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CASE REPORT

Successful wound healing by autologous peripheral blood mononuclear cell therapy in a diabetic patient on hemodialysis with no-option critical limb ischemia: a case report

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Abstract

Peripheral artery disease is a common condition in patients on chronic dialysis treatment, end-stage kidney failure itself being a risk factor. The most severe stage of peripheral artery disease, critical limb ischemia, causes marked chronic pain and is associated with risk of limb loss. Despite improvements in revascularization procedures, the results of limb salvage procedures among dialysis patients remains poor, and lower extremity amputation is associated with high mortality and grim socio-economic implications. We report on a limb salvage approach that was successfully employed in a 74-year-old woman on hemodialysis suffering from no-option critical limb ischemia complicated by diabetic foot infection, i.e. otherwise a candidate for major amputation. The approach consists in implanting in the wound bed of the affected limb a concentrate of autologous peripheral blood mononuclear cells collected from the peripheral blood of the patient using a selective filtration separation system. The procedure, performed by a vascular surgeon in an outpatient setting and sterile conditions, was repeated three times at intervals of 15 days, and was well tolerated; no adverse safety signals were observed. Complete wound healing was obtained, with successful limb rescue.

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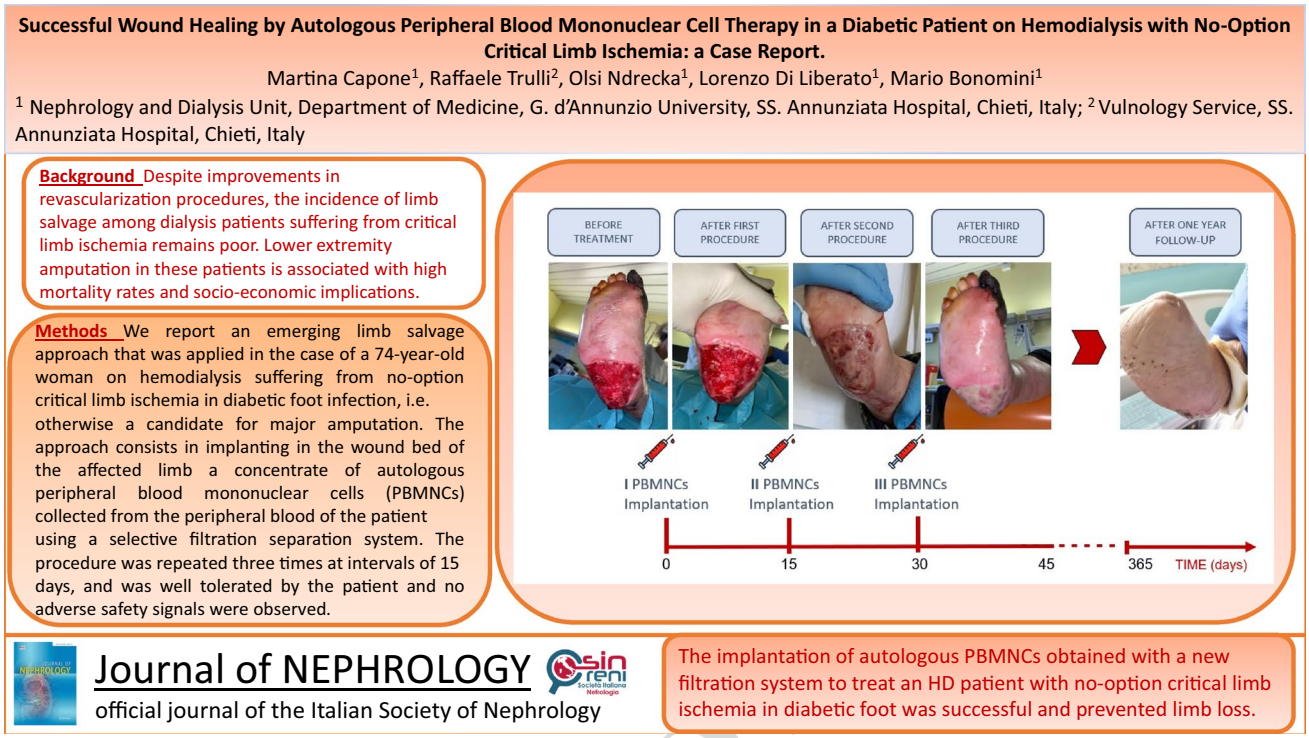
Graphical abstract



Keywords No option-critical limb ischemia · Diabetic foot · Hemodialysis · Peripheral blood mononuclear cell therapy

Lower extremity peripheral artery disease is an atherosclerotic lesion involving vessels from the aorto-iliac segment to the pedal arteries, which can result in critical limb ischemia. Marked chronic rest pain, ulcers or gangrene are typical features of critical limb ischemia, leading to poor outcomes. Peripheral artery disease affects over 230 million adults worldwide; its incidence increases in patients over the age of 70, and prevalence appears to be equal among aging men and women [1].

End-stage kidney disease (ESKD) has been recognized as a risk factor for peripheral artery disease. The coexistence of ESKD and diabetes is associated with complex vascular dysfunction that significantly worsens the prognosis in patients with diabetes-related foot ulcer, causing decreased healing rates, recurrence of ulceration, and leading to major limb amputations [2]. Despite improvements in revascularization techniques, the probability of limb salvage among dialysis patients remains poor. Furthermore, many patients are not amenable to endovascular, surgical, or other treatments (no-option), making amputation inevitable. Lower extremity amputation induces disability, decreases the quality of life, and contributes to high morbidity, mortality and health care costs in patients on dialysis [3].

We report an emerging limb salvage therapy approach in a diabetic patient on hemodialysis suffering from no-option critical limb ischemia it consists in locally injecting the wound with autologous peripheral blood mononuclear cells (PBMNCs) obtained using a selective filtration separation system.

The case

A 74-year-old diabetic female was referred to our Center in December 2021 for initiation of hemodialysis (HD). Clinical history included type 2 diabetes mellitus on insulin therapy, hypertension, ischemic heart disease needing angioplasty revascularization (March 2021), diastolic heart failure, and chronic peripheral artery disease (Leriche-Fontaine stage IV) with painful diabetic ulcers in the right foot. The patient had undergone several endovascular revascularization procedures, in the right lower extremity (femoro-popliteal axis, tibial-peroneal-trunk, posterior tibial artery, and plantar artery), with poor results. She was receiving standard therapy including surgical debridement, antiplatelet drugs, statins, and pain relief treatment with paracetamol and opioids. In April 2022, she underwent double angioplasty to

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treat steno-obstruction of the right superficial femoral artery and occlusion of the right posterior tibial artery, the morphological outcome being satisfactory. In May 2022, she was admitted to our unit for worsening of ischemic rest pain and local infection (osteomyelitis treated with a 2-dose regimen of weekly dalbavancin). Duplex ultrasonography showed patency of the femoro-popliteal axis. At clinical evaluation, an ulcerative lesion with dry necrosis of the right heel (10 cm × 8 cm) and of the right hallux was found, together with mild perilesional inflammation. Escharectomy with placement of negative pressure wound therapy was performed but proved ineffective. The vascular surgeon's conclusion was non-healing ulcer in diabetic foot infection, with a high risk of major amputation. The patient was not eligible for surgery or endovascular procedure, considering the high comorbidity and because obstructive lesions below the knee and below the ankle arteries were considered as no-option critical limb ischemia.

The patient gave informed consent and was treated with an innovative therapeutic approach that consists in the implantation, in the perilesional area of the affected lower limb, of a concentrate of autologous PBMNCs obtained using a selective filtration separation system (MonoCytes, Tiss'You Srl, San Marino). The procedural steps are shown in Fig. 1.

One hundred and twