Topical Application of Autologous Peripheral Blood Mononuclear Cells in Patients with Small Artery Disease and Diabetic Foot Ulcers: Efficacy, Safety and Economic Evaluation

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Article

Topical application of Autologous Peripheral Blood Mononuclear Cells in Patients with Small Artery Disease and Diabetic Foot Ulcers: Efficacy Safety and Economic Evaluation

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Abstract: BACKGROUND: diabetic foot ulcers (DFU) represent the main cause of major amputations and hospitalizations in diabetic patients. Aim of this study was to assess safety and cost-efficacy of intramuscular injection of peripheral blood mononuclear cells (PBMNCs), in diabetic patients with no-option chronic limb-threatening ischemia (CLTI) and small artery disease (SAD). METHODS: a retrospective study was carried out on a series of types 2 diabetic patients with DFU grade Texas 3 and no-option CLTI and SAD. All patients had undergone at least a previous revascularization and were allocated in a surgery waiting-list for major amputation. The principal endpoint evaluated at 90 days was a composite of $TcPO_2$ values at the first toe ≥30 mmHg and/or $TcPO_2$ increase of at least 50% from baseline and/or ulcer healing. Secondary endpoint were individual components of the primary endpoint, any serious and non-serious adverse events, direct costs at one year. RESULTS: the composite endpoint was achieved in 9 patients (60.0 %); one patient (6.7%) healed within 90 days and 26.7% and 46.7% showed $TcPO_2$ ≥30 mmHg and a $TcPO_2$ increase of at least 50% at 90 days, respectively. At 1-year, three (20.0%) patients underwent a major amputation (all diagnosed SAD grade III). One patient died after seven months, and seven patients (46.7%) healed. The overall median and mean cost per patient were 8,238±7,798€ and 4,426[3,798;8,262]€, respectively. CONCLUSIONS: the use of PBMNCs implants in no-option CLTI diabetic patients with SAD seems to be of help in reducing the risk of major amputation.

Keywords: diabetes mellitus; foot ulcer; cell-therapy; small artery disease; chronic limb-threatening Ischemia; Economic evaluation

1. INTRODUCTION

DFU (Diabetic Foot Ulcers) represent the main cause of major amputations and hospitalizations in diabetic patients (1). Major amputation rate in diabetic patients is 15 times superior to that of non-diabetic patients (2) and about 85% of limb amputations are preceded by a foot ulcer (3). The most important risk factors for the development of DFU are diabetic neuropathy and peripheral artery disease, which are frequently concomitant (4).

Chronic limb-threatening ischemia (CLTI), affecting about 25% of diabetic patients (5), represents the most advanced form of peripheral artery disease (PAD), responsible for a considerably higher rate of major amputation (6) and mortality (7).

The gold standard for the treatment of CLTI is percutaneous or surgical revascularization. However up to 25% of diabetic patients with CLTI are not eligible for revascularization due to technical difficulties to overcome vessel obstruction and/or high number of comorbid conditions (8, 9). CLTI is defined as 'no-option' ischemia in case of absence of a suitable target arterial path with no visible distributing arterial circulation in the foot (*"desert foot"*) (10).

Diabetic patients with no-option CLI (NO-CLTI) are at higher risk of major amputation (30% vs. 4.5%, $p = 0.0001$) and mortality (50% vs. 8.9%, $p < 0.0001$) in comparison to patients undergoing revascularization (9). This risk is even higher in presence of small artery disease (SAD), which is an often-neglected condition affecting patients with diabetes and/or renal insufficiency and dialysis (11). SAD is a complex vascular disorder defined as a disease of small vessels of plantar arch (11). Despite

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its relevant prevalence and clinical significance, current therapeutic options for SAD are limited and often ineffective, leading to high morbidity, amputation, mortality, and healthcare direct and indirect costs.

In recent years, cell therapy has emerged as a promising approach to address NO-CLTI by promoting angiogenesis, vasculogenesis, and tissue repair. Autologous cell therapy, in fact, has shown favourable effects on several outcomes, such as pain, transcutaneous oxygen tension, ulcer healing, major amputation, and mortality (12). Stem cells increase peripheral circulation by stimulating neo-angiogenesis achieved through paracrine activities of growth factors, cytokines, and messenger molecules, as well as through exosomes (13, 14).

