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Chapter 3

Intramyocardial Injection of Bone Marrow Mononuclear Cells in Chronic Myocardial Ischemia Patients after Previous Placebo Injection Improves Myocardial Perfusion and Anginal Symtpoms: an Intra-patient Comparison

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ABSTRACT

Background We recently demonstrated in a randomized, double-blind, placebo-controlled trial that intramyocardial bone marrow cell (BMC) injection is associated with improvements in myocardial perfusion and anginal symptoms in chronic myocardial ischemia patients. In the present study the results of the cross-over phase of this trial, in which patients previously treated with placebo received autologous BMC injections are reported. This allows a unique intra-patient comparison on the effect of BMC versus placebo injection with elimination of patient-related confounding factors.

Methods In sixteen patients (14 male, 64±10 years), who previously received intramyocardial placebo injections in the setting of a randomized trial, 100×10^6 BMC were injected using the NOGA-system. Canadian Cardiovascular Society (CCS) angina score and quality of life were evaluated at baseline, 3 and 6 months. Tc-99m single-photon emission computed tomography (SPECT) and magnetic resonance imaging were performed at baseline and 3 months to assess myocardial perfusion and left ventricular (LV) function.

Results CCS score and quality of life improved significantly after BMC injection as compared to placebo (P=0.01 and P=0.02, respectively). SPECT revealed a significant greater improvement (p=0.03) in summed stress score after BMC injection as compared to placebo. LV end-systolic volume significantly decreased after BMC injection but not after placebo injection. LV end-diastolic volume and LV ejection fraction did not change.

Conclusion Intramyocardial BMC injection in patients with chronic myocardial ischemia who previously received intramyocardial placebo treatment resulted in significant improvement in angina symptoms and myocardial perfusion. These results confirm the outcome of our previously reported randomized trial.

INTRODUCTION

Despite optimal medical treatment and advanced revascularization strategies, a growing number of patients suffer from severe coronary artery disease resulting in chronic myocardial ischemia and disabling angina, not amenable to conventional treatment options. Bone marrow cell (BMC) injection has emerged as a potential therapeutic option to improve myocardial perfusion, left ventricular (LV) function and accompanying anginal symptoms, in patients with chronic ischemic heart disease[1].

We previously reported the results of a double-blind placebo-controlled randomized trial in which we evaluated the efficacy of autologous bone marrow-derived mononuclear cell injection into the ischemic myocardium of patients with refractory angina[2]. At 3 months follow-up improvements in myocardial perfusion and LV function were observed as well as a decrease in anginal symptoms[2]. In line with these findings, a recently published randomized placebo-controlled trial reported improvements in angina frequency and exercise tolerance at 12 months follow-up after intramyocardial injection of CD34+ cells[3]. Furthermore, at 6 and follow-up improvements in stress perfusion were observed. Although the results of these medium-sized trials confirmed the findings of non-randomized pilot studies[4-7], 2 small-sized randomized studies yielded discordant results and documented only limited or no improvement in myocardial perfusion and anginal complaints[2, 8, 9].

The observed differences may be related to a variety of factors, including cell isolation protocols[10], cell type[11] and dose[8, 12]. In addition, patient-specific characteristics such as comorbidity, genetic profile and cellular response to BMC administration may influence treatment effect. Therefore, unknown confounding factors may influence treatment outcome, especially since the exact mechanism by which bone marrow cells may improve myocardial perfusion and function is only partially understood.

After completion of the randomized trial, patients which were initially treated with placebo injection were offered to enter the cross-over phase of the trial to receive intramyocardial BMC injection. Therefore, this cross-over phase allows an intra-patient comparison of BMC injection and placebo treatment with a minimum of patient-specific confounding factors. Consequently, the aim of the current study was to assess the effect of BMC injection in the same patients with chronic myocardial ischemia that previously received placebo treatment Furthermore, the results of the cross-over phase were compared with the results of the initial randomized trial.

METHODS

Patient population

The study population consisted of patients who received placebo injection in the previously described randomized, double-blind, placebo-controlled trial.[2] All patients had severe angina (Canadian Cardiovascular Society (CCS) class II, III or IV) despite optimal medical therapy, and stress-inducible ischemia on technetium-99m tetrofosmin single-photon emission computed tomography (SPECT) without options for conventional revascularization. Exclusion criteria were left ventricular ejection fraction (LVEF) less than 35%, acute myocardial infarction within 6 months before enrollment, history of malignancy, renal dysfunction or unexplained haematological or biochemical abnormalities. The institutional ethics committee approved the protocol, and all patients provided written informed consent. A detailed study protocol and results of the randomized, double-blind, placebo-controlled trial have been reported previously[2]. The study was registered at the Dutch trial registry (www.trialregister.nl, no. NTR400/ ISRCTN58194927).

Study design and protocol

The current study was designed as an intra-patient comparison to compare the effect of autologous mononuclear BMC injection with the effect of a previously administered placebo injection in patients with chronic myocardial ischemia. As such, each patient served as his own control. Placebo injections were performed in the setting of a randomized, double-blind, placebo controlled trial of which the results have been recently published[2].

The study protocol of the trial has been previously reported[2]. In the cross-over phase, clinical and functional assessment at baseline and follow-up, as well as the injection procedure, were identical to the main trial. In brief, the study protocol was as follows: At least twelve months after placebo injection, eligibility for cross-over treatment was determined using the inclusion and exclusion criteria of the original trial. At baseline, the clinical status was assessed according to the CCS classification, ranging from class I (mild) to IV (severe)[13]. The disease specific Seattle Angina Questionnaire was used to evaluate the patients' quality of life[14, 15]. Technetium-99m tetrofosmin SPECT was performed to assess myocardial perfusion, and LV function and volumes were assessed by magnetic resonance imaging (MRI).

Bone marrow was aspirated from the iliac crest under local anesthesia, followed by Ficoll density centrifugation to isolate the mononuclear cells. With the use of the NOGA system (Biologics Delivery Systems, Johnson & Johnson, Irwindale, California), approximately 100×10^6 autologous bone marrow-derived mononuclear cells were injected at myocardial regions with stress-inducible ischemia on SPECT[4].

At 3 months and 6 months follow-up, the clinical status (CCS and quality of life) was reassessed. Technetium-99m tetrofosmin SPECT and MRI were repeated after 3 months to evaluate myocardial perfusion and LV function, respectively. To monitor the occurrence of arrhythmias, 24-hour Holter electrocardiogram recordings were obtained at 6 weeks and 6 months follow-up.

SPECT imaging

Technetium-99m tetrofosmin SPECT imaging was performed using a two-day stress-rest protocol as previously described.[4] Briefly, the stress protocol included adenosine infusion (0.14 mg/kg/min) for 6 minutes and intravenous injection of 500MBq Tc-99m tetrofosmin after 3.5 minutes of adenosine. Rest images were obtained using an injection of 500 MBq technetium-99m tetrofosmin. In order to analyze the myocardial perfusion, a standard short- and long-axis projection, perpendicular to the heart axis, was reconstructed, which were adjusted for peak myocardial activity (100%).

The myocardium was divided into 17 segments according to the American Heart Association/American College of Cardiology recommendations[16]. To analyze myocardial perfusion, Quantitative Gated SPECT software (QGS software, Cedars-Sinai Medical Center, Los Angeles, California) was used and segmental tracer activity was categorized on a 4-point scale: 1 = normal tracer activity >75%; 2 = tracer activity 50% to 75%; 3 = tracer activity 25% to 49%; and 4 = tracer activity <25%.

Significant fill-in (>10%) of perfusion defects, observed on the images at rest, was classified as ischemic myocardium. By summation of the patients' segmental scores at stress and rest, the summed stress score and summed rest score, respectively, were calculated[17].

