Experimental therapeutic strategies in restenosis and critical limb ischemia

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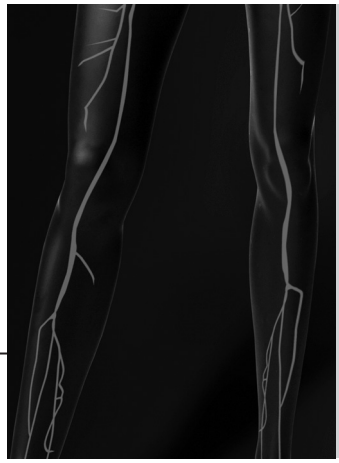

Chapter 06

Intramuscular or combined intramuscular/ intra-arterial administration of bone marrow mononuclear cells: a clinical trial in patients with advanced limb ischemia

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Abstract

Background: Recent evidence indicates that bone marrow mononuclear cells (BMC) promote collateral vessel formation in patients with severe peripheral arterial disease (PAD). However, aspects concerning optimal administration mode, durability and long-term safety require consideration.

Combined intra-arterial plus intramuscular BMC delivery may be more effective than exclusive intramuscular injections. We evaluated feasibility, safety and effect of exclusive intramuscular (IM) versus combined intramuscular/intra-arterial (IA+IM) delivery of autologous BMC in patients who were not candidates for surgical or endovascular treatment.

Methods: 27 patients were treated with either combined IA+IM (n=12) or sole IM (n=15) administration of autologous BMC. Efficacy was assessed after 1, 6 and 12 months. Limb salvage, pain-free walking distance, ankle-brachial pressure index (ABI) and pain scores were evaluated.

Results: There were no adverse reactions related to injection of the cells. Three patients died within the first year of follow-up due to non-procedure related causes. Two patients in the IA+IM group required limb amputation because of ongoing critical ischemia versus seven patients in the IM group (P=0.17). BMC treatment in the remaining patients resulted in a significant and sustained (>12 months) improvement. Pain-free walking distance improved from 81 ± 56 meters at baseline to 257 ± 126 meters at t=6 months (P=0.0002). Mean ABI increased 23% after 6 months (P=0.01) and pain score reduced for up to 50% as shown by Brief Pain Inventory (P=0.001).

Conclusion: Both intramuscular and combined intramuscular/intra-arterial delivery of autologous BMC are safe, and result in relevant and sustained improvement in a considerable proportion of patients with severe PAD who are not amenable for conventional treatment.

Registration number: ISRCTN76049483 (http://www. controlled-trials.com)

Introduction

Despite improvements in conventional surgical and endovascular treatment for revascularization, approximately 50% of patients with critical limb ischemia (CLI) or severely disabling claudication still remain not amenable for revascularization.[1] Prognosis for these patients in terms of quality of life and life expectancy is poor. Recently, there has been considerable interest in potential therapeutic use of bone marrow-derived mononuclear cells (BMC) to promote formation of collateral arteries (arteriogenesis) in patients with peripheral arterial disease (PAD). Animal studies demonstrate that injection of BMC results in augmented collateral development and clinically improved vascularisation.[2,3]

In their pioneering study, Tateishi and colleagues showed that local injection of BMC in the gastrocnemius muscle of patients with limb ischemia is feasible and safe. [4] During a 6 months follow-up period clinical parameters like ankle-brachial indices and pain free walking distances improved significantly. Currently, similar encouraging results have been reported in a number of studies of limited size, [5-9] and in some institutions, BMC therapy seems to have got a more or less established place.[10,11] Yet, long term safety[12] and durability[13] of the effect have recently been questioned. Moreover, questions like selection of patients, optimal fraction of BMC and method of delivery have not been solved. It can be argued that exclusive intramuscular delivery in the calf muscle is not the most effective mode of delivery. Most patients with advanced PAD present with multi-level disease including extensive pathology of the femoropopliteal tract and pedal arteries. These zones, the femoropopliteal segment in particular, may not be addressed by exclusively intramuscular calf injections. It can be argued that intramuscular (IM) implantation combined with selective intra-arterial (IA) delivery of the cells could be more efficacious than sole injections in the calf muscle.

The aim of our study was to test the safety and feasibility, as well as the efficacy of exclusively intramuscular, as compared with combined intramuscular/intra-arterial administration of BMC in patients with PAD who were without conventional options for surgical or endovascular treatment.

Patients and Methods

Participants

Patient records were reviewed by a panel of experienced vascular surgeons and interventional radiologists. Technical options for revascularisation by percutaneous transluminal angioplasty (PTA) or reconstructive surgery were extensively evaluated and disqualified. Patients were eligible for transplantation of autologous bone marrow-derived mononuclear cells if they suffered from critical leg ischemia (ischemic rest pain or ulcers), or in case of persistent (> 12 months) profound disabling claudication and a maximum walking distance of 100 meter. Life expectancy should at least be 1 year. Patients with a

history of malignant disease in 5 years prior to treatment were excluded. Written informed consent for participation was obtained from all subjects before enrolment into the study. The medical ethics committee of the Leiden University Medical Center approved the protocol.

Procedure

Hospital admittance was planned in a short-stay setting (24-48 hrs). The harvest procedure was performed according to standard protocols for bone marrow donation for allogenic transplantation in our hospital. For this purpose, 750 milliliter bone marrow was collected in flasks containing Hanks balanced salt solution and heparin from the posterior iliac crest under epidural or general anesthesia. The suspension was filtered and subsequently concentrated on the COBE Spectra Apheresis System (Gambro, Stockholm, Sweden) in a final volume of 40 mL in our GMP (Good Manufacturing Practice) facility. Upon concentration of the BMC-fraction, the erythrocyte fraction was collected separately and reinfused intravenously to the patient.

The mononuclear cells were injected approximately 4 h after bone marrow aspiration. The method of administration was randomly assigned to the patients using a random number table: either by local injection into the gastrocnemius muscle or by combined IA+IM delivery. The investigators were not blinded for the assignment. In case of total IM delivery, we implanted 1 ml using a 26-gauge needle on 40 sites, 1.5 cm deep, using the full surface of the gastrocnemius muscle. In patients assigned to the combined treatment arm, the volume of each IM injection was 0.5 ml. The remaining 20 ml was slowly infused after selective catheterization of the superficial femoral artery (or profunda femoral artery in case of occlusion of the SFA), performed according to the standard procedures within the department of radiology.

Assessment

Patients were evaluated at 1, 6 and 12 months after implantation. Clinical endpoints were limbsalvage or the pain-free walking distance in case of intermittent claudication (standardised treadmill test, 3 km/h, no incline). Full wound recovery and/or doubling of walking distance were defined as clinical response. Amputation was considered a failure of therapy. At 12 months, a yearly control visit was planned to check long-term effects.

Ankle-brachial pressure index (ABI) and the Brief Pain Inventory score (BPI-SF) were considered secondary follow-up parameters. The BPI-SF is a validated questionnaire to assess both severity of pain and the impact of pain on daily functions. [14,15] Eleven items were scored on a scale of 0–10.

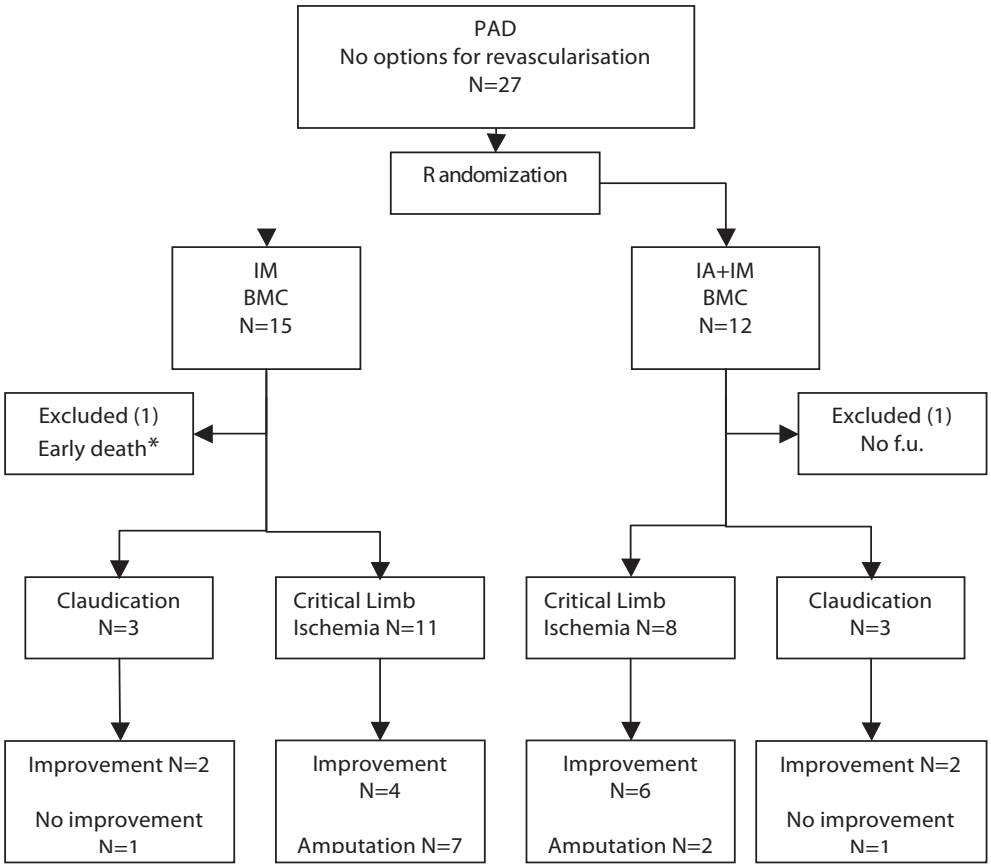
Collateral vessel formation was analysed by digital subtraction angiography (DSA, Seldinger technique) 1-2 weeks before and 6 month after the procedure. In both tests, catheter position, contrast and vasodilator doses (tolazoline) were similar. Four contrast phases of the proximal and distal part of the lower leg, as well as the foot, were recorded.

The angiographic scores for the formation of new collateral vessels were evaluated as +1 (new collateral development), 0 (no new collateral development), and -1 for angiographic progression of the disease. Two evaluating radiologists and 2 vascular surgeons were blinded for the pre and post procedural angiographies.
The absolute number of injected BMC and CD-34 positive cells was determined as well as the amount of colony forming units (CFU, qualitative assessment of the cells) to examine any effect of these parameters on the outcome.

Statistical evaluation

Data are expressed as mean ± SD. Changes in the variables from the baseline to 6 months after BMC implantation were studied using the analysis of variance with repeated measurements (ANOVA). Inter group comparisons were performed by independent t-test, Mann-Whitney test or Fisher's exact test when appropriate. Values of $P<0.05$ were considered significant. Tests were performed using SPSS 12.0.1 for windows (SPSSinc., Illinois, CA, USA).

Figure 1. *Enrolment and outcomes of study patients.*



* Non related death 2 months after the procedure.

Results

From June 2004 until March 2006, 27 patients were treated. Twenty one patients had critical limb ischemia (patients with ischemic rest pain or ischemic ulcers / gangrene), while 6 patients had claudication. Patient baseline characteristics are listed in table I. Fifteen patients were assigned to exclusive IM injections; twelve others received half of the BMC dose intra-arterially and the other half directly into the calf.

The procedure was carried out successfully in all patients. The mean time needed for the bone marrow aspiration was 40±12 minutes. The mean numbers of implanted BMC and CD-34 positive cells were $1.23 \pm 0.49 \times 10^9$ and $3.07 \pm 2.02 \times 10^6$ respectively. Two patients suffered from post-operative cardiac failure due to fluid retention and were treated with diuretics with uneventful recovery. No local complications of the intramuscular and/or intra-arterial injections related complications were observed.

The mean follow-up was 24 ± 8 months. One patient died 2 months after the treatment due to pneumonia at the age of 77 years. One other patient developed severe co-existing conditions that interfered with the ability of the patient to comply with the follow-up protocol (no limb-loss). These patients could not be evaluated and were therefore excluded from further analyses. Therefore, 25 remaining patients could be analysed (figure I). Two other patients died during follow-up; a 86 year old male, 6 months after IM treatment, due to deteriorating general condition after respiratory infection and a 67 year old male, 1 year after IM treatment, due to myocardial infarction. Further, 3 other patients suffered from major events. The first patient of the study was diagnosed with locally advanced colon carcinoma 41 months after treatment. Patient 5 underwent laparotomy because of a perforated gastric ulcer 3 months after treatment. He had taken analgetics (NSAIDs) for a longer period. Patient 10 was diagnosed with larynx cancer after 7 months and recently non-small cell lung carcinoma after 25 months. Patient 13 had to undergo surgical removal of an earlier occluded PTFE femoropopliteal bypass due to chronic low-grade infection, several months after lower leg amputation.

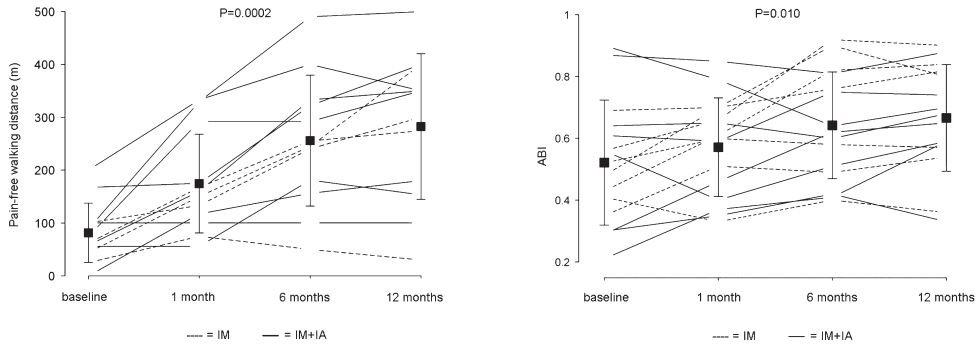
Within 3 months after the BMC treatment, 9 of the 25 patients required major amputation (8 lower legs, 1 upper leg) and could therefore not be analyzed at 6 months. Baseline ABI values in the amputated patients varied significantly from the non-amputated patients: 0.29 ± 0.13 versus 0.52 ± 0.20 (P=0.02), indicating more advanced disease. ABI immediately prior to amputation was unchanged as compared with ABI before BMC injection. In the group receiving IAM+IM treatment, 2 of the 8 patients who suffered from CLI had to undergo amputation as compared to 7 of the 11 patients with CLI in the exclusively IM treated patients. (Fischer's exact test for a difference between the modes of delivery P=0.17)

Table I. *Baseline patient characteristics.*

Characteristics	IA+IM (n= 12	IM (n=15)
Age (yr)	66.9 (SD 16.3)	69.8 (SD12.4)
Male sex	9	10
History of smoking	10	13
Hypercholesterolemia	4	4
Diabetes mellitus	3	5
Hypertension	5	7
Myocardial infarction	2	4
Claudication	3	3
Ischemic rest pain	2	3
Tissue loss	7	9
Bilateral disease	10	12

In the 16 non-amputated patients, BMC administration substantially improved pain-free walking distance from 81 ± 56 meters at baseline to 257 ± 126 and 282 ± 139 meters after 6 and 12 months respectively (P=0.0002; figure II). There appeared to be no difference between the two delivery methods (P=0.85). In all patients treatment resulted in full wound closure in case of ischemic ulcers. Before treatment pain scores in those who were later amputated and those who were not, were similar. However, pain scores improved significantly in the latter group, particularly in the first 6 months after treatment (table II).

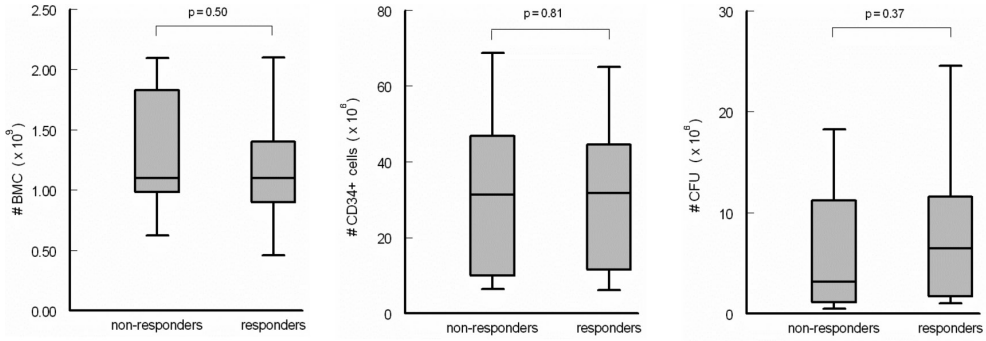
Figure 2. *Change in pain-free walking distance and ankle-brachial pressure index (ABI).*



Results are shown as mean ± SD. The p-values refer to the analysis of variance with repeated measurements (ANOVA).). In 1 patient with diabetes mellitus, no ABI could be measured due to non-compliance of the lower crural vessels. Also, 4 patients with painful foot ulcers were unable to undergo baseline treadmill testing. There was no difference between the two delivery methods (pain-free walking distance: P=0.85, ABI: P=0.24).

Ankle-brachial pressure index increased from pre-treatment 0.52 ± 0.20 to 0.64 ± 0.17 after 6 months and to 0.66 ± 0.17 after 1 year follow-up (P=0.01; figure II). In all these patients, the ABI of the contra lateral (non-treated) limb remained unchanged: 0.80 ± 0.25 at baseline, 0.77 ± 0.26 at 1 year (P=0.26). Again, no difference was found between the IA+IM group and the IM group (P=0.24). Rather high ABI values at baseline in two cases could be explained by diminished vessel compliance due to diabetes. Consequently, PAD was more severe in these patients than suggested by their ABI.

Figure 3. *Relationship between number of implanted cells and clinical response.*



The absolute number of injected BMC and CD-34 positive cells, as well as the amount of colony forming units (CFU) in relation to the outcome (Mann-Whitney test).

The results of the follow-up DSA's were not consistent. An increase in collateral vessels was scored in 7 patients. In 4 patients no difference was found and in 4 patients angiographic deterioration was observed. In one case the DSA's could not be properly compared. No association with the clinical outcome was observed.
We sought for a possible relationship between BMC and outcome. No association was found between the number of injected cells and clinical response. Furthermore, no difference was found in the number of colony forming units between the responding and non-responding (amputated) patients (figure III).

Discussion

This study confirms that transplantation of BMC in patients with severe symptoms of PAD is feasible and safe. Furthermore it appears that this approach is associated with a clinically relevant and persistent response in a considerable number of patients.
Aiming at safety and feasibility, the study was not primarily designed to demonstrate a

presumed difference between IA+IM and IM administration. However, the trend towards a lower amputation rate in the combined treatment group (p=0.17) is remarkable and warrants evaluation in a sufficiently powered study.

Stimulation of revascularization in ischemic zones would be a very attractive therapeutic strategy for these patients provided that adverse effects are acceptable. Important is that these were absent in this study. Improving collateral circulation is since many years a cornerstone of conservative treatment. So far, walking has been the only successful strategy.[16] The concept of BMC administration originated from the hypothesis that supply of a cocktail of cytokines, growth factors and cells to ischemic tissue could be a more physiological and effective approach of achieving an arteriogenic effect. Although the feasibility of this approach has been shown in several animal models and preliminary clinical studies, [4-9] various questions concerning optimal delivery mode, safety, [10] and durability[11] remain unanswered. We reasoned that exclusive intramuscular injection in the calf might be not the most optimal mode of delivery. Most patients with advanced PAD present with multi-level disease including extensive pathology of the femoropopliteal tract and pedal arteries. These zones are in theory not addressed by the intramuscular calf injections but may be reached by selective IA administration. The concept of intra-arterial (coronary) delivery of cells BMC accomplished positive and encouraging results of cardiac function after myocardial infarction.[17,18] BMC biodistribution studies after IA (coronary artery) transfer of BMC however, show limited BMC retainment in the infarcted myocardium.[19] Consequently, we hypothesized that IM implantation with simultaneous IA delivery of the cells combines the advantages of both delivery strategies.

In view of the safety concerns, the primary goal of our open phase I/II study was to assess the safety and feasibility comparing both approaches. Adverse events were observed (two patients developed transient congestive heart failure following bone marrow aspiration), but these were not related to the IM delivery method or the combined IA+IM procedure. Mean leukocyte concentration in the injected 40 ml cell concentrates was 80.4 ± 35.1 x 10^9 /L and therefore embolic complications due to leucostasis are conceivable, yet no clinical signs of leucostasis were observed.[20-23] Follow-up indicated significant morbidity and mortality in the patients' group, yet these complications appear unrelated to the BMC treatment but rather reflected the poor health status and reduced life expectancy of patients with advanced PAD.

With more mechanistic clues, less bone marrow might be required obviating the need for general or epidural anesthesia. If bone marrow aspiration would be feasible under local anesthesia, adverse events like heart failure would be prevented.

It is encouraging that this study confirms that in a substantial number of no-option patients, clinical parameters as pain-free walking distance, pain scores and ABI improved significantly without any other specific therapy. Although it can be argued that these improvements reflect an intrinsic study effect, no signs of improvement were observed in

the contra-lateral leg in which ABI appeared to be unchanged. The success rate of almost 60% is lower than the findings of the original TACT report,[4] however it is comparable to the results of a larger series of patients treated by the TACT group.[24] BMC-treatment appears to have a similar effect in other diseases than PAD due to atherosclerosis. In a recent article Durdu et. al specifically studied patients with thromboangiitis obliterans. None of the total of 26 patients in this study had to undergo a major amputation after a mean follow-up time of 16.6 months. Total healing of the most important lesion occurred in 15 out of 18 patients with ischemic ulcers after IM injections with BMC.[9] These apparently even better results may be explained by a different underlying disease and/or an impaired age-related bone marrow cell activity (mean age of the patients 42.6 versus 68.5 years in our study).[25]

Observations of Matsui and colleagues challenge the durability of the approach: in spite of favorable short term outcome, a return to the baseline of several clinical parameters in 16 patients occurred after one year of follow-up.[11] Our results for the responding patients on the other hand show a persistent functional improvement (pain free walking distance, ABI and pain inventory) beyond the 12 months follow-up. A time course of several weeks to improvement is suggested in our study with a maximal and steady effect occurring after 3-6 months.

In 11 of our patients symptoms did not improve or worsened, leading to amputation in 9 cases. It remains unclear why some patients favorably responded to this cell therapy whilst others showed no improvement at all. Certainly, many patients were frequently referred to us in an end-stage of their disease. An obvious explanation would be that biological differences among patients or stage of disease might well be decisive in determining the therapeutic response. Immunological differences might be of considerable importance in collateral artery development as our group recently reported in mouse models.[26] We found no relation between the total number or CD-34 positive transplanted cells and the efficacy of BMC (figure III). Saigawa and co-workers earlier suggested a strong correlation between the number of CD-34 positive cells and the change in ABI.[7] Furthermore, in line with our results, no association was observed between the total amount of implanted BMC and ABI-change.

In the present study clinical improvement was not associated with unequivocal improved collateral circulation on angiography. Only in half of the patients with a clear clinical improvement, enhanced angiographic collateral vessel formation was demonstrated. Therefore, it seems likely that either undersized formed collaterals could not be visualized by angiography or improvement of symptoms is not primarily caused by collateral circulation. Nonetheless, other mechanisms may play a role.

Table II. *Brief Pain Inventory (BPI).*

Scales	Items	Mean 0 mth		Mean 1 mth		Mean 6 mth		Mean 12 mth	
Pain intensity	Worst pain	6.5	(SD 2.1)	5.4	(SD 2.6)	2.3	(SD 2.4)	2.4	(SD 1.8)
	Least pain	2.5	(SD 2.4)	0.9	(SD 1.3)	0.4	(SD 0.9)	0.6	(SD 1.4)
	Average pain	4.8	(SD 1.5)	3.4	(SD 1.7)	1.9	(SD 1.8)	1.6	(SD 1.7)
	Pain right now	3.2	(SD 2.2)	1.7	(SD 1.8)	0.8	(SD 1.4)	0.9	(SD 1.5)
Pain interference	General activity	6.3	(SD 2.4)	4.8	(SD 3.0)	2.2	(SD 2.8)	1.5	(SD 1.8)
	Mood	4.6	(SD 3.0)	3.1	(SD 3.0)	1.6	(SD 2.7)	0.7	(SD 1.6)
	Walking ability	7.1	(SD 2.1)	5.7	(SD 2.8)	4.4	(SD 2.4)	2.8	(SD 2.8)
	Normal work	6.8	(SD 2.7)	5.1	(SD 2.9)	4.1	(SD 3.3)	2.1	(SD 2.6)
	Relationship	3.3	(SD 3.6)	2.1	(SD 2.6)	0.9	(SD 1.8)	0.4	(SD 0.8)
	Sleep	4.6	(SD 3.6)	3.1	(SD 3.3)	1.0	(SD 1.9)	1.0	(SD 2.1)
	Enjoyment of life	4.9	(SD 3.0)	2.8	(SD 2.9)	1.6	(SD 2.6)	0.9	(SD 1.7)
Total		54.7	(SD 19.0)	38.8	(SD 21.8)	24.4	(SD 22.5)	20.9	(SD 23.1)
Amputated patients (n=9)		51.7	(SD 23.2)						

The change in BPI score (P=0.001). The improvement did not vary between the IA+IM and the IM group (P=0.65). There was no significant difference in baseline score between the amputated- and non-amputated patients (T-test P=0.75). Lower scores reflect less impairment.

Conclusions

The findings of this study show that BMC treatment is safe and feasible. Significant improvement was obtained in 14/25 of the patients and we observed a trend towards a lower amputation rate after IA+IM administration of bone marrow mononuclear cells. Although promising, the preliminary results of the present study require confirmation in a placebo controlled study.

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