Cell therapy (i.e.: mesenchymal stem cells, blood marrow mononuclear cells) can be delivered through different routes and methods depending on the cell type, stage of the disease, and treatment goal. The most common routes of administration include intramuscular injection, intravenous infusion, direct injection into target tissue or muscle, and delivery through a biomaterial or scaffold (15,16). In recent years, some authors have proposed the intramuscular injection of peripheral blood mononuclear cells (PBMNCs), which has shown similar efficacy in comparison with "traditional" autologous stem cells. Notably, this new approach presents several advantages, such as less invasive extraction techniques not requiring hospitalisation, less painful and time consuming procedures, etc. (15,16).

No data on the efficacy and safety of cell therapy for diabetic patients with SAD have been published so far and therefore the present retrospective study is aimed to evaluate cost-effectiveness and safety of PBMNCs implant, in diabetic patients with no-option CLTI and SAD allocated in a surgery waiting-list for a major amputation.

2. PATIENTS AND METHODS

The present analysis was performed on a consecutive series of NO-CLTI patients with DFUs and SAD who underwent the implantation of PBMNCs from peripheral blood at the Diabetic Foot Unit of Careggi Hospital, Florence, Italy, between January 1st, 2020 and June 30rd, 2021. All patients were candidates for elective major amputations and allocated in a surgery waiting-list.

The study protocol was approved by the local ethical committee (Protocol number SPE_22580) and informed consent was obtained from all patients before the inclusion in the analysis.

Patients were included if fulfilling the following criteria:

1) Diagnosis of diabetes mellitus
2) Age > 18 years
3) DFUs grade Texas 3
4) No-option CLTI and SAD (see below for definitions)
5) Allocation in a surgery waiting-list for major amputation
6) At least one previous revascularization procedures (endoluminal or open surgery)
7) Absence of severe infection according to the PEDIS classification system (PEDIS<2; 17)
8) Absence of severe anaemia (Hb > 8 g/dL)
9) Absence of coagulation disorder/thrombocytopenia (PLT > 50,000/L)
10) Absence of active cancer/leukaemia or lymphoma or haematological disease
11) Being able to sign informed consent.

CLTI was diagnosed in case of ischemic pain at rest or ischemic ulcer/gangrene at foot level associated with systolic blood pressure at ankle level < 70 mmHg or systolic blood pressure at first toe < 50 mmHg or TcPO2 values at foot level < 30 mmHg (18).

SAD is defined as a disease of plantar arch small arteries (tarsal, metatarsal digital, calcaneal branches), detected by an angiographic study (13). A vascular surgeon and an interventional cardiologist confirmed the presence of SAD disease by reviewing all angiographic procedures. SAD was defined according to a global evaluation of the arch and the small foot arteries as grade 1: absence of disease or mild disease with a well-represented network of forefoot and calcaneal arteries; grade 2: diffuse disease with narrowing and poverty of metatarsal, digital and calcaneal arteries; grade 3: extreme poverty of arch, metatarsal, digital and calcaneal arteries (11)

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All patients received a multidisciplinary evaluation with vascular surgeons and interventional cardiologists to explore the possibility of a new lower limb revascularization. Patients were therefore included in the present analyses only if considered 1) not eligible for a new revascularization according to ESVS - ESC 2017 criteria (18), or 2) in case of no run-off pedal vessels or 3) failure after infra-genicular bypass grafting. The indication to perform implantation of perilesional and perivascular monocytes has been discussed collegially by the multidisciplinary team (diabetologist, vascular surgeon, and interventional cardiologist) as an attempt of limb savage.

2.1. Baseline data collection

Demographic and clinical data were collected from clinical records, including a medical history with detailed information on the duration of diabetes, complications and concomitant medical conditions, current pharmacological treatment, cardiovascular risk factors, self-reported smoking habits and any other relevant medical condition. At the first visit, following an established standard procedure of the Clinic, all patients underwent a physical examination, during which their weight, height, and blood pressure were recorded. All patient underwent a blood sample after a minimum 8 hours fasting (i.e. HbA1c, glicemia, creatinine, total cholesterol, HDL-cholesterol, triglycerides, transaminase, bilirubinemia, ɣ-GT, potassium, sodium).

Ulcers dimensions were evaluated with MolecuLight i:X ®. When more than one lesion was present, only the largest ulcer was taken into account. Diagnosis of diabetic neuropathy was performed measuring vibratory perception threshold with a biothesiometer (METEDA, San Benedetto del Tronto, Italy) and monofilament testing 10g. Ulcers were classified according to the University of Texas score (16).