Magnetic resonance imaging

Assessment of the parameters of global systolic function was performed by MRI studies, using a 1.5-Tesla system (Philips medical Systems; Best, the Netherlands) with a 5-segment synergy coil and vector electrocardiographic gating. A steady state free precession (fields

of view 400x400 mm^2, matrix size 256x256 pixels) was used to image the heart in the short-axis view during 15 second breath holds. To determine LV volumes and LVEF, previously validated software (QMass MR, Medis Medical Imaging Systems; Leiden, the Netherlands)[18] was used. The images were analyzed by two experienced observers (J.v.R. and S.F.R), blinded to all clinical data. The intra- and inter-observer variability were 1±3 ml and 2±4 ml for LV end-systolic volume (LVESV), 1±4 ml and 2±6 ml for LV end-diastolic volume (LVEDV), and 0.2±1.6% and 0.5±2.1% for LVEF[4].

Statistical analysis

All analyses were performed according to the intention-to-treat principle. Complete case analysis was performed for all paired tests. Data are reported as mean±SD. An intra-patient comparison was conducted comparing patients after placebo treatment and cross-over BMC treatment. Continuous data were compared using a Wilcoxon signed rank test. Categorical data were compared using a χ^2-test or Fisher exact test. We applied repeated measures analysis of variance and the Friedman test to compare the change in distribution of continuous outcome data after cross-over and placebo treatment, at baseline and follow-up. A Mann-Whitney test was used to compare changes in semiquantitative data. A P-value of less than 0.05 was considered statistically significant. All statistical analyses were performed with SPSS software version 17.0 (SPSS, Chicago, Illinois).

RESULTS

In the previously reported trial[2], twenty five patients were randomly assigned to placebo treatment. Of these patients, 9 patients were not eligible for cross-over treatment since they did not meet the inclusion criteria. Therefore, 16 patients (age 64±10, male 14) were enrolled in the current evaluation. Since the current study comprised an intra-patient comparison, baseline patient characteristics were identical in both treatment groups apart from baseline age and changes which occurred after placebo treatment (Table 1). In all patients, the type and dose of antianginal medication remained unchanged during the study period of a given treatment. Between both treatments only minimal changes in medication had taken place.

Table 1. Baseline characteristics

Baseline characteristics	Placebo (n=16)	Cross-over (n=16)	P-value
Age, years	62±10	64±10	<.00
Men	14(88)	14(88)	1.00
Body mass index, kg	30±5	30±5	1.00
Cardiovascular risk factors, n(%)			
Smoking	5(31)	5(31)	1.00
Hypertension	8(50)	8(50)	1.00
Diabetes	5(31)	5(31)	1.00
Dyslipidemia	8(50)	8(50)	1.00
Family history of CAD	8(50)	8(50)	1.00
Current medication, n(%)			
Nitrates	14(88)	14(88)	1.00
B-blockers	15(93)	15(93)	1.00
Calcium channel Blockers	12(75)	13(81)	0.32
Statins	16(100)	16(100)	1.00
ACE-inhibitors	10(63)	10(63)	1.00
Clopidogrel	6(38)	5(31)	0.32
Aspirin	13(81)	13(81)	1.00
Oral anticoagulants	2(13)	2(13)	1.00
History, n(%)			
Prior MI	12(75)	11(75)	1.00
Prior CABG	10(63)	10(63)	1.00
Prior PCI	8(50)	9(56)	0.32

Procedural and safety data

Mean procedural time for mapping and injection was 53±15 minutes during cross-over treatment and 50±14 minutes during placebo treatment (P=0.67). During cross-over patients received 8.5±1.4 injections, whereas during placebo treatment, patients received 8.1±0.9 injections (P=0.31). Injections of approximately 0.2 to 0.3 ml cell suspension or placebo were delivered at each injection site. The cell suspension as injected during cross-over treatment contained a total of 100 x10^6 bone marrow mononuclear cells, with a CD34-positive cell fraction of 1.9%±1.2%. Compared to cross-over treatment, the primary treated cell group underwent a comparable cell harvesting procedure followed by 8.5±1.3 injections (vs. cross-over P=NS) with a similar cell suspension (98 x10^6 bone marrow cells [vs. cross-over P=NS], CD34-positive cell fraction 2.4±0.9% [vs. cross-over P=NS]).

In all patients, intramyocardial injection was performed without major periprocedural complications. During 6 months follow-up, no arrhythmias were observed in any of the 24-hour Holter recording or during exercise testing.

Two weeks after BMC injection, 1 patient died due to sepsis caused by bilateral pneumonia. Safety data from placebo treatment have been published previously.[2]

Myocardial perfusion and ischemia

For both treatment modalities, SPECT images at baseline and 3 months follow-up were available in all surviving patients. After cross-over treatment, summed stress score improved from 24.7±4.5 at baseline to 21.9±3.9 at 3 months follow-up (P<0.01). After placebo treatment, a modest improvement from 24.6±4.8 at baseline to 23.8±5.0 had been observed. When the two treatment strategies were compared, the improvements in summed stress score were significantly larger after cross-over treatment (treatment effect, -2.32; 95% CI, -3.64 to -0.23; P=0.03). As compared to the effect of BMC injection in the original trial, the improvement in summed stress score in the cross-over study group was similar to the improvement documented in the cell treatment group in the initial randomized trial (treatment effect, -0.79; 95% CI, -2.39 to 1.05; P=0.44).

Summed rest score showed a small but significant improvement after cross-over treatment (from 21.1±4.1 at to 20.3±3.7, P=0.03), whereas no changes were observed after placebo treatment (20.9±4.6 vs. 20.6±4.2 (P=0.18). The changes in summed rest score were not significantly different between both treatment phases (P=0.24).

The number of ischemic myocardial segments per patient decreased significantly from 3.1±2.1 to 1.6±1.4; P<0.01), after cross-over treatment. After placebo treatment, the number of ischemic segments also decreased from 3.1±1.6 at baseline to 2.5±1.7 at 3 months (P=0.02). However, the decrease in ischemic segments was significantly larger after cross-over treatment as compared to placebo treatment (treatment effect, -2.36; 95% CI, -2.37 to -0.17; P=0.03) (Figure 1).

Myocardial perfusion in injected and noninjected segments

A total of 127 injections was targeted at 60 myocardial segments during cross-over (4.0±0.8 injected segments/patient). During placebo treatment, a total of 120 injections targeting 57 ischemic segments had been performed (3.8±0.4 injected segments/patient). After cross-over treatment, myocardial perfusion score had increased at least 1 point in stress or rest perfusion in 50% (30 segments) of the total of 60 injected segments and in 8% (15 segments) of the total of 195 noninjected segments, after 3 months. After placebo treatment, 7 of 57 injected segments (12.3%) revealed improvement after 3 months follow-up, whereas 13 of the 198 noninjected segments (6.6%) improved in perfusion.

