Comparison of bone marrow mesenchymal stem cells with bone marrow-derived mononuclear cells for treatment of diabetic critical limb ischemia and foot ulcer: A double-blind, randomized, controlled trial

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Aims: To identify better cells for the treatment of diabetic critical limb ischemia (CLI) and foot ulcer in a pilot trial.

Methods: Under ordinary treatment, the limbs of 41 type 2 diabetic patients with bilateral CLI and foot ulcer were injected intramuscularly with bone marrow mesenchymal stem cells (BMMSCs), bone marrow-derived mononuclear cells (BMMNCs), or normal saline (NS).

Results: The ulcer healing rate of the BMMSC group was significantly higher than that of BMMNCs at 6 weeks after injection ($P = 0.022$), and reached 100% 4 weeks earlier than BMMNC group. After 24 weeks of follow-up, the improvements in limb perfusion induced by the BMMSCs transplantation were more significant than those by BMMNCs in terms of painless walking time ($P = 0.040$), ankle-brachial index (ABI) ($P = 0.017$), transcutaneous oxygen pressure (TcO2) ($P = 0.001$), and magnetic resonance angiography (MRA) analysis ($P = 0.018$). There was no significant difference between the groups in terms of pain relief and amputation and there was no serious adverse events related to both cell injections.

Conclusions: BMMSCs therapy may be better tolerated and more effective than BMMNCs for increasing lower limb perfusion and promoting foot ulcer healing in diabetic patients with CLI.

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1. **Introduction**

Although billions have been spent for controlling the blood glucose levels of diabetics [1], one in four diabetics still develop peripheral arterial disease (PAD) [2]. In more severe diseases, critical limb ischemia (CLI) may develop, which is more likely to result in incurable ulceration, gangrene, and even limb loss than in non-diabetic patients [3,4]. Diabetics with PAD have a fivefold higher rate of amputation and threefold higher mortality rate than non-diabetic patients with PAD over a
Due to diffuse vascular disease, the distal location of obstructions, and the presence of multiple comorbidities in diabetics, a significant portion of the patients are not amenable to revascularization by surgical bypass, endovascular stenting, or balloon dilatation [6–8]. Consequently, there is an increasing body of research on the use of autologous stem cell administration for treating diabetic CLI and foot ulcers [7].

In many preclinical studies, the therapeutic angiogenesis of bone marrow mononuclear cells (BMMNCs) and bone marrow mesenchymal stem cells (BMMSCs) had been widely proven [8–15]. Some animal experiments have shown that transplanted BMMSCs, which can be easily isolated and rapidly expanded in vitro through many generations while retaining the capacity to differentiate [16], could induce more benefits than BMMNCs for the treatment of CLI, as well as brain or myocardial infarction [10,17,18]. In diabetes, basic studies have shown that BMMSCs could not only relieve hind limb ischemia [19] but also accelerate wound healing [20]. Although the neovascular potential of both BMMNCs and BMMSCs from diabetic rats was impaired [21,22], one clinical trial has shown that applied BMMNCs could restore angiogenesis and promote wound healing in diabetic patients [23]. However, few reports have shown the safety and efficacy of ex vivo expanded BMMSCs, or have compared BMMSCs with BMMNCs in the treatment of diabetic CLI and foot ulcers. This double-blind, randomized, placebo-controlled exploratory trial was conducted to evaluate and compare the safety and efficacy of autologous BMMSC transplantation with BMMNCs for treating diabetic CLI and foot ulcers.

### Table 1 – Inclusion and exclusion criteria of the trial.

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<tr>
<th>Inclusion criteria</th>
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<td>Age from 40 to 70 years</td>
<td>Dry gangrene above the ankle or moist gangrene</td>
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<td>Type 2 diabetic patients</td>
<td>Malignant tumor</td>
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<td>Bilateral critical limb ischemia</td>
<td>Severe coronary, cerebral and renal vascular disease</td>
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<td>(ABI from 0.30 to 0.60)</td>
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<td>At least with one foot ulcer</td>
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![Fig. 1 – Flow chart of this study. ABI, ankle-brachial pressure index; MRA, magnetic resonance angiography; TcO2, transcutaneous oxygen pressure; BMMSCs, bone marrow mesenchymal stem cells; BMMNCs, bone marrow-derived mononuclear cells; NS, normal saline.](image-url)
1.1 Subjects

This clinical study was approved by the Ethical Committee Board of the Third Military Medical University Southwest Hospital and complied with the recommendations of the Declaration of Helsinki. Written informed consent was obtained from all subjects. Patients were screened for eligibility according to the criteria listed in Table 1. Eligible patients were admitted to our hospital from October 2009 to January 2010 and participated in this trial on a voluntary basis. The subjects were randomized into groups A and B using a randomization table. In addition, using another randomization table, one lower limb in group A or B was selected randomly for BMMSC or BMMNC transplantation, which were designated as the BMMSC and BMMNC groups; the other lower limb in the same patient was selected for placebo (NS) injection (NS group). The procedures of this clinical trial are presented (Fig. 1) and the patients and investigators were blinded to the treatments.

2. Materials and methods

2.1 Procedures

The primary outcome of the study was safety and feasibility of treatment, defined as improvements in rest pain, pain-free walking time, ulcer healing rate, limb salvage rate, ABI and TcO2 and enhancement of vessel formation as judged by the MRA. All patients received the same ordinary treatment for the duration of this trial (from participation to the end of follow-up), including control of blood glucose, blood pressure, and blood lipids, debridement to remove extensive callus and necrotic tissue, pressure-relief after wound dressing, and application of antibiotics. Smoking cessation was encouraged during the trial. The patients assigned to the NS, BMMSC, and BMMNC groups received injection with NS or the corresponding BMMSCs or BMMNCs.

2.2 Preparation of BMMSCs

The preparation, expansion, and characterization of BMMSCs were performed via following previously published procedures with slight modification [24]. Under strict aseptic conditions and local anesthesia, bone marrow (30 mL) was obtained from the iliac crest of each patient in group A. The obtained bone marrow was transferred to a clean room (built in compliance with the standard of ISO 14644) and subjected to Percoll (Pharmacia, 1.073 g/mL) density gradient centrifugation. Then, the mononuclear cell layer was harvested and cultured in the flasks containing alpha-modified minimum essential medium (α-MEM; Invitrogen-Life Technologies Corp., Carlsbad, CA, USA) supplemented with 10% autologous serum. [From each patient in group A, approximately 20 mL of whole blood was quickly transferred into 10 mL plain vacutainer tubes (BD, Plymouth, UK) and allowed to clot for 4 h at 4–8 °C. Then, the blood was centrifugated at 1800 × g at 4 °C for 15 min, and the serum was collected and filtered through a 0.2 μm membrane (Sarstedt, Nümbrecht, Germany). The sterile autologous serum was stored at −20 °C. This procedure was done once a day for 10 days.] On day 1, non-adherent cells were discarded, and adherent cells were washed with phosphate-buffered saline (Gibco) and then cultured in α-MEM with 10% autologous serum. The medium was replaced every 2–3 days. Upon approximately 70–80% confluence, the cells were resuspended in trypsin–EDTA and the cell density was adjusted to approximately 4000 cells per cm². The cells were passaged once every 4–5 days. When the targeted numbers of expanded BMMSCs were attained, cells were thoroughly washed and resuspended in NS.

2.3 Preparation of BMMNCs

After 18 days of the ordinary treatment, bone marrow (~300 mL) from the patients in group B were aspirated from the ilium under epidural anesthesia and gathered into plastic bags containing heparin. The bone marrow aspirate was processed by density gradient centrifugation as previously indicated and the resulting fraction of BMMNCs was suspended in NS.

2.4 Microbiological tests

Aliquots of the cells from each patient were taken for: bacteria and eumycete culture and mycoplasma polymerase chain
reaction test using a kit (LookOut Mycoplasma PCR Detection Kit, Sigma). A 2 μL sample was mixed with 23 μL DNA polymerase/rehydration buffer and reacted under the following cycler program: 1 cycle 94 °C for 2 min, 40 cycles 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s. Then, 1.2% standard agarose gel with 5 mm comb was used, and 8 mL were loaded into each PCR into a separate lane. After a migration of 2.5 cm, electrophoresis was stopped, and photos were taken through a computer. At the same time, the following tests were performed: gram staining and dynamic turbidimetric limulus testing for endotoxin content.

2.5. Flow cytometry of BMMSCs

For flow cytometry of the surface molecule expression of BMMSCs, the following monoclonal antibodies (Mabs) directly conjugated with fluorochromes were used: CD29-PE, CD71-FITC, CD90-PE, CD105-FITC (Biolegend), CD34-PE, and CD45-FITC (SBA) (Becton Dickinson, USA). Irrelevant control Mabs were included for all fluorochromes. Cells were directly coated with the conjugated Mabs at room temperature for 15 min, washed, and fixed in 1% paraformaldehyde. Then, the cells were analyzed using a FACS Calibur flow cytometer (Becton, Dickinson and Company, San Jose, CA).

2.6. Measurements of angiogenic factors from BMMSCs and BMMNCs in vitro

To compare the secretion of angiogenic factors from the BMMSCs with that from BMMNCs, the BMMSCs and BMMNCs (2 × 10^6 cells) were plated onto 10 cm dishes in complete culture medium [a-MEM supplemented with 10% autologous serum (the autologous serum of each patient in group B was obtained as previously described)] and incubated under normoxic (21% O2 and 5% CO2) or hypoxic conditions (1% O2 and 5% CO2). After 36 h of incubation, the conditioned medium was collected and levels of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and angiopoietin-1 were measured using enzyme immunoassay kits (Quantikine, R&D Systems).

2.7. Release criteria and transplantation of the cell product

The cell suspensions in both groups were filtered through a cell strainer (BD Falcon, nylon, 100 μm). Aliquots of the cells were transferred into storage vials for quality testing. Release criteria for cell transplantation was based on the following: (1) negative results of the microbiological tests, (2) endotoxin

Fig. 3 – Immunophenotype analysis of human bone marrow mesenchymal stems (BMMSCs). Human BMMSCs at passages 3 expressed identical cell surface antigens when analyzed by fluorescence-activated cell sorting. BMMSCs were positive for the cell surface markers CD29, CD71, CD90 and CD105 and negative for the markers CD45 and CD34. Data are expressed as a histogram plot, with white representing isotype control and gray representing experimental.
content of ≤5 EU/kg patient body weight, and (3) cell viability (trypan blue exclusion) of ≥95% [25].

Under strict aseptic conditions, 100 mg tramadol hydrochloride was injected intramuscularly to relieve pain. After 20 min, cells suspended in 20 mL NS [contained in 20 injectors (1 mL per injector), which were covered with tin foil sheet by one researcher and then transferred to another researcher to perform the transplantation] were injected intramuscularly into the lower limb (20 sites, 3 cm × 3 cm in intervals, 1–1.5 cm in depth, and 0.5–1 mL BMMSCs or BMMNCs per site). Cells of 2 mL were injected into the basilar part of each foot ulcer and the surrounding subcutaneous tissue. In a similar manner, the control limbs in NS group were injected with an equal volume of NS.

2.8. Clinical assessment after cell transplantation

Most clinical and laboratory data were prospectively collected, and follow-up visits were performed at 1 day before and 2, 4, 8, and 24 weeks after transplantation. Ulcer healing rate (i.e., number of patients whose ulcers healed/total number of patients with ulcers in a group) was calculated every week after transplantation until the end of this study or the cure of all ulcers. Rest pain scores on rating scales ranged from 0 for the best (completely resolved) to 4 points for the worst condition (severe pain unresolved with paracetamol or non-steroidal anti-inflammatory drugs) [26]. Painless walking time was assessed at a constant speed (2.5 km/h) with no inclination. Resting ABI and TcO2 were measured by a laser Doppler (Lisca Developments, Linkoping, Sweden) and a TCM400 (Radiometer Medical, Denmark) at 25°C, respectively. MRA analysis of lower limb blood vessels was performed at 1 day before and 24 weeks after transplantation by AVANTO 1.5 T magnetic resonance imaging scanners (Siemens Company, Germany). The scores of the blood vessel images for the formation of new collateral vessels were assessed as +0 (no collateral circulation), +1 (slight collateral circulation), +2 (moderate collateral circulation), and +3 (abundant collateral circulation) [26,27]. The number of amputation was counted at the end of the study, with a focus on any potential adverse effects resulting from the transplantation during follow-up.

2.9. Determination of sample size

Given that magnetic resonance angiography (MRA), a major noninvasive tool, could image the vascular tree of the lower limbs as accurately as digital subtraction angiography could [28], MRA analysis was used to estimate the sample size. With a possible standard deviation score of 1 (increment of new collateral circulation) in both treatment groups [26], a minimum of 32 evaluable patients (16 per group) were required to detect a possible score difference of 1 at \( P = 0.05 \) (2-tailed), with a power of 80%, between treatment groups.

2.10. Data and statistical analysis

Continuous variables were presented as mean ± SD. Paired-samples t-test was performed to determine the differences between before and after interference. A chi-square test was used to compare the rates and the likelihood ratio of the chi-square test for the differences among these groups. Statistical significance was assumed at a value of \( P < 0.05 \). All statistical analyses were performed using SPSS (Version 13.0 for Windows, SPSS, Chicago, IL).

3. Results

3.1. Characterization of the cell products for transplantation

Ex vivo expanded human BMMSCs were characterized by their ability to proliferate in culture with a fusiform shape (Fig. 2A), by the presence of the mesenchymal antigens CD29, CD71, CD90, and CD105 and negative/dim expression of the myeloid surface antigens CD45 and CD34 (Fig. 3). For all patients, the targeted number of ex vivo expanded BMMSCs was attained at 3 passages, equivalent to 18 ± 3 days after the primary bone marrow aspiration. In contrast, BMMNCs presented a variety of forms, which were mainly circular or semicircular, and showed a big difference in size (Fig. 2B). The targeted number of BMMNCs was attained immediately after density gradient centrifugation of the primary bone marrow. The adopted release criterion for cell transplantation was adequacy because no events of contamination were reported in all patients after cell transplantation.

![Fig. 4 – Angiogenic factor released from BMMSCs and BMMNCs. Protein levels of angiogenic factors were measured in the medium of the cell culture system under different experimental conditions. Human BMMSCs secreted significant higher levels of VEGF, bFGF and angiopoietin-1 than BMMNCs have done under normoxia as well as hypoxia condition. Data are expressed as mean ± SD (pg/10^6 cells, n = 12, *P < 0.001 BMMSCs vs. BMMNCs under normoxia condition; #P < 0.001 BMMSCs vs. BMMNCs under hypoxia condition). Compared with normoxia condition, hypoxia induced BMMSCs and BMMNCs secreted significant higher levels of all angiogenic factors (*P < 0.001 BMMSCs under normoxia condition vs. BMMSCs under hypoxia condition; #P < 0.001 BMMNCs under normoxia condition vs. BMMNCs under hypoxia condition), except angiopoietin-1 secreted by BMMSCs.](image)
3.2. Angiogenic factors released from BMMSCs and BMMNCs

Compared with BMMNCs, BMMSCs secreted significantly greater amounts of VEGF, bFGF, and angiopoietin-1 (Fig. 4). The amount of VEGF secretion from BMMSCs under normoxic and hypoxic conditions were 2.3-fold (140.2 ± 19.7 pg/10⁶ cells vs. 62.1 ± 16.3 pg/10⁶ cells, $P < 0.001$) and 4.2-fold (368.5 ± 39.9 pg/10⁶ cells vs. 87.9 ± 19.2 pg/10⁶ cells, $P < 0.001$) those of BMMNCs, respectively. The amount of bFGF secretion from BMMSCs under normoxic and hypoxic conditions were 2.0-fold (169.8 ± 18.3 pg/10⁶ cells vs. 85.7 ± 23.0 pg/10⁶ cells, $P < 0.001$) and 3.0-fold (335.7 ± 39.9 pg/10⁶ cells vs. 113.4 ± 21.5 pg/10⁶ cells, $P < 0.001$) those of BMMNCs, respectively. Although the increase in angiopoietin-1 released by BMMSCs from normoxic to hypoxic conditions was not significant (109.6 ± 10.9 pg/10⁶ cells vs. 113.9 ± 13.6 pg/10⁶ cells, $P = 0.305$), they were significantly higher than those

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<td>Female</td>
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<td>Age (years, mean ± SD)</td>
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<td>Body mass index (figure, mean ± SD)</td>
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<td>Waist/Hip ratio (figure, mean ± SD)</td>
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<td>Patients smoking at the time of recruited</td>
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<td>Patients using calcium antagonists</td>
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<td>Glycosylated hemoglobin (% mean ± SD)</td>
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<td>Rest pain in limbs injected with BMMSCs, BMMNCs or NS (score, mean ± SD)</td>
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<td>Pain-free walking time in limbs injected with BMMSCs, BMMNCs or NS (min, mean ± SD)</td>
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<td>Ulcer size in limbs injected with BMMSCs, BMMNCs or NS (cm², mean ± SD)</td>
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<td>Ulcer duration before inclusion (days, mean ± SD)</td>
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<td>ABI in limbs injected with BMMSCs, BMMNCs or NS (mean ± SD)</td>
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<td>TcO₂ in limbs injected with BMMSCs, BMMNCs or NS (mm Hg, mean ± SD)</td>
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<td>Angiographic score of MRA in limbs injected with BMMSCs, BMMNCs or NS (score, mean ± SD)</td>
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<td>Implanted cell number (10⁸, mean ± SD)</td>
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Notes: Data are number (%) unless otherwise indicated. BMMSCs: bone marrow-derived mesenchymal stem cells; BMMNCs: bone marrow-derived mononuclear cells; NS: normal saline; ABI: ankle-brachial index; TcO₂: transcutaneous oxygen pressure. All of patients are Fontaine grade IV. Six patients have two ulcers, which located at their two limbs, respectively. So the total number of ulcer is 43.
from BMMNCs under normoxic and hypoxic conditions (109.6 ± 10.9 pg/10^6 cells vs. 59 ± 7.7 pg/10^6 cells, and 113.9 ± 13.6 pg/10^6 cells vs. 91.6 ± 9.7 pg/10^6 cells, P < 0.001, respectively).

3.3. Characteristics of patients

Four of the 41 enrolled patients withdrew from this trial because three died from sudden cardiac death and one from severe pulmonary infection within 4 weeks after they were enrolled into the trial. No amputation and other outcomes occurred in these 4 patients. In groups A and B, the ischemic status, as assessed by ABI, TcO2, and MRA analyses, were well matched among the limbs implanted with BMMSCs, BMMNCs, or NS (Table 2). Ulcer status, as assessed by ulcer size and the stage of Fontaine classification, were also well matched (Table 2).

3.4. Procedural safety

After bone marrow aspiration, one and two patients bled at the iliac crest in groups A and B, respectively. No infection, bleeding, or other complications related to the microbiological condition of the cells were detected in all patients after transplantation, which was well tolerated with no or only mild discomfort. In three limbs in the BMMSC group and two in the BMMNC group, the patients experienced short-term episodes of slight pain 2 h after cell transplantation. No complications, such as rejection, allergic reactions, and tumorogenesis, were detected because of the intramuscular injections of the cell products.

3.5. Clinical efficacy of transplantation

At 24 weeks after transplantation, although rest pain in the limbs treated with both cells was significantly improved...
levels of ABI and TcO2 in the BMMSC group were significantly higher than those in the BMMNC group (ABI: 0.17 ± 0.06 vs. 0.12 ± 0.06, P = 0.017; TcO2: 24.4 ± 7.6 vs. 16.4 ± 6.4, P = 0.001) (Fig. 5C and D).

At 24 weeks after transplantation, the results of the MRA analysis showed that the number of notably increased collateral vessels (increased score ≥2) in the BMMSC group was significantly higher than that of the BMMNC group (15 of 18 vs. 8 of 19, P = 0.008). The increased score of MRA from baseline to 24 weeks in BMMSCs was significantly higher than that in the BMMNC group (1.9 ± 0.5 vs. 1.5 ± 0.6, P = 0.018). The increased scores in both the BMMSC and the BMMNC groups were markedly higher than the score in the NS group (Fig. 5E). Representative results of angiography are shown in Fig. 6.

Ulcer healing rate of the BMMSC group was always highest, and that of the BMMNC group was always average since 2 weeks after transplantation (Fig. 5F). The number of healing ulcers in the BMMSC group was significantly higher than that in the NS group (6 of 11 vs. 2 of 21, P = 0.006), which appeared earlier at 4 weeks after transplantation, whereas the number in the BMMNC group was significantly higher than that in the NS group, which appeared at 12 weeks (11 of 11 vs. 11 of 21, P = 0.001). Six weeks after transplantation, the number of healing ulcers in the BMMSC group was significantly higher than that in the BMMNC group (10 of 11 vs. 5 of 11, P = 0.022). The ulcer healing rate of the BMMSC group reached 100% 4 weeks earlier than the BMMNC group (8–12 weeks). Representative results of ulcer healing are shown in Fig. 7.

No lower limb amputation occurred in both cell transplanted groups, but 6 patients in the NS group had to undergo lower limb amputation (0 of 18 in the BMMSC group vs. 6 of 37 in the NS group, P = 0.024; 0 of 19 in the BMMNC group vs. 6 of 37 in the NS group, P = 0.021).

4. Discussion

BMMSC and BMMNC implantation effectively increased blood flow in all 37 appreciable limbs, as assessed by the substantial improvement in rest pain, pain-free walking time, ABI, TcO2, or by formation of new collateral vessels under MRA analysis. The increased blood flow may contribute to the improvement of ulcer healing and limb salvage in the BMMSC and BMMNC groups. BMMSC implantation was more effective than BMMNCs for the treatment of type 2 diabetic patients with CLI and foot ulcers. Safe use of BMMSC and BMMNC therapy was demonstrated by the absence of immediate or long-term clinical complications related to the cell infusion.

Since 2002, BMMNCs have been used for treatment of patients with CLI [26], but transplantation of BMMNCs requires at least a 3-h systemic/epidural anesthesia and aspiration of large amounts of marrow (300–500 mL) to prepare enough BMMNCs [26]. Many patients could not tolerate the BMMNC cytotherapy. In this trial, only 30 mL bone marrow under local anesthesia was needed to achieve the targeted number of BMMSCs and 300 mL bone marrow under epidural anesthesia to achieve the targeted number of BMMNCs. Patients are more likely to select the BMMSC cytotherapy.

