Both autologous bone marrow mononuclear cell and peripheral blood progenitor cell therapies similarly improve ischaemia in patients with diabetic foot in comparison with control treatment

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Abstract

Background The aim of our study was to compare the effect of bone marrow mononuclear cell and peripheral blood progenitor cell therapies in patients with diabetic foot disease and critical limb ischaemia unresponsive to revascularization with conservative therapy.

Methods Twenty-eight patients with diabetic foot disease (17 treated by bone marrow cells and 11 by peripheral blood cell) were included into an active group and 22 patients into a control group without cell treatment. Transcutaneous oxygen pressure and rate of major amputation, as the main outcome measures, were compared between bone marrow cells, peripheral blood cell and control groups over 6 months; both cell therapy methods were also compared by the characteristics of cell suspensions. Possible adverse events were evaluated by changes of serum levels of angiogenic cytokines and retinal fundoscopic examination.

Results The transcutaneous oxygen pressure increased significantly ($p < 0.05$) compared with baseline in both active groups after 6 months, with no significant differences between bone marrow cells and peripheral blood cell groups; however, no change of transcutaneous oxygen pressure in the control group was observed. The rate of major amputation by 6 months was significantly lower in the active cell therapy group compared with that in the control group (11.1% vs. 50%, $p = 0.0032$), with no difference between bone marrow cells and peripheral blood cell. A number of injected CD34+ cells and serum levels of angiogenic cytokines after treatment did not significantly differ between bone marrow cells and peripheral blood cell.

Conclusions Our study showed a superior benefit of bone marrow cells and peripheral blood cell treatments of critical limb ischaemia in patients with diabetic foot disease when compared with conservative therapy. There was no difference between both cell therapy groups, and no patient demonstrated signs of systemic vasculogenesis. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords stem cell therapy; diabetic foot; critical limb ischaemia

Introduction

Peripheral arterial disease (PAD) is an important predictor of outcome of ulcer healing in patients with diabetic foot ulcers and often leads to major amputations [1]. According to the multicentre EURODIALE study, the rate of major amputation was significantly higher in patients with PAD in comparison with
those without PAD (8% vs 2%), and significantly (p < 0.001) worse ulcer healing rates were observed in patients with than in those without PAD (69% vs 84%, respectively) [2]. Up to one third of patients with PAD are not suitable for revascularization by percutaneous transluminal angioplasty or bypass and could achieve therapeutic benefit from autologous stem cell therapy as an adjunctive technique in the management of PAD [3–6]. There are two main sources of vascular precursors eligible for limb revascularization – bone marrow-derived mononuclear cells (BMMNC) and peripheral blood progenitor cells (PBPC) separated from peripheral blood after previous stimulation by the granulocyte colony-stimulating factor (G-CSF).

Little is known about the exact mechanism of action of injected stem cells at tissue level. Capillary growth, a sprouting of endothelial tubes, is stimulated by local hypoxia and is not sufficient to offset tissue ischaemia; arteriogenesis is a process stimulated by increased shear stress in pre-existing collateral arterioles [7,8]. Endothelial progenitor cells (EPC), a fraction of precursor cells responsible for therapeutic angiogenesis, are characterized mainly by CD34+ and CD133+ markers [9]. It is still unclear if stem cells are directly involved in the angiogenesis process or act as cytokine factories with paracrine effect [10]. Fadini et al. showed that PAD is associated with an extensively low number of EPC [11], and they also observed that type 2 diabetes is associated with a significant reduction in total circulating plasmatic CD34+ cells [12].

Tissue ischaemia is believed to trigger the mobilization of EPC from bone marrow to peripheral blood through the release of angiogenic cytokines, which strongly influence angiogenesis [13]. The most important of the angiogenic cytokines are the vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF), whose production is also regulated by comprehensive metabolic processes, for example, interferon-gamma and interleukin-10 [14].

Several meta-analyses concluded that stem cell therapy is effective in treatment of ischaemia, subjective symptoms, wound healing and decrease of amputation rate [7,13,15] and also more efficient than therapy with isolated G-CSF [16]. Little is known about the comparisons of the degree of positive effects between different precursor cell types and doses [7]. It is not clear which subsets of stem cell therapy are optimal for PAD treatment in diabetic foot disease (DFD). Potential disadvantages include concern over off-target angiogenesis that could result in progression of proliferative retinopathy or affect tumour growth. These potential side effects depend on incorporation of stem cells in different tissues and also on systemic release of angiogenic cytokines [3,17,18]. A direct sign of systemic vasculogenesis could be vascular changes in the retina [19]. The aim of our study is to compare the effect of treatments with BMMNC and PBPC in patients with DFD and persistent ischaemia unresponsive to revascularization with conservative therapy.

Research design and methods

Twenty-eight patients treated by cell therapy (17 in the BMMNC group and 11 in the PBPC group) and 22 patients treated conservatively at our foot clinic between January 2008 and March 2012 were consecutively recruited into an active or control group in this nonrandomized, single-site, controlled comparative study and followed up for 6 months. Both groups were treated by standardized methods in our foot clinic – those needing pressure offloading received therapeutic shoes, orthotics or a total contact cast, as indicated; for infected ulcers, we prescribed appropriate antibiotic therapy, and in case of emergency, the admission was immediately provided.

Inclusion criteria for both groups were identical: DFD and chronic critical limb ischaemia (CLI) defined as PEDIS stage 3 with transcutaneous oxygen pressure (TCPO2) < 30 mmHg or ankle brachial index (ABI) < 0.6 [20], Rutherford category 4–6 [21] and very severe angiographic finding not eligible for revascularization. All patients fulfilled Trans-Atlantic Inter-Society Consensus II criteria for chronic CLI, which should be used for all patients with chronic ischaemic rest pain, ulcers or gangrene attributable to objectively proven arterial occlusive disease [22]. Patients in the control group underwent examination according to protocol but could not be treated by the cell therapy group because of transient changes of local and European medicine agency recommendations during the inclusion period. During our study, the European Medical Agency assessed the role of treatment with transplantation of human tissues and cells or advanced therapy medical products, which influenced the possibility of cell treatment in our centre and resulted in its interruption for 11 months. Therefore, during this period, patients who were eligible for the treatment with cell therapy could not receive the treatment and formed the control group. However, there was no difference in patient characteristics between control and treatment groups (Table 1).

Exclusion criteria were severe limb oedema, severe haematologic abnormalities (high leucocytes or platelets and haematono-ontological diseases), deep vein thrombosis in the last 6 months, progressive retinopathy or diagnosed neoplasm of any organ. The study was performed in accordance to the declaration of Helsinki (World Medical Association, 2008) and was approved by the local ethics committee, and all patients gave written informed consent.

The protocol of the study included detailed oncological, haematological and angiiological screening. Consecutive patients from the active cell therapy group were allocated alternately for BMMNC and PBPC therapies with an effort to introduce both methods.

Major amputations in patients in the active cell therapy group in our study were performed for progression of foot infection, despite improvement in ischaemia. All these patients were ineligible for standard revascularization – they were discussed at the multidisciplinary meeting including experienced interventional radiologists and vascular surgeons, and no intervention was indicated because of very distal arterial occlusion, unsuccessful
previous percutaneous transluminal angioplasty or bypass and multi-organ disease (renal and cardiac insufficiency). An assessment of the study was carried out per protocol in patients who completed 6 months of follow-up (Figure 1).

The effect of treatment of ischaemia was assessed by TcPO2 measurement. The ABI was not used because in 74.1% of patients, this index was unreliable because of the influence of mediocalcinosi (index >1.2). The TcPO2 measurement was performed by the standardized method on a TCM400 monitor (Radiometer Medical ApS, Denmark) and assessed by the same foot care specialist.

