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Clinical Research

Peripheral Blood Mononuclear Cells Therapy for Treatment of Lower Limb Ischemia in Diabetic Patients: A Single-Center Experience

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Background: The aim of this study is to analyze the effects of peripheral blood mononuclear cells (PBMNCs) therapy in diabetic patients with critical limb ischemia (CLI), with particular regard to its application, as adjuvant therapy in patients underwent endovascular revascularization.

Methods: Fifty diabetic patients affected by CLI were enrolled. All patients underwent PBMNCs therapy. Thirty-two patients underwent PBMNCs therapy associated with endovascular revascularization (adjuvant therapy group). In 18 patients, who were considered nonrevascularizable or underwent unsuccessful revascularization, regenerative therapy with PBMNCs was performed as the therapeutic choice (PBMNCs therapy group).

Results: The median follow-up period was 10 months. The baseline and end point results in adjuvant group were as follows. The mean transcutaneous partial pressure of oxygen ($TcPO_2$) improved from 25 ± 9.2 mmHg to 45.6 ± 19.1 mmHg ($P < 0.001$), and visual analogue scale (VAS) score means decreased from 8.6 ± 2.1 to 3.8 ± 3.5 ($P = 0.001$). In PBMNCs therapy group, the mean $TcPO_2$ improved from 16.2 ± 7.2 mmHg to 23.5 ± 8.4 mmHg ($P < 0.001$), and VAS score means decreased from 9 ± 1.1 to 4.1 ± 3.3 ($P = 0.001$). Major amputation was observed in 3 cases (9.4%), both in adjuvant therapy group and in PBMNCs therapy one (16.7%) ($P = 0.6$).

Conclusions: The role of cellular therapy with PBMNCs is decisive in the patients that are not susceptible to revascularization. In diabetic patients with CLI and healing resistant ulcers, the adjuvant PBMNCs therapy could represent a valid therapeutic option.

INTRODUCTION

Peripheral arterial occlusive disease (PAOD) is estimated to occur in 4.2−35% of the general population, and among them, up to 9.6% will develop critical limb ischemia (CLI).[1] In CLI, 1-year mortality is approximately 25%, and mayor limb amputation is 30%.[2]

Diabetic PAOD patients show the highest risk to progress toward CLI, with an increased mortality (25% 1-year mortality and still higher by 45% after amputation) and a majority prevalence of gangrene.[3]

The initial clinical presentation is rarely symptomatic and characterized by the ischemic lesions or gangrene, with a rapid evolution. Many factors can be associated with negative prognosis, such as anatomical distribution, infection, neuropathy, renal insufficiency, and concomitant coronary disease.[4]

In PAOD patients, the revascularization is indicated when clinical presentation is characterized

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Ann Vasc Surg 2018; ■: 1–7
https://doi.org/10.1016/j.avsg.2018.05.036

Manuscript received: January 10, 2018; manuscript accepted: May 1, 2018; published online: ■ ■ ■

by disabling claudication and/or rest pain and trophic lesion with foot transcutaneous oximetry ($TcPO_2$) < 30 mmHg, or any sign of healing after 1 months.[5,6]

Nowadays, for revascularization in these patients, both endovascular and surgical techniques that improve perfusion cause a reduction of pain and limb preservation. However, 25% to 40% of CLI patients are not good revascularization candidates because of clinical or anatomical reasons.[2]

Cell-based regenerative therapies aiming at enhancing neovascularization and improving limb perfusion have been proposed as novel treatment strategies in patients who do not fit for surgical or endovascular revascularization.

Peripheral blood mononuclear cells (PBMNCs) and bone marrow–derived mononuclear cells (BMMNCs) are the most frequently used cell types in CLI.

Efficacy and safety of cell-based therapeutic angiogenesis have been demonstrated in many clinical trials.[4,7,8]

However, therapeutic outcomes are still limited, and further improvements are required for extensive clinical applications.

Several studies demonstrated that implantation of PBMNCs, such as BMMNCs, into ischemic limbs induces collateral vessel formation (therapeutic angiogenesis).[7,9–12] However, PBMNCs have also local effects mediated by macrophages.[13]

Defective transition from proinflammatory (M1) to anti-inflammatory (M2) macrophages behavior has been implicated as a potential source of sustained inflammation that prevents healing of chronic wounds, such as diabetic ulcers.[8,13] The implantation of PBMNCs has a role in tissue repair and regeneration, allowing the M1 polarization with a transition to an M2 phenotype.