There were no acute or chronic serious adverse events related to the BMMSC or BMMNC injection during the 24-week follow-up period. Similarly, many trials reported that BMMSC transplantation was safe in long-term follow-up [25,29–32]. Some trials even suggested that there were no serious adverse events related to the intravenous allogeneic BMMSC transplantation in patients [29,30,33]. In a recent study involving combination BMMSCs with EPC therapy for severe limb ischemia, no serious adverse events related to the cell injection were reported after the follow-up period (10 ± 2 months) [25]. The safety of BMMNCs for therapy of CLI also has been recognized [26,34].

Moreover, the infusion of BMMSCs was more effective than that of BMMNCs in increasing lower limb perfusion. The efficacy was suggested by both hemodynamic (ABI, TcO2) and clinical changes, such as pain-free walking time and ulcer healing rate. Furthermore, MRA data also presented encouraging modifications of blood flow in the limbs injected with BMMSCs. MRA scores before and after the infusion of the cells revealed the increased blood flow, probably mediated by collateral vessel development.

Fig. 6 – MRA analysis of collateral vessel formation in limbs injected with BMMSC, BMMNC and NS. A moderate increased collateral circulation in a limb of BMMNCs group from +0(A left) to +2(B left) appeared 24 weeks after implantation. An abundant increased collateral circulation in a limb of BMMSCs group from +0(C left) to +3(D left) appeared 24 weeks after implantation. By contrary, there was no collateral circulation (+0) in the limb of NS group before (A and C, right) and 24 weeks after (B and D, right) implantation.
There were no differences in terms of rest pain and limb salvage between the BMMSC and the BMMNC groups. However, the similar outcome of rest pain may be caused by the decreased sensitivity of pain with diabetic peripheral neuropathy. On the other hand, because cell treatment could decrease the incidence of amputations in these two groups, the incidence of amputations was largely lower than that of the other outcomes during the 24 weeks of follow-up. Thus, to detect a possible significant difference between the amputation rates of these two cell treatments, larger sample sizes and longer-term follow-ups are needed.

To explore the different possible mechanisms of therapeutic angiogenesis between the BMMSCs and BMMNCs, angiogenic factors, which promote blood vessel growth and maturation, released from both cells in vitro were detected. In addition, BMMSCs from diabetic patients were found to secrete more VEGF, FGF-2, and angiopoietin-1 than BMMNCs under normoxic and hypoxic conditions. A previous research also reported that rat BMMSCs release more VEGF and bFGF than BMMNCs in serum-free medium [10] and revealed that BMMSCs locally transplanted into the hind-limbs of rats improve regional blood flow during ischemia much better than transplanted BMMNCs, partly by differentiating into angioblasts such as vascular endothelial cells and vascular smooth muscle cells [10]. On the other hand, when BMMSCs are locally injected around the impaired tissue, they may promote ulcer healing through differentiation and angiogenesis [35,36]. These effects may partly contribute to the difference in the clinical outcomes between the BMMSC and the BMMNC groups.

Given that there was no limb amputation in both the BMMSC and the BMMNC groups, there are no histologic data in

Fig. 7 – Limb ulcer status after BMMSCs, BMMNCs and NS implantation in three patients. Non-healing ulcer on medial border of foot 1 day before (A) and 24 weeks after NS implantation (B). An ulcer and sinus (C) in a patient was cured 3 weeks after BMMNCs implantation (D). Two ulcers (E) in a patient healed up 2 weeks after BMMSCs implantation (F).
this trial. Thus, further studies are needed to reveal the different possible mechanisms of therapeutic angiogenesis between BM-MSCs and BM-MNCs in diabetic patients with CLI and foot ulcers.

In summary, the autologous transplantation of BM-MSCs may be as safe as BM-MNC therapy, with the former being more effective in increasing lower limb perfusion and promoting foot ulcer healing in diabetic CLI. To our knowledge, this is the first trial to explore the possible differences in safety, feasibility, and efficacy between BM-MSC and BM-MNC therapy for diabetic patients with CLI and foot ulcers.

Acknowledgements

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Conflict of interest

There are no potential conflicts of interest relevant to this article.

References