The percentage of injected segments with an improved perfusion was significantly higher after cross-over treatment (P<0.01). No significant difference between both treatments was observed in the percentage of noninjected segments with improved perfusion (P=0.68) (Figure 2).
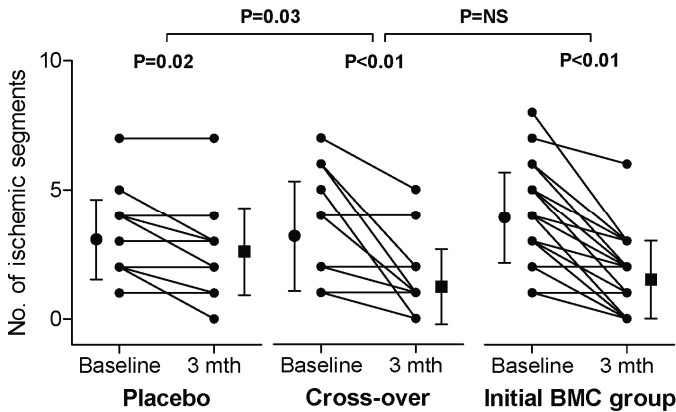
Figure 1. Number of ischemic segments as assessed by SPECT
Number of ischemic segments at baseline and 3 months as assessed by SPECT. Comparing placebo treatment and cross-over treatment demonstrates a significantly larger improvement after cross-over treatment.
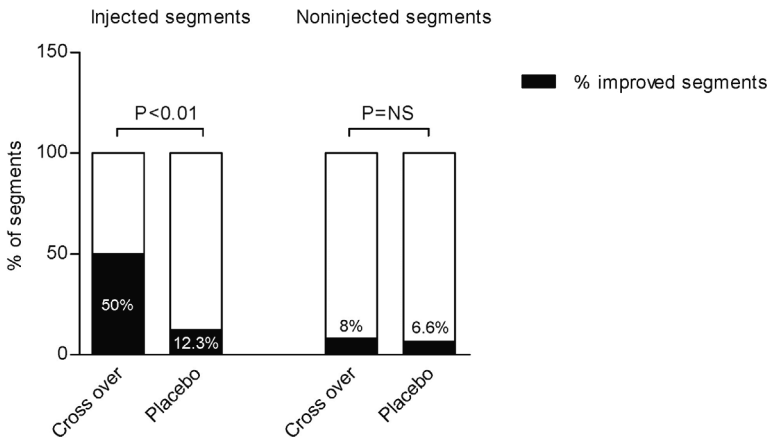
Figure 2: Segmental improvement in myocardial perfusion
Segmental improvement in myocardial perfusion score comparing cross-over treatment and previous placebo treatment in the same patients. The number of injected segments that improved was significantly larger after cross-over treatment.

Left ventricular function and volumes

Paired MRI was performed in 12 patients after cross-over treatment and in 11 patients after placebo treatment. After cross-over treatment, a non significant improvement of LVEF was observed from 55%±11% at baseline to 57%±12% at 3 months follow-up (P= 0.10). After placebo treatment, no substantial changes in LVEF were detected (54±10% vs. 53±10%, P=0.42). The improvement in LVEF was not larger after cross-over treatment as compared to the placebo treatment (P=0.12). LVESV decreased significantly after cross-over treatment but not after placebo. However, the difference in pre- vs. postinjection values between treatment modalities was not significantly different (P=0.29). No significant changes in LVEDV and LV stroke volume were observed after both treatments (Table 2).

Table 2. Left ventricular volumes as assessed by MRI at baseline and follow-up after placebo and crossover treatment.

	Placebo			Crossover			δ
	Baseline	**3 mo**	**P**	**Baseline**	**3 mo**	**P**	**P**
MRI measurements							
LVEF, %	54±10	53±10	0.42	55±11	57±12	0.10	0.12
LVEDV, mL	187±56	182±52	0.53	170±42	159±38	0.31	0.57
LVESV, mL	91±44	89±42	0.53	80±35	73±36	0.04	0.29
LVSV, mL	97±16	93±14	0.33	90±15	86±12	0.51	0.98

Clinical outcome

Clinical status was assessed according to the CCS classification at baseline, 3 months and 6 months follow-up. After cross-over treatment, a significant improvement was observed from 3.1±0.6 at baseline to 2.4±0.8 at 3 months, and to 2.4±0.9 at 6 months (P=0.02). No significant improvement in CCS class was observed after placebo injection (2.7±0.6 to 2.6±0.7 at 3 months, to 2.5±0.6 at 6 months, P=0.52).

Quality-of-life score increased after cross-over treatment from 59±13% to 66±14% at 3 months, and 69±16% at 6 months (P<0.01). After placebo injections, an improvement in quality of life was noted from 59±9% to 62±11% at 3 months and to 62±11% at 6 months (P<0.01). The improvements in CCS class and quality of life were significantly greater after cross-over treatment as compared to placebo treatment (P=0.01 and P=0.02, respectively). The effect of cross-over treatment on both CCS class and quality of life were not significantly different from the effects of BMC injection in the initial randomized trial (P=0.80 and P=0.27, respectively, Figure 3).
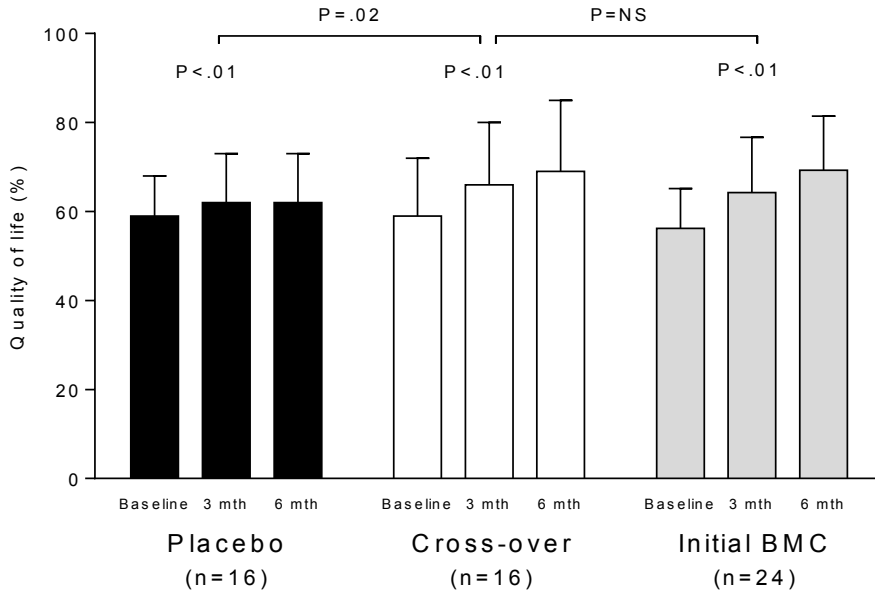
Figure 3. Quality-of-life score
Quality-of-life score at baseline and 3 and 6 months follow-up. Comparing the improvement in quality of life between cross-over treatment and placebo treatment within the same 16 patients demonstrated a significant greater improvement after cross-over treatment. The improvement in quality of life after cell injection in the cross-over phase and the randomized trial (24 patients) are similar.

DISCUSSION

Key findings of the present study were: 1) autologous BMC injection in patients previously treated with placebo is associated with improvements in myocardial perfusion and clinical parameters as compared to the previous placebo injection. And 2), these results are in line with the findings of the recently reported randomized, double-blind, placebo-controlled trial which evaluated the efficacy of BMC treatment in patients with chronic myocardial ischaemia[2].

Since current revascularization strategies do not suffice in treating the increasing population of patients with end-stage ischemic heart disease, cell therapy has been introduced as a new treatment modality[19, 20]. Therapeutic administration of bone marrow cells has been suggested to stimulate angiogenesis by the release of growth factors[21] and/or by direct incorporation of cells into new capillaries[22], resulting in improvements in cardiac perfusion and function. As in no-option patients with chronic myocardial

ischemia severe obstructive coronary artery disease often precludes intracoronary delivery of cells, intramyocardial injection of bone marrow cells is the preferred approach in these patients.

Previous studies have indicated the safety and feasibility of intramyocardial BMC administration in patients with chronic myocardial ischemia[4-7, 23, 24] In line with these studies, overall safety assessment in the current trial did not reveal any complications considered to be related to cell administration.

The efficacy of BMC treatment in patients with chronic myocardial ischemia has been evaluated in several randomized, placebo-controlled trials. Although in general the reported findings demonstrate positive effects on myocardial perfusion, function, exercise capacity and anginal symptoms, a number of inconsistent findings are present[2, 8, 9] In the small-sized randomized study of Losordo et al[8]., intramyocardial injection of granulocyte-colony stimulating factor (GCSF)-mobilized CD34+ cells did not result in significant improvements in angina frequency, exercise capacity or myocardial perfusion. However, a larger randomized placebo-controlled trial of the same study group showed significant improvements in angina pectoris frequency and exercise tolerance 12 months after cell injection[3]. In addition, myocardial perfusion during stress showed significant improvements at 6 months follow-up. The results of the PROTECT-CAD trial[9] showed modest improvements in exercise capacity and LVEF after injection of bone marrow-derived mononuclear cells. Nonetheless, no improvements in anginal complaints or myocardial perfusion were observed, although it must be noted that post-hoc analysis demonstrated regional improvements in myocardial perfusion in the injected segments. In the randomized trial from our group, of which the current study describes the cross-over phase, 50 patients with chronic myocardial ischemia were assigned to receive intramyocardial injection of either bone marrow-derived mononuclear cells or placebo[2]. In this study, BMC injection was associated with improvements in myocardial perfusion, LV function and anginal complaints.

Theoretically, a number of methodological factors may account for the discordant results of the aforementioned studies. First, differences in cell isolation and storage protocols may affect the functional capacity of the cells, as recently suggested by Seeger et al[10]. Second, cell type and dose varied considerably between these studies, ranging from 0.5 to 5×10^5 GCSF-mobilized CD34+ cells in the studies of Losordo to 100×10^6 bone marrow-derived mononuclear cells in our trial. Therefore, the potential impact of a dose-response relationship, as previously proposed for BMC infusion after acute myocardial

infarction[25], may have influenced treatment effect. Third, group size was substantially different in these studies, with the largest treatment groups in the larger randomized trial from Losordo et al. (n=53) and our group (n=25), compared to a group size of 9 or 10 patients in the PROTECT-CAD trial and a group size of 6 patients in the small-sized study of Losordo et al. Besides the risk of underpowering in the assessment of treatment effect, these relatively small group sizes can result in imbalances in baseline characteristics. For example, in the PROTECT-CAD trial, diabetes was more prevalent in the placebo group. Since it has been suggested that BMC therapy is more effective in diabetic patients[26], this imbalance in patient characteristics might theoretically confound study results. Similarly, numerous other patient-specific characteristics such as genetic variations[27], response to medication[28] as well as co morbidity[28] are known to influence treatment outcome in cardiac disease. It is likely that some of these factors will impact BMC function, the response of host tissue, and patient prognosis after BMC administration, thus modifying the treatment results of BMC injection. As a result, outcomes may have been confounded by known and unknown patient-specific factors, especially since treatment groups in the described studies were relatively small.

In the current study, an intra-patient comparison model is used, allowing elimination of known and unknown patient-specific factors which may possibly confound analysis of the treatment effect. Using this design, we observed improvements in myocardial perfusion, quality of life and CCS class consistent with the findings of our initial randomized study (Figure 3). Of note, the increases in LV ejection fraction and exercise capacity were not significant, although there was a trend towards improvements comparable to the effect in the initial randomized study. Interestingly, in contrast to findings of the initial trial, there was a trend towards a decrease in LVEDV with a decrease in LV stroke volume after cross-over treatment. Therefore, the increased LVEF results from a greater decrease in LVESV as compared to the decrease in LVEDV. However, because the changes in MRI parameters between cross-over and the initial cell-treated group are not significantly different, no conclusions can be drawn. (Figure 4.) Overall, the findings of the present study confirm the findings of our initial randomized trial using a study design in which the number of potential confounding factors is minimized, thereby strengthening the concept of BMC injection for the treatment of chronic myocardial ischemia.
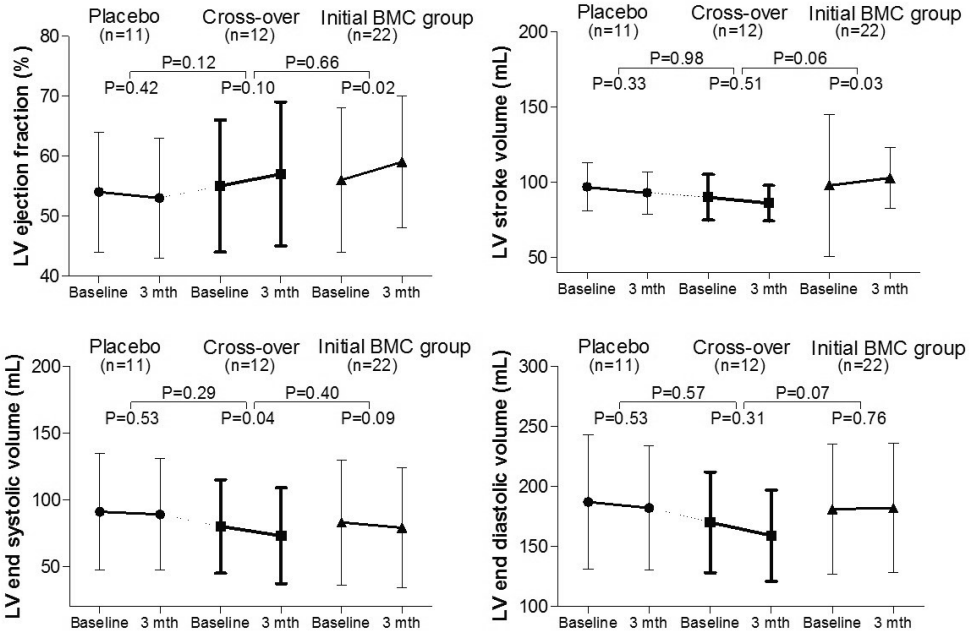
Figure 4: Left ventricular volumes
MRI-derived left ventricular (LV) ejection fraction, LV end systolic volume, LV end diastolic volume and LV stroke volume at baseline and 3 months after placebo treatment (11 patients), cross-over treatment (12 patients) and of the initial bone marrow cell (BMC) treated group (22 patients). Comparing the change in MRI parameters between placebo treatment and cross-over treatment demonstrates no significant difference. Similarly, the cross-over group shows comparable changes as compared to the initial BMC group.

Limitations

One of the limitations of the current study design was the lack of blinding during the cross-over phase that could have led to a placebo effect after BMC injection. Since patients were aware of treatment assignment during the cross-over phase, a placebo effect cannot be ruled out. However, the placebo effect was at least partially taken into account by comparing the results of BMC injection with the effect of (blinded) placebo treatment during the initial trial. In addition, there might be a patient bias since in the 16 cross-over patients placebo treatment resulted in 12.3% improvement of injected cells, as compared to 20% the total placebo treated group in the primary study. Therefore, the placebo-treated patients included for cross-over may be slightly different from the original placebo group. Furthermore, the patient population was small, which may have resulted in a lack of statistical power to observe significant differences in LV function and exercise capacity between BMC and placebo injections. Finally, the current study does

not provide data of long term follow-up on the effects of BMC injection on morbidity and mortality.

In conclusion, the results of the study demonstrate that intramyocardial BMC injection is associated with a beneficial effect on myocardial perfusion, CCS class and quality of life as compared to placebo treatment. Using an intra-patient comparison design, the current study confirms the findings of the initial randomized trial, thus strengthening the concept of BMC therapy for chronic myocardial ischaemia.

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