Other endpoints were the rate of major amputation, number of fully healed ulcers, qualitative marker of applied cell suspensions (assessed by CD34 count) and possible systemic vasculogenesis after cell therapy (assessed by serum levels of VEGF and b-FGF and fundus examination). Angiogenic cytokines were assessed after 1 day, 1 week and 1, 3 and 6 months following stem cell treatment in both groups; fundoscopic examination of both eyes was blindly assessed by an experienced ophthalmologist before and 6 months after stem cell treatment.

There was no difference between both active groups and control group in basic demographic characteristics and also in objective evidence of CLI – baseline values of TcPO2 and clinical staging system for describing PAD by Rutherford category (Table 1). Angiographic findings were assessed in accordance with Graziani classification (morphological categorization mainly focused on infrapopliteal lesions applicable for diabetic patients with CLI) [23]. DFD was characterized as active foot ulcers in 25/28 (89%) patients in the active cell therapy group and 20/22 (90.2%) patients in the control group or as history of previous ipsilateral minor amputation (two patients in the active group and one patient in the control group), after contralateral major amputation (one patient in the active group) and history of ipsilateral foot ulcers (one patient in the control group). Foot ulcers were characterized by stage 2C–2D in accordance with the Texas classification [25], mean size of 5.4±1.5 and 5.5±1.7 and 5.9±2 cm² (BMMNC versus PBPC versus control group, respectively), with no signs of severe infection according to the International Consensus on the Diabetic Foot 2011 [25]. Healed ulcer was defined as fully epithelized wound that remained closed for at least 6 weeks. There was no difference in the presence of other co-morbidities – ischaemic heart disease, end-stage renal

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BMMNC group (n = 17)</th>
<th>PBPC group (n = 11)</th>
<th>Control group (n = 22)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.7 ± 9.4</td>
<td>63.4 ± 10.4</td>
<td>63.3 ± 9.1</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (% men)</td>
<td>88.2</td>
<td>81.8</td>
<td>77.3</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>23.1 ± 15.2</td>
<td>21.5 ± 9.4</td>
<td>19.8 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (DCCT %)</td>
<td>7.2 ± 1.1</td>
<td>7.7 ± 1.1</td>
<td>7.7 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Ischaemic heart disease (%)</td>
<td>56.3</td>
<td>45</td>
<td>59</td>
<td>NS</td>
</tr>
<tr>
<td>Chronic renal failure dialysis (%)</td>
<td>6.3</td>
<td>9</td>
<td>13.6</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>81.2</td>
<td>91</td>
<td>81.8</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking</td>
<td>63.5</td>
<td>64</td>
<td>68.2</td>
<td>NS</td>
</tr>
<tr>
<td>TcPO2 at baseline (mmHg)</td>
<td>16.3 ± 11</td>
<td>16.4 ± 9.8</td>
<td>14.6 ± 9.6</td>
<td>NS</td>
</tr>
<tr>
<td>Rutherford category (mean ± SD)</td>
<td>4.88 ± 0.33</td>
<td>4.91 ± 0.30</td>
<td>4.91 ± 0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Angiographic findings – Graziani (mean ± SD)</td>
<td>4.3 ± 1.2</td>
<td>4.9 ± 1.6</td>
<td>5.1 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Area of defect before therapy (cm²)</td>
<td>5.2 ± 1.6</td>
<td>5.5 ± 1.7</td>
<td>5.9 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>TEXAS classification</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C–3C (%)</td>
<td>66.7</td>
<td>70</td>
<td>72.2</td>
<td>NS</td>
</tr>
<tr>
<td>2D–3D (%)</td>
<td>33.3</td>
<td>30</td>
<td>27.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMMNC, bone marrow mononuclear cells; DCCT, Diabetes Control and Complications Trial; NS, not significant; PBPC, peripheral blood progenitor cells; SD, standard deviation; TcPO2, transcutaneous oxygen pressure.

Figure 1. Scheme of the study. BMMNC, bone marrow mononuclear cells; PBPC, peripheral blood progenitor cells.

disease and hypertension (Table 1). All patients were diagnosed with severe peripheral neuropathy (vibration perception threshold > 35 V).

Bone marrow was harvested from the iliac crest of both sides by the Jamshidi technique [standardized procedure of bone marrow (trephine biopsy) with a Jamshidi needle] in the operating theatre, and BMMNC were separated by using a Smart PReP2 (Harvest Technologies Corporation) or sedimented using succinate gelatine (Gelofusine) [26]. PBPC were separated by leukapheresis on Haemonetics MCS+ using 16–20 cycles for achievement of minimal concentration of CD34+ cells in peripheral blood 2 × 10^7/mL after previous 3–6 days of stimulation by 5–8 μg/kg/day of G-CSF. The main criterion for the number of cycles was the value of the first flow, which was influenced by patients’ parameters (haematocrit and weight). A final cell suspension of 40–90 mL gained by both methods was injected into the calf, dorsal and plantar muscles of the affected lower limb (deep injection close to the obstructed artery trunks) in a series of about 40–50 punctures of 1–2 mL each.

All data were expressed as mean ± standard deviation. The statistical significance was analysed using Kruskal–Wallis analysis and Wilcoxon pair test with a level of p < 0.05 being considered statistically significant. The Holm–Sidak method was used for multiple comparisons.

**Results**

Baseline characteristics of the study group are shown in Table 1. There was no difference in age, duration of diabetes, microvascular and macrovascular complications, baseline TcPO_2, staging for PAD and ulcer size and grade.

Of the 28 patients recruited to the active cell therapy group, one patient died and 27 patients finished the 6-month follow-up (three underwent major amputation). Of the 22 patients recruited to the control group, two patients died and 20 patients completed the 6-month follow-up (ten patients underwent major amputation). The rate of major amputation till 6 months was significantly lower in the active cell therapy group compared with that in the control group [3/27 (11.1%) vs 10/20 (50%), p = 0.009]. Two patients in the BMMNC group and one patient in the PBPC group underwent a major amputation (3–5 months after treatment), performed for progression of foot infection. In contrast, ten major amputations (50%) were required in the control group (2–5 months after inclusion); indications for amputations were progressive ischaemic necrosis, severe rest pain or sepsis. The number of healed ulcers in 6 months was significantly higher in the active cell therapy group (14/25, 56%) in comparison with that in the control group [14/25 (56%) vs 3/18 (16.7%), p = 0.0093].

The TcPO_2 at baseline in nonamputated patients in the control group was not significantly different from that in the BMMNC and PBPC groups (19.5 ± 8.0, 16.6 ± 11.5, 15.9 ± 10.2 mmHg, respectively). TcPO_2 increased significantly 4 weeks after treatment in nonamputated patients in both active groups (16 in BMMNC and 11 in PBPC), and this increase persisted till 6 months, with no difference between BMMNC and PBPC therapies (Figure 2); Holm’s correction of multiple comparisons was significant in all measured intervals (BMMNC p < 0.01 and PBPC p < 0.05). However, TcPO_2 decreased slightly in nonamputated patients from the control group (n = 10) after 6 months (from 19.5 ± 8 to 17.7 ± 8.1 mmHg, p = not significant). TcPO_2 increase after 6 months in both active groups (ΔTcPO_2 25.9 in the BMMNC group and 23.2 mmHg in the PBPC group) was significantly higher (p = 0.03 and p = 0.004, respectively) in comparison with TcPO_2 change in the control group (ΔTcPO_2 −1.8 mmHg in the control group).

Characterization of cell suspensions (Table 2) by the use of the number of totally injected CD34+ cells was similar in both groups; however, there were significantly higher numbers of totally injected mononuclear cells (p = 0.03) and platelets (p = 0.0001) in the PBPC group compared with those in the BMMNC group.

Possible systemic vasculogenesis assessed by serum levels of angiogenic cytokines was similar in the BMMNC and PBPC groups at baseline and did not significantly increase at 6 months after treatment (VEGF: 4.7 vs 6.7 and b-FGF: 27.7 vs 37.8 pg/ml, respectively; Figure 3a,b). No vascular changes in the retina were observed after 6 months.

We observed no difference in adverse events between the BMMNC and PBPC groups: leg oedema was observed in 4/16 (25%) patients in the BMMNC group and 3/11 (27.3%) patients in the PBPC group, transient elevation of C-reactive protein was seen only in one patient in the BMMNC group and temporary worsening of ischaemic pain due to leg oedema was observed in one patient in the PBPC group. One patient in the BMMNC group died after 5 months because of cardiac failure but with improved leg ischaemia; no deaths in the PBPC group were seen, and two deaths were observed in the control group: one patient died 2 months after endarterectomy.

**Figure 2. Transcutaneous oxygen pressure before and after therapy of bone marrow mononuclear cell (BMMNC), peripheral blood progenitor cell (PBPC) and control groups (white square represents the control group, white triangle represents the BMMNC group and black square represents the PBPC group)**

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of femoral artery because of severe arterial bleeding, and the other patient died 3 months after inclusion because of cardiac failure.

**Discussion**

This study was performed to evaluate the effects of BMMNC and PBPC on the circulation in the lower limbs of diabetic patients with CLI not amenable to surgery or following failed revascularization and to compare the effect of cell therapy with a control group treated conservatively by standardized methods [1] in our foot clinic.

The TcPO2 significantly increased in both the active cell therapy groups, while a slight decrease in TcPO2 was observed in the control group at the end of 6 months. Similar results were published by Walter et al. [27] – combining TcPO2 measurements of the BMMNC patients of the randomized-start phase with those of the placebo group patients after crossover to BMMNC revealed that within 3 months after a single BMMNC administration, TcPO2 significantly increased in the BMMNC-treated patients, whereas a significant decrease was observed in the placebo group. The mean increase in TcPO2 following cell therapy (23.2 and 25.9 mmHg, respectively) in our study of diabetic patients with CLI was greater than the mean TcPO2 increase (15 mmHg) in a meta-analysis of 13 studies of patients with thromboangiitis obliterans and atherosclerotic PAD published by Fadini et al. [27–30].

We observed no significant difference in TcPO2 changes between BMMNC and PBPC therapy groups in our study; similarly, Huang et al. [15] also did not observe a difference in clinical effect between both methods; however, in contrast to our study, only 66% patients had diabetes in the latter study. Additionally, TcPO2 improvement 3 months after cell therapy was greater in our study than in Huang’s, which could be caused by a substantially lower baseline TcPO2 in our study (29 vs 16.4 mmHg).

Cell therapy in our study was injected into the calf muscles; the rationale behind intramuscular injection is to create a depot of cells with paracrine activity in the ischaemic area, and survival times of mononuclear cells injected into peripheral muscle up to 14 days were reported; this application method was preferred in most human trials [7]. A recently published study however did not confirm the difference between intramuscular and intra-arterial delivery of BMMNC; the TcPO2 increased significantly in both groups when compared with baseline [31].

We used TcPO2 as the main parameter for ischaemia assessment. We did not use ABI as an endpoint in our study because of the presence of mediocalcinosis in most of our diabetic patients. ABI was used in the PROVASA study because of the presence of mediocalcinosis in most of our diabetic patients. ABI was used in the PROVASA study as a primary endpoint and was not significantly different between BMMNC-treated and placebo-treated patients, whereas the TcPO2 measurement in that study was significantly increased in the cell therapy group [32].

In our study, patients in the control group had a greater number of amputations compared with the cell therapy group patients (50% vs 11.1%). We did not observe any

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**Table 2. Characteristics of cell suspensions**

<table>
<thead>
<tr>
<th>Cell populations</th>
<th>Concentration/total injected amount</th>
<th>BMMNC group (n = 17)</th>
<th>PBPC group (n = 11)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of suspension (mL)</td>
<td>53.4 ± 11.4</td>
<td>63.6 ± 19.9</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>CD34+ cells</td>
<td>Concentration (×10⁶/L)</td>
<td>386.2 ± 225.2</td>
<td>375.3 ± 325.8</td>
<td>0.95</td>
</tr>
<tr>
<td>Total injected amount (×10⁷)</td>
<td>2.2 ± 1.5</td>
<td>2.4 ± 2.2</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Mononuclear cells</td>
<td>Concentration (×10⁷/L)</td>
<td>25.7 ± 15.4</td>
<td>143.2 ± 66.8</td>
<td>0.03</td>
</tr>
<tr>
<td>Total injected amount (×10⁸)</td>
<td>1.8 ± 1.3</td>
<td>10.4 ± 7.1</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>Concentration (×10⁹/L)</td>
<td>1.7 ± 1.4</td>
<td>17 ± 10.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Total injected amount (×10⁹)</td>
<td>0.1 ± 0.08</td>
<td>1.6 ± 1.1</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>Concentration (×10⁹/L)</td>
<td>17.7 ± 9.5</td>
<td>69.5 ± 30.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Total injected amount (×10⁸)</td>
<td>0.8 ± 0.5</td>
<td>4.4 ± 2.6</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Concentration (×10⁹/L)</td>
<td>65.1 ± 44.7</td>
<td>50.5 ± 21.4</td>
<td>0.59</td>
</tr>
<tr>
<td>Total injected amount (×10⁹)</td>
<td>3 ± 2.6</td>
<td>3.2 ± 1.4</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>Concentration (×10⁷/L)</td>
<td>296.3 ± 291.2</td>
<td>3299.4 ± 1060.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total injected amount (×10⁶)</td>
<td>17.7 ± 16.4</td>
<td>202.1 ± 69.4</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

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**Figure 3. Changes of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) in time in both methods compared with baseline.** (a) Levels of VEGF. (b) Levels of b-FGF.
difference in major amputation rate between the BMMNC and PBPC groups, which is similar to the results of a recent study published by Onodera et al. [33]. The high rate of major amputation in our control group is in accordance with published data in patients ineligible for revascularization treated conservatively – the Trans-Atlantic Inter-Society Consensus II suggests that only about half these patients will be alive without a major amputation a year after the onset of CLI [22]. The long-term effect of stem cell therapy on limb salvage in 60% of patients with severe PAD was proven by Matoba et al., in the 3-year follow-up study of the Therapeutic Angiogenesis by Cell Transplantation study [6] [17].

A greater number of patients’ ulcers healed after 6 months in those treated by active cell therapy compared with controls (56% vs. 16.7%). According to the Proceedings of the 6th International Symposium on the Diabetic Foot, it is recommended to achieve ulcer healing by revascularization also when mild or questionable ischaemia is diagnosed and conservative treatment does not promote ulcer healing in 6 weeks [35]. Fadini et al. showed that the incidence of ulcer healing after stem cell therapy was significantly better in the active treatment group as compared with the control group [29].

Human stem cell systems have to be extensively tested to define media culture and characterized with highly validated antibodies and detection reagents; the CD34 surface marker is mostly used as a qualitative marker for haematopoietic and vascular precursor cells [7,9,29]. The gain of CD34+ cells in our study was comparable in both methods of isolation of stem cells, and a similar effect of BMMNC and PBPC on ischaemia by repeated measure of TcPO2 during 6 months was found. The equivalent benefit to treatment might be due to the similar concentration of vasculogenic precursors (CD34+) in both groups. It has been suggested that a comparison of stem cell vasculogenesis by the use of surface markers does not define the exact subpopulation responsible for revascularization effect. A combination of cell types may provide enhanced benefit, by providing precursors of various lineages or by the interaction between them [36]. It seems that cell therapies of severe ischaemia based on the intramuscular application of whole bone marrow-derived monocyctic cells or on whole stimulated PBPCs are more successful than methods that use subfractionated cell preparations [7], for example, only CD133+ fraction [37] or highly purified CD34+ cells from peripheral blood after G-CSF mobilization only [38].

We found a significantly higher number of mononuclear cells and platelets in the suspension of PBPC in comparison with BMMNC, but these differences did not have an influence on the clinical outcomes (assessed by TcPO2). Mononuclear cells of ‘unstimulated’ peripheral blood can theoretically influence the clinical outcomes, but the number of CD34+ cells (including EPC) in peripheral blood mononuclear cell is about 500-fold lower than in BMMNC [6]. The higher number of platelets and mononuclear cells in the PBPC group compared with that in the BMMNC group could be caused by different methods of cell separation – peripheral blood separator techniques and bone marrow collection and isolation [15]. The whole plasmatic volume is washed out through the separator several times during isolation of PBPC and contains a higher number of platelets and mononuclear cells than by the using the BMMNC separation technique, where cells are concentrated only in the harvested bone marrow blood. Prochazka et al. [39] concluded that BMMNC therapy resulted in 79% limb salvage in patients suffering from CLI and diabetic foot ulcers; lymphopenia and thrombocytopenia were identified as potential causative factors for amputation in 21% of patients.

We used stem cell suspension isolated from the whole peripheral blood after previous G-CSF stimulation or isolated from harvesting of bone marrow blood without any manipulation. New studies that compare the clinical effect of stem cell therapy by the use of cells isolated by different procedures have come out recently [8,40], with similar results concerning limb salvage.

Increased risk of systemic vasculogenesis defined as an increase in angiogenic cytokines (VEGF and b-FGF) and/or vascular changes in the retina after stem cell therapy were not noticed in our study. Kajiguchi et al. assessed also angiogenic cytokines (VEGF, b-FGF and angiopoietin-1) after stem cell therapy and did not observe any significant difference in their serum levels between the responders and nonresponders to cell therapy [41].

There were no major adverse events after stem cell therapy and no significant difference between BMMNC and PBPC. The most frequent adverse event in the active cell therapy group was leg oedema, which improved 2 weeks after bandage and antiplatelet therapy. Any possible complications during bone marrow harvest such as bleeding, bowel perforation or puncture wound contamination [42] were not observed in our study. All procedures were carried out under aseptic conditions in the operating theatre, and no perioperative infection was seen.

One death occurred in the active cell therapy group (BMMNC subgroup) with no relation to stem cell treatment, and two deaths were observed in the control group with no statistical difference between both groups. The patient who died in the active cell therapy group because of cardiac failure had prior renal and pancreas transplant and ischaemic heart disease with low ejection fraction (25%), but his diabetic foot ulcer was healed before death.

Long-term safety and clinical outcomes of autologous stem cell therapy were assessed in the Therapeutic Angiogenesis by Cell Transplantation trial [17], where the 3-year mortality was relatively low (20%). According to published data, the mortality of patients with CLI and diabetic foot ulcer is about 50% at 5 years and 50% at 2 years in patients after major amputation [43].

A main limitation of our study was that it was controlled, but not randomized. From published data and our first clinical experience, we felt that it would be unethical not to offer cell treatment to patients who had

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CLi, and no further vascular intervention was possible. Our control group was created from all patients with the same inclusion criteria as the active cell therapy group treated in our foot clinic during the study period (transient changes of local and European medicine agency recommendations regarding stem cell therapy of CLi were the reason for the conservative treatment in the control group). However, the advantages of our study were well-defined patient characteristics, inclusion criteria and methods used for assessment with relatively long follow-up. We also monitored the safety of this procedure in terms of systemic vasculogenesis.

The next step for research in cell therapies is whether these therapies are used only in severe ischaemia when there are no revascularization options or also in moderate ischaemia. The problem with diabetic patients with CLi is that these patients have a higher risk of cardiovascular mortality and these risk factors have to be simultaneously managed to improve patient survival rate [44].

In conclusion, our study showed comparable benefits of BMNC and PBPC treatments of CLi after unsuccessful standard revascularization in patients with diabetic foot ulcers and significant improvement of ischaemia and limb salvage in those treated by stem cells compared with conservative therapy. The gain of precursor cells was the same in BMNC and PBPC, and no effect on systemic vasculogenesis was documented by angiogenic cytokines and vascular changes in the eye fundus. Further trials are urgently needed if this treatment is to be recommended to improve wound healing, reduce amputations and improve quality of life in diabetic patients with critical limb ischaemia.

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Conflict of interest
None declared.

References