The aim of this study is to analyze the effects of PBMNC therapy in diabetic patients with CLI, with particular regard to its application, as adjuvant therapy in patients underwent endovascular revascularization.

METHODS

Patients and Study Design

This monocentric observational study is based on the analysis of collected data of patients who underwent PBMNCs therapy from November 2015 to May 2017.

All patients are diabetic with CLI and show tissue loss according to Rutherford categories.[5,6]

Demographic data were collected, and clinical status at the time of presentation and at postoperative revaluation was determined according to the Rutherford classification.[14]

The implantation of PBMNCs was performed in all patients. In most cases, it was used as adjuvant therapy associated with endovascular revascularization (adjuvant therapy group). On the contrary, for patients who were considered nonrevascularizable or who underwent unsuccessful revascularization, regenerative therapy with PBMNCs was performed as a therapeutic choice (PBMNCs therapy group).

Exclusion criteria included the following: active infection, active malignancy, bone marrow, or hematologic disorders.

Diagnostics

All the patients were evaluated with Echo Doppler ultrasound examination. A computed tomographic angiography or magnetic resonance angiography was performed, while a conventional angiography was performed in patients who underwent endovascular revascularization.

CLI was defined according to Inter-Society Consensus for the Management of Peripheral Arterial Disease.[3]

Endovascular Revascularization

Endovascular revascularization consisted in percutaneous transluminal angioplasty of tibial arteries. The endovascular revascularization was performed under local anesthesia, and the homolateral percutaneous femoral access was cannulated.

PBMNCs Isolation

Intraoperative procedure consists of 100–120 mL volume of blood taken from peripheral vein of the patients; mononuclear cells were isolated from peripheral blood (PB) through the use of the Mono-Cells system (Athena–Biomedical Innovations), an effective point-of-care device designed to obtain a high concentration of PBMNCs by means of whole-blood filtration by electrostatic charge.

The filtration was carried out in the operating room.

PBMNCs Treatment Protocol

To facilitate the injections, the procedure was carried out under transient deep sedation, and none required intubation. The ready-to-injection peripheral cells fraction has a volume of 10 mL. The pattern of injection sites is linear, overlying the areas of arterial flow (anterior and posterior tibial arteries). Injections were placed also around the perimeter of the ulcer/wound (0.25 mL in 30–40 sites, 1–2 cm

deep). All injections were performed immediately after filtration procedure under sterile conditions.

The patients received also conventional care for their ulcers, and wound debridement was performed.

The clinical study includes the withdrawal and injection of PBMNCs for 2 times at 30 and 60 days after the first enrollment.

For patients who are candidates for revascularization, the first implantation of PBMNCs was performed immediately after endovascular procedure. As for PBMNCs therapy group, only PBMNCs injection was repeated at 30 and 60 days after endovascular procedure.

Clinical Parameters for PBMNCs Treatment Evaluation

Patients were followed up at 1, 3, 6, and 12 months after the third cycle of PBMNCs therapy.

Visual analogue scale (VAS) was used to quantify ischemic-related pain, and transcutaneous partial pressure of oxygen ($TcPO_2$) was used to measure the amount of O_2 that has diffused from the capillaries.

VAS score and $TcPO_2$ value were evaluated before and after 30 days from the first procedure, 30 days from the second PBMNCs injection, and 30 days after the third procedure. During follow-up, they were recorded at 1, 3, 6, and 12 months.

The primary end points of this study were the $TcPO_2$ values and limb salvage. Additional parameter studied was the ischemic pain score.

Statistical Analysis

Categorical variables were presented as frequencies with percentages and continuous variables, normally distributed, as means with SDs and as median and 25°−75° percentile, when not normally distributed. Comparisons of categorical data were pursued with χ^2 and Fisher exact tests. Wilcoxon signed-rank test was applied to compare preoperative and postoperative TcPO2 values and VAS score. A P value less than 0.05 was considered statistically significant.

RESULTS

Fifty patients were enrolled. Demographic and clinical features are shown in Table I. All patients were diabetic and affected by CLI. Thirty-four patients were male (68%), and mean age was 71.3 ± 12 years.

In 32 cases (64%), peripheral cell treatment had been performed in combination with endovascular procedure (adjuvant therapy group), and in the remaining 18 cases (36%), the patients underwent only to PBMNCs therapy since they were evaluated preoperatively as nonrevascularizable or underwent an unsuccessful attempt of revascularization (PBMNCs therapy group) (Table II).

Table I. Demographic and clinical features

Characteristics	Patients ($n = 50$)
Gender (M)	34 (68%)
Age (years)	71.3 ± 12.6
Diabetes mellitus	50 (100%)
Hypertension	40 (80%)
Hyperlipidemia	21 (42%)
Renal insufficiency	13 (26%)
CAD	12 (24%)
Non-IHD	11 (22%)
Cerebral vascular insufficiency	4 (8%)

CAD, coronary artery disease; IHD, ischemic heart disease.

Table II. Procedural characteristics

Characteristics	Patients ($n = 50$)
Adjuvant therapy	32 (64%)
PBMNCs therapy	18 (36%)
Unsuccessful attempt of revascularization	4 (8%)
Nonrevascularizable	14 (28%)

The median follow-up period was 10 months (25°−75° percentile, 3−13 months). Two patients (4%) were drop out of the protocol. One patient (2%) died, and another one (2%) slipped into a diabetic coma.

In adjuvant therapy group, at baseline, the mean $TcPO_2$ was 25 ± 9.2 mmHg, and at the follow-up period, the value showed a significant increase, 45.6 ± 19.1 mmHg ($P < 0.001$) (Fig. 1A). In PBMNCs therapy group, we observed a significant increase of the mean $TcPO_2$ between preprocedural period and the follow-up one, 16.2 ± 7.2 mmHg and 23.5 ± 8.4 mmHg, respectively ($P < 0.001$) (Fig. 1B).

It is intuitive as comparing the variation of $TcPO_2$ between the 2 groups, the values collected in adjuvant therapy group were significantly higher than that in PBMNCs therapy group ($P = 0.003$).

Analyzing preoperative and follow-up VAS score means in adjuvant therapy group, we observed a significant reduction of values, 8.6 ± 2.1 and 3.8 ± 3.5, respectively ($P = 0.001$) (Fig. 2A).

Furthermore, even in PBMNCs therapy group, the variation of VAS score means was statistically significant between preoperative and follow-up period, 9 ± 1.1 and 4.1 ± 3.3, respectively ($P = 0.001$) (Fig. 2B).

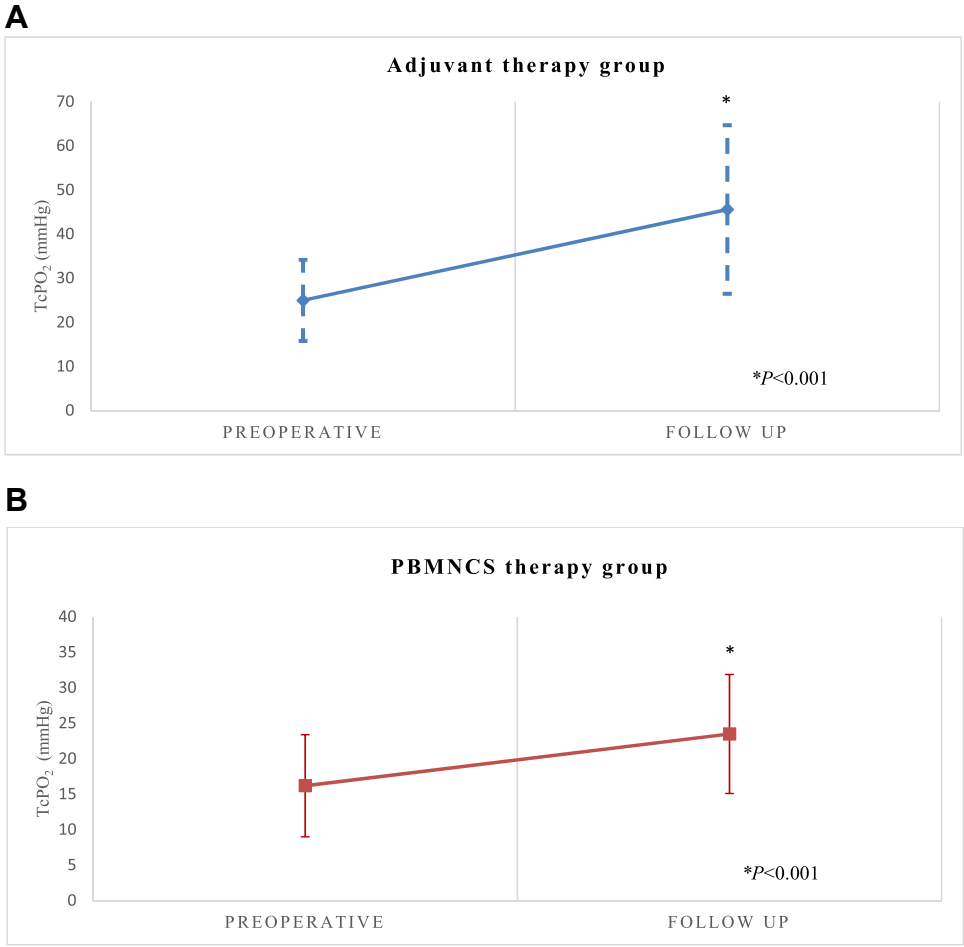

Fig. 1. Comparison between preoperative and follow-up $TcPO_2$ means (mean ± SD). **(A)** In adjuvant therapy group. **(B)** In PBMNCs therapy group (mean ± SD). SD, standard deviation.

Comparing the variation of VAS score between the 2 groups, the values collected in adjuvant therapy group were no significantly lower than in PBMNCs therapy group.

Major amputation was observed in 3 cases (9.4%), both in adjuvant therapy group and in PBMNCs therapy group (16.7%). The difference between these 2 groups of patients was not statistically significant ($P = 0.6$) (Fig. 3).

Minor amputation rate was 28.1% (9 cases) in adjuvant therapy group and 33.3% (6 cases) in PBMNCs therapy group ($P = 0.7$).

DISCUSSION

The present study analyzes our overall experience with PBMNCs therapy applied to diabetic patients affected by CLI, and to the best of our knowledge, it is the first study that investigated the role of PBMNCs therapy associated with endovascular revascularization.

PAOD is an independent risk factor for ulceration and limb loss in diabetes. It is present in 50% of patients with diabetic foot ulceration.[15,16]

CLI is clinically defined as the chronic and severe stagnation of limb perfusion, its ultimate outcomes being tissue ulceration and gangrene. It can cause diabetic microangiopathy and vasculitis and is associated with a high risk of cerebrocardiovascular events, including myocardial infarction and stroke.[17] Accordingly, it presents poor prognosis and high mortality: 20% within 6 months and 50% within 5 years of the diagnosis.[18]

The revascularization in diabetic patients with CLI can be more challenging due to the distal distribution of disease, impaired collateral formation, and vessel calcification.

The revascularization through endovascular approach is the first strategy in diabetic patients

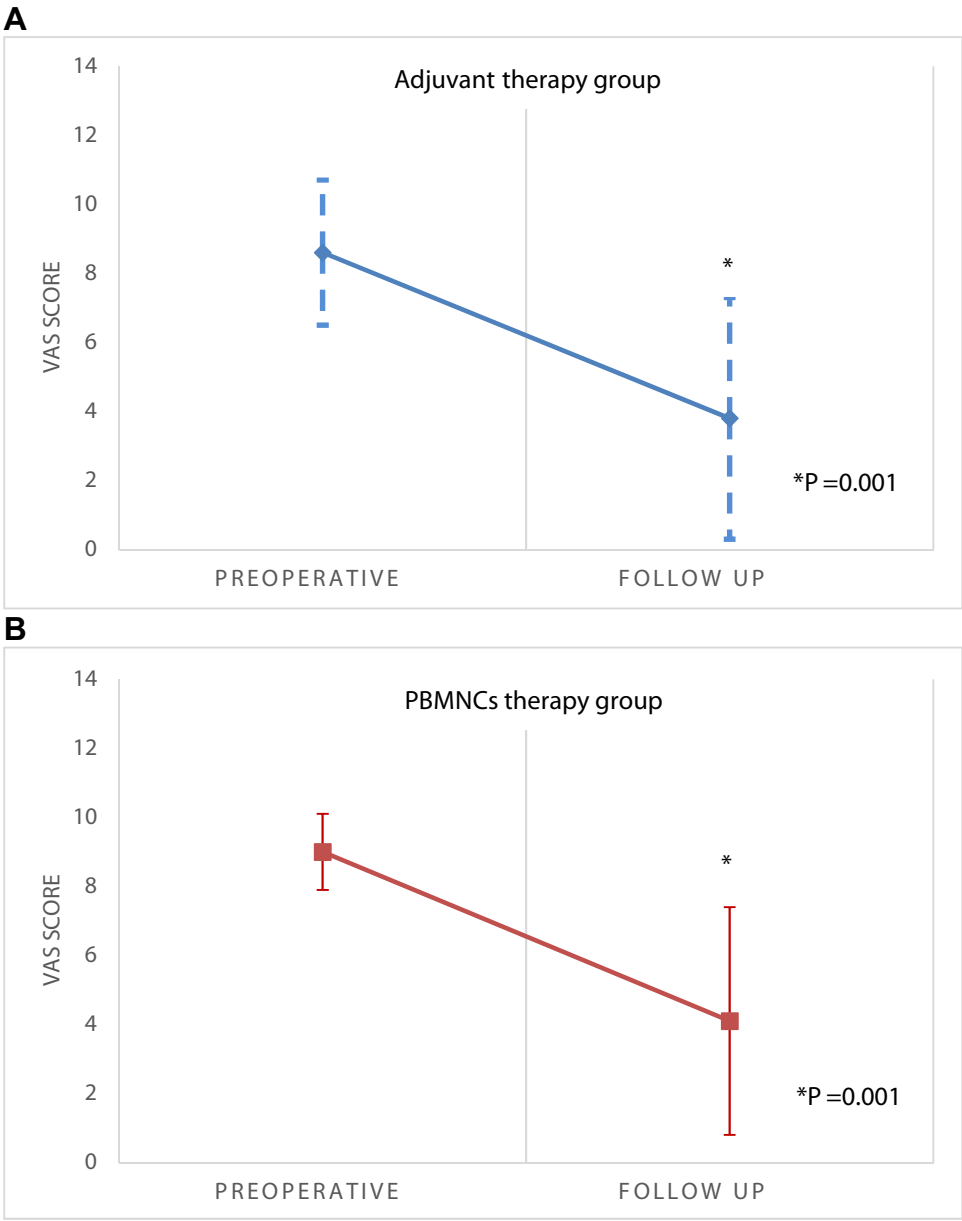

Fig. 2. Comparison between preoperative and follow-up VAS means (mean ± SD). **(A)** In adjuvant therapy group. **(B)** In PBMNCs therapy group. SD, standard deviation.

with CLI.[19] Many patients with CLI are elderly, affected by multiple comorbidities, and at high operative risk.[6,20]

Despite the endovascular approach can be increasingly proposed even in extreme situations and assures the better long-term patency of the treated vessels, there is a fraction of 25 to 40% of CLI that are not candidate to revascularization.[2]

Consequently, the development of alternative therapeutic strategies for these high-risk patients is strongly desired.[21]

Mononuclear cells from bone marrow and PB (e.g., BMMNCs and PBMNCs) appear to be the most realistic choice in clinical settings among different cell types already used as therapeutic choice. Common characteristics of these cell types are the presence of endothelial progenitor cells (EPCs) and the ability to secrete various proangiogenic factors. Although cellular heterogeneity and differentiation capacity vary between BMMNCs and PBMNCs, their clinical outcomes are not significantly different.[4,22,23]

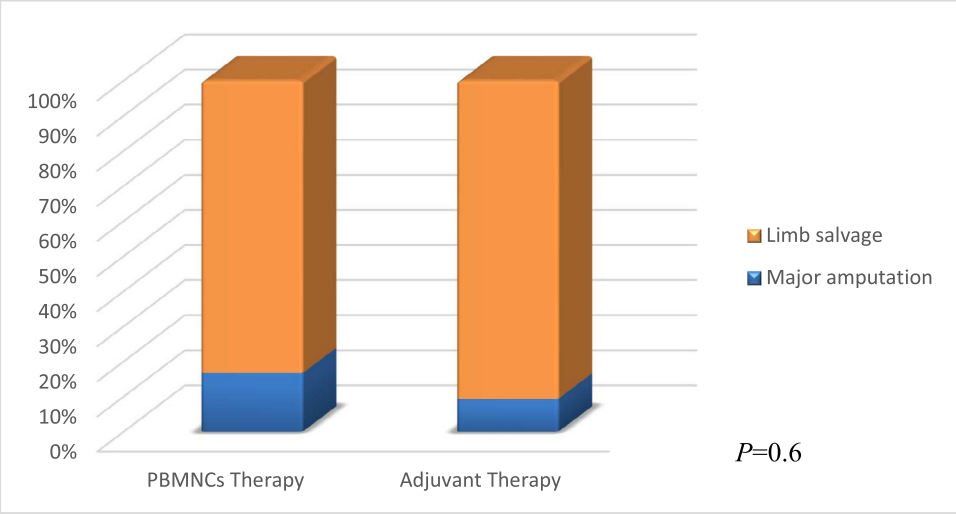

Fig. 3. Major amputation rate between adjuvant therapy group and PBMNCs therapy group.

We have decided to treat our patients with PBMNCs as therapeutic neovascularization, for its benefits by different mechanisms.[24]

First, PBMNCs induce collateral vessel formation by supplying angiogenic factors and cytokines in ischemic limb.[7,10–12,24]

Second, PBMNCs therapy has a role in tissue repair and regeneration, allowing the macrophages M1 polarization to an M2 phenotype, that are implicated in wound healing processes.[8,13]

Many studies demonstrate that macrophages, the primary cell of the innate immune system, act on a spectrum of phenotypes that correspond to diverse functions. Macrophage phenotype dysregulation is associated with many diseases. In particular, defective transition from proinflammatory (M1) to anti-inflammatory (M2) behavior has been implicated as a potential source of sustained inflammation that prevents healing of chronic wounds such as diabetic ulcers.[14]

Furthermore, in the critical diabetic patients, PB monocytes $CD14^+$ seem to have better reactivity to the hypoxic stimuli and a better paracrine angiogenic action at the site of the ischemic area, compared with $CD34^+$ cells (EPCs).[25,26]

Finally, the use of this cell population also allows a reduced invasiveness of the procedure and its repeatability over time.

Endovascular revascularization is the first strategy in diabetic patients with CLI, but in some cases, the only revascularization is not enough for the lesion healing. Probably, there are molecular mechanisms that can change the result of distal revascularization especially in diabetic patients.[27]

From these observations, we decided to associate a cell-based regenerative therapy with peripheral revascularization in diabetic patients with ulcer lesions resistant to revascularization to combine the positive effects of both methodologies.

The aim of this study was to evaluate the effects of PBMNCs therapy in diabetic patient with CLI and also to analyze the data obtained by PBMNCs therapy as adjuvant therapy of endovascular treatment.

Collective clinical findings of small case series that analyzed the effect of PBMNCs therapy showed significant improvements in pain scale, walking distance, and limb salvage compared with the baseline.[28] Randomized trials of PBMNCs have confirmed these early clinical improvements (3 months) in pain, although amputation rates did not differ between those treated with unselected PBMNCs therapy versus the control group.[29]

The outcomes considered are the $TcPO_2$, pain score, and limb salvage. Our results showed a significant increase in $TcPO_2$ and a decrease in VAS after PBMNCs therapy.

This study represents also the first analysis of results obtained by the association between PBMNCs therapy and endovascular revascularization in a CLI-related diabetic patient.

Study limitations are the short period of time for the follow-up and the inclusion in this study of a single center. Therefore, multicenter trial could be useful to underline the benefits of PBMNCs treatment applied ad adjuvant therapy in comparison with only peripheral endovascular procedure.

CONCLUSIONS

In our study, as observed by other reports, we found that the role of cellular therapy with PBMNCs is decisive for patients who are not susceptible to revascularization.

PBMNCs therapy has a positive benefit-to-risk ratio in CLI, and it presents a favorable safety profile with a low adverse event rate. This therapy improves $TcPO_2$ values and decreases ischemic pain and the need for amputation.

In diabetic patients with CLI and healing resistant ulcers, the adjuvant PBMNCs therapy could represent a valid therapeutic option that improves obvious benefits of revascularization, without increasing the risks of the procedure.

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