first injection (\( P_{\text{slope}} = 0.001 \), first versus repeated; \( P = 0.444 \) for angina frequency scale and \( P_{\text{slope}} < 0.001 \), first versus repeated; \( P = 0.816 \) for disease perception scale). After the first injection, patients also had improved on the physical limitation scale (\( P_{\text{slope}} < 0.001 \)). However, after repeated injection, there was only a positive trend on the physical limitation scale (\( P_{\text{slope}} = 0.075 \), first versus repeated; \( P = 0.025 \)). The domains angina stability and treatment satisfaction did not significantly change after both injection procedures (Figure 2).

Discussion
The key finding of this study is that repeated bone marrow mononuclear cell injection in patients with residual or recurrent angina pectoris and myocardial ischemia after a previous efficacious bone marrow cell injection procedure is safe and improves myocardial perfusion and anginal complaints, with an effect size comparable with that after the first injection procedure. Furthermore, these improvements are sustained ≤12 months of follow-up.

During the past decade, intramyocardial bone marrow cell injection has been investigated as a therapeutic option for patients with chronic ischemic heart disease. On the basis of animal studies, it is hypothesized that, because of a paracrine effect or by direct incorporation of cells into new capillaries, administration of bone marrow mononuclear cells stimulates neovascularization. Encouraged by these findings, clinical studies have investigated the effect of bone marrow–derived cell treatment in various cardiac patient populations, including patients with refractory angina because of myocardial ischemia. These studies have demonstrated that intramyocardial bone marrow cell injection is safe and improves myocardial perfusion, LV function, exercise capacity, and anginal complaints.

The duration of sustained improvement, however, is unknown. A sustained effect after intramyocardial cell injection in patients with refractory angina was reported at 12 months of follow-up. However, we recently reported that 4 years after intramyocardial cell injection, anginal complaints had reoccurred in the majority of patients with chronic ischemia. It is hypothesized that the duration of the treatment effect might be determined by the balance between the progression of atherosclerosis and angiogenesis. Therefore, repeated bone marrow cell injection could be a promising approach to boost neovascularization of ischemic tissues and reduce cardiac complaints in these patients.

A small number of studies have evaluated the effect of repeated cell treatment shortly after the first infusion (within 6 months). The Danish Stem Cell Study-Congestive Heart Failure (DanCell-CHF) investigated the effect of repeated intracoronary bone marrow cell treatment 4 months after an initial cell infusion in patients with congestive heart failure. This nonrandomized study concluded that, after 12 months,
repeated infusion was safe and resulted in improvements in clinical symptoms and LV filling patterns, without demonstrating improvements in LV ejection fraction. However, in patients with recent acute myocardial infarction, repeated intracoronary cell infusion was associated with positive effects on LV ejection fraction and perfusion. For example, in a randomized study by Yao et al,6 repeated intracoronary bone marrow cell infusion at 3 months after the initial infusion was evaluated in patients with acute myocardial infarction and reduced LV function. When compared with a single bone marrow cell infusion and placebo infusion, repeated bone marrow cell treatment improved LV ejection fraction and decreased infarct size at 12 months of follow-up. In line with these findings, Gu et al7 reported that repeated intracoronary infusion of peripheral blood stem cells at 6 months after the initial infusion in refractory heart failure patients with recent myocardial infarction resulted in improved LV ejection fraction, myocardial perfusion, and cardiac complaints when compared with a single infusion or a control group.

To our knowledge, the current trial is the first study to evaluate the effect of repeated intramyocardial bone marrow cell injection in patients with refractory angina. In accordance with the aforementioned trials and other previously published studies, this study demonstrates a positive safety profile of repeated intramyocardial bone marrow cell injection without the occurrence major clinical events, periprocedurally or during 12 months of follow-up.1,4,6,8–11,13,15,17,18 Furthermore, repeated bone marrow cell injection resulted in clinical improvements and increased myocardial perfusion, comparable with the observed improvements after the first injection.

The patients included in the current trial have previously received bone marrow cell injection in a prospective study or randomized controlled trial. Importantly, the method used for cell isolation, processing and intramyocardial injection, as well as inclusion criteria, and functional and imaging protocols were identical to the methods used during the first injection.4,10,11 However, when compared with the first injection, patients had decreased myocardial perfusion before the repeat injection, indicating the progression of flow limiting coronary atherosclerosis over time.

This study is the first to show that repeated injection of bone marrow cells into ischemic myocardium can again improve myocardial perfusion and anginal complaints. Improvement in quality of life seems attributable to a decrease of angina frequency and of the burden on patients’ life. Notably, the extent of the improvements after the repeated injection was comparable with the improvements after the first bone marrow cell injection. Thus, repeated bone marrow cell injection might be a viable therapeutic option for patients having progressive non-option obstructive coronary artery disease.

Despite the observed improvements in myocardial perfusion and anginal complaints, no increase in LV ejection fraction or exercise capacity was observed after repeated injection. There are several potential explanations for these findings. The absence of changes in LV ejection fraction may be related to patient selection because this selected patient group had a normal baseline ejection fraction before repeated injection (55±9%) and before the first injection (61±9%), with no improvement after the first injection. Another explanation may be related to the relatively small patient sample size in this study, resulting in inability to detect subtle differences. Similarly, the domain scores of the Seattle Angina Questionnaire are difficult to interpret because of these small patient numbers. Furthermore, a repeated injection theoretically might have a lower treatment effect, although the observed improvement in myocardial perfusion and clinical parameters after the repeated injection procedure suggest a comparable effect with the first injection.

This study has several limitations. Because of the small sample size, the study was not powered to detect small changes in secondary end points. Moreover, because of numerous hypothesis tests for secondary end points, the rate of false positives might be inflated. Because of the lack of a control group, a placebo effect cannot be ruled out. Furthermore, because only patients who showed improvements after a previous bone marrow cell injection were included, it remains unknown whether previously nonresponding patients would benefit from a second injection.

In conclusion, the results of this study suggest that repeated bone marrow cell injection in previously responding patients with refractory angina is safe and is associated with improvements in myocardial perfusion and anginal complaints ≤12 months of follow-up. These results confirm previous findings on clinical and objective improvements after bone marrow cell injection in end-stage patients with ischemic heart disease.

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Disclosures
None.

References


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