Pain at the first visit and quality of life were assessed using a visual analogue scale (VAS) ranging from 0 to 10 (VAS for pain) and from 0 to 100 (VAS for quality of life), respectively.

Number of previous surgical and percutaneous omolateral revascularizations were registered.

As per local standard of care, transcutaneous pressure of oxygen (TcpO2; Radiometer Medical ApS; Brønshøj, Denmark) at the basis of the first toe and at the ankle and ankle-brachial index (ABI; or toe brachial index) were measured, and an echo-colour doppler examination of lower-limb arteries was performed.

Renal failure was defined as a reported previous diagnosis of renal failure, or as serum creatinine >1.5 mg/dl. Ischemic Heart Disease (IHD) and cerebrovascular disease were diagnosed when patients reported previous myocardial infarction/angina or stroke/transient ischemic attack. Comorbidity was assessed through the calculation of Charlson's comorbidity score (CCS).

2.2. Ulcer treatment

All patients received the same standard therapy according to IWGDF guidelines (4): surgical debridement, local dressings and foot offloading, antiplatelets drugs, antibiotic therapy in case of infection and pain relief therapy.

All patient underwent a procedure of local infiltration of autologous mononuclear cells through multiple perilesional and intramuscular injections of 10 mL PBMNCs cell suspensions (0.2–0.3 mL in boluses) performed below the knee along the relevant vascular axis (anterior tibial artery and posterior tibial artery) at intervals of 1–2 cm and to a mean depth of 1.5–2 cm, using a 21 G needle. The procedures were performed according to the instructions of the manufacturer and were repeated at least two times for each patient at intervals of 30 days. All procedures were performed in an operating room with anesthesiologic support (midazolam iv and/or peripheral block). For these procedures Athena Monocells Solution kits were used following the manufacturer's instructions (19).

2.3. Follow-up data

Patients were evaluated at baseline, one, three, six and twelve months after the first implantation and the following parameters were recorded:

1) TcPO2 at the 1st toe

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2) Pain (using VAS scale from 0 to 10)
3) Vital status of patients
4) Healing rate
5) Major amputation rate
After 6 months:
1) Quality of life

2.4. Endpoints

The primary endpoint of the study was a composite of the following items at 90 days:
- $TcPO_2$ at the first toe ≥30 mmHg and/or
- increase of at least 50% of TcPO2 in comparison with baseline values and/or
- healing of the ulcer.
Secondary outcomes evaluated at each time-point were:
- individual components of the primary endpoint
- any serious and non-serious adverse events
- direct costs at one year.

Complete healing was defined as full epithelialization of the wound (also obtained after minor amputation) confirmed after 7 days. Minor amputations were performed, as recommended by international guidelines (4) only with distal TcPO2 >= 30 mmHg or in case of a 50% increase of TcPO2 compared with basal values; minor amputation was considered as limb rescue and was defined as any amputation performed below the ankle. Major amputation was defined as a surgical procedure performed above the ankle.

2.5. Economic assessment

The economic assessment was performed considering the perspective of the local health system, thus considering only direct healthcare costs and including costs associated with healthcare resources used all over the follow-up and extracted from clinical records. In detail, direct costs included specialist visits, diagnostic procedures, hospital admissions (related to diabetic foot), major and minor amputations, antibiotic therapy, grafts, and off-loading orthesis. Costs for hospitalizations were estimated on the basis of established regional tariffs (https://www.salute.gov.it/portale/temi/p2_6.jspid=3662&area=programmazioneSanitariaLea&menu=vuoto), i.e. tariffs established for the diagnosis related group (DRG) associated with each episode for hospital admissions (either day-hospital or full-length stay) and recorded in clinical records; similarly for costs related to specialistic visits and outpatient procedures performed (e.g. RX, MRI, laboratory exams, ecc.). The cost of antibiotic therapy was estimated considering ex-factory prices (https://www.salute.gov.it/portale/temi/p2_6.jsp?id=3662&area=programmazioneSanitariaLea&menu=vuoto),while current market prices were used to value costs for orthopaedic shoes/orthesis. The health economic analysis performed tried to estimate costs born to the healthcare system, mainly using tariffs related to different healthcare services, over one year. As discounting typically require collection of data over different time point to give different value to both costs and health outcomes that are predicted to occur in the future because they are usually valued less than present costs, given the time frame considered in our analysis we decided to not apply any discount rate. All costs were referred to 2020 and are reported in Table 1S and 2S.

2.6. Statistical analyses

Statistical analysis was performed on SPSS 25.0. Data were expressed as mean ± standard deviation (Std.dev), or as median (25th-75th percentile), depending on their distribution. Comparisons between groups were performed using Student's t-test for independent samples or Mann–Whitney U test as appropriate. Chi-square and Fisher exact tests were used for between-group comparisons of categorical variables as appropriate. The Kaplan–Meier method was used to derive the probability of healing over time.

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3. Results

The whole cohort was composed of 15 patients (4 women, 26.7%), aged 69.8±13.0 years, and affected by ischemic DFU. The principal characteristics of patients are summarised in **Table 1**.

Table 1. – Main anthropometric and demographic characteristics of the enrolled cohort and of observed ulcers.

	Case **(n = 15)**
Age(years)	69.8±13.0
Gender (women, %)	4 (26.6%)
Body Mass Index(kg/m²)	25.4±4.2
Diabetes type 2 (%)	14 (93.3)
Diabetes duration(years)	28.2 ±10.6
Medical history and risk factors (n, %)	
Diabetes mellitus type 1	1 (6.6%)
Charlson's score index	6.0[3.0-7.0]
Peripheral artery disease	15 (100.0%)
Neuropathy	15 (100.0%)
Retinopathy	6 (40.0%)
Chronic renal insufficiency	9 (60.0%)
Dialysis	1 (6.7%)
Ischemic heart disease	10 (66.7%)
Hearth failure	4 (26.7%)
Ictus	2 (13.3%)
Charcot disease	4 (19.0%)
Connective tissue diseases	2 (13.3%)
Malignancies (< 5 years)	1 (6.7 %)
Cognitive impairment	2 (13.3 %)
Smokers	1 (6.6)
Laboratory parameters	
HbA1c(%)	57.7± 14.6
Creatinine(mg/dl)	1.09 [0.86; 1.59]
LDL-Cholesterol(mg/dl)	58.7±34.9
Pharmacological treatment (n, %)	
Insulin	11 (73.3%)
Glucose-lowering agents	15 (100%)
Antiaggregants	11 (73.3%)
Anticoagulants	7 (46.7%)
Statins	15 (100%)
Main ulcers' characteristics	
Duration (days)	365 (114; 546)
Site	
Forefoot	12 (80.0)
Midfoot	1 (6.6)
Hindfoot	2 (13.3)
TEXAS (%)	
3B	5 (33.3)
3D	10 (66.6)
Gangrene (%)	5 (33.3)
Osteomyelitis (%)	12 (80.0)

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TcPO2 (mmHg)	3.8 (1.2; 22.1)
Pain (VAS 0-10)	5.0 (3.0; 8.0)
Quality of life (VAS 0-100)	50 (27; 60)
Number of previous revascularization (%)	
1	7 (46.6)
2	3 (20.0)
3	2 (13.3)
4	2 (13.3)
5+	1 (6.6)

Most DFU involved the forefoot (80%) and gangrene was present in 33% of cases; median $TcPO_2$ at the first toe level at baseline was 3.8 (1.2; 22.1) mmHg and SAD grade 2 and 3 was detected in 10 and 5 patients, respectively.

The primary 90-day composite endpoint was achieved in 9 patients (60.0 %). One patient (6.7%) healed within 90 days and 4 (26.7%) and 7 (46.7%) showed $TcPo_2$> 30 mmHg and/or a $TcPo_2$ increase of at least 50% from baseline, respectively. No patients underwent major amputation in the first three months of follow-up.

Median values of $TcPO_2$ (at the basis of the first toe) at baseline, 1, 3, 6, and 12 months are reported in **Table 2**; a significant increase of $TcPO_2$ values were observed at 3, 6, and 12 months (**Table 2**) from baseline. A statistically significant reduction of pain was observed at any time-point and quality of life measured at six months showed a nonsignificant trend toward increase.

Table 2. – Average costs during the follow-up of 1 year.

	Mean Std. Dev.	**Median [interquartiles]**
Minor amputations/grafts	374±562	0 [0;731]
HA for FRP	1,705±2,508	0 [0;4,904]
Outpatient visits and laboratory exams	571±261	563 [324;780]
Major amputations	4,213±8,722	0[0;0]
Antibiotics	272±976	0[0;24]
PBMNCs	3,240±1,009	3,600[1,800;3,600]
Total costs	**8,238±7,798**	**4,426[3,798;8,262]**

HA: hospital admission; FRP: foot related problems; Std: Standard; dev: deviations.

At 1-year, three (20.0%) patients underwent a major amputation (all diagnosed SAD grade III). One patient died after seven months, and seven patients (46.7%) healed (4 after minor amputations) within twelve months.

Following our internal protocol all patients, except four, underwent two infiltrations of PBMNCs; one patient received three infiltrations due to an incomplete response to the treatment, and the other three patients underwent major amputation before undergoing the second infiltration for clinical reasons.

No major adverse events were observed during follow-up and only four patients reported pain immediately after the procedure (median value 3.5) which completely disappeared in a few minutes without requiring any treatment.

A formal analysis of direct costs sustained during the 1-year follow- are reported in **Table 3.** The overall median and mean cost per patient were 8,238±7,798€ and 4,426[3,798;8,262]€, respectively, which were significantly ($p < 0.001$) lower than (direct) costs which would have been sustained for major amputation (21,065€).

Table 3. – Median values of distal $TcPO_2$, perceived pain and quality of life at 0, 1, 3, 6 and 12 months.

Month	**0**	**1**	**3**	**6**	**12**

Distal TcPo2	3.8[1.2;22.1	5.4[1.0;26.3]	20.2[5.1;32.2]*	26.0[16.4;52.4]*	24.1[19.0; 32.4]*
Pain	5[3;8]	3[0;6]*	0[0;3.5]*	0[0;2]*	0[0;0.2]*
Quality of life	50[27;60]	-	-	60[30;70]	-

* $p < 0.05$ from baseline.

4. DISCUSSION

Chronic limb-threatening ischemia is a challenging condition for clinicians involved in the treatment of DFU. The therapeutic approach to CLTI depends on several factors such as patient-specific vascular anatomy, availability of vascular conduits for revascularization, and comorbid conditions, such as cardiac disease and renal insufficiency (8, 10, 11). Peripheral artery disease in patients affected by diabetes is characterised by multisegmental distribution and distal involvement of the artery at the foot level. In these conditions, traditional endovascular techniques as well as open revascularization procedures are frequently less effective than in nondiabetic patients (20). Moreover, revascularization procedures in diabetic patients are also challenging due to technical reasons (e.g., absence of autologous venous conduit for bypass or lack of a suitable pedal or plantar artery target, intima-media calcification etc.) (20).

Moreover, diabetes and renal insufficiency (often co-exiting) are independent risk factors for SAD, which is a further condition limiting the feasibility and efficacy of revascularization. In this complex scenario, a not negligible fraction of type 2 diabetic patients is at high risk for no-option CLTI and major amputation.

Since, there is no definitive treatment for SAD, and existing therapies, such as lifestyle modifications, pharmacotherapy, and revascularization procedures have limited efficacy and significant side effects, there is a growing interest in the potential use of cell-based therapies (12). However, to our knowledge, no studies have been performed in patients (candidates to major amputation) with diabetic foot ulcers and SAD.

Despite the growing interest and the present preliminary results on the potential of cell-based therapies in SAD, there are several challenges that still need to be addressed to optimise their safety and efficacy, including the selection of the most appropriate cell type, dose, and delivery method, as well as the optimization of the therapeutic window, timing, and endpoints. In addition, there are several concerns regarding the safety and immunogenicity of allogeneic cell products, the potential risk of tumorigenesis or ectopic tissue formation, and the regulatory and ethical issues related to the manufacturing, labelling, and approval of cell therapy products (21).

Our study, although limited by its retrospective nature and the small sample size, can provide some insights on this topic and be of help for clinicians involved in the treatment of NO-CLTI patients with SAD. In fact, the obtained results (i.e., the increase of TcPO2 values, the reduction of pain and the avoidance of major amputation in a large fraction of patients) are encouraging and of help as a hypothesis-generating research. In the present study, we have also assessed direct costs sustained for the treatment of these patients, which are relevant, but significantly lower than that needed for major amputations, avoided in a large fraction of patients included in the present analysis.

In conclusion, despite these promising results, further studies (in particular randomised controlled trials) are needed to elucidate the mechanisms, optimise the procedures, assess the cost-effectiveness and validate the safety and efficacy of cell-based therapies for NO-CLTI complicated by SAD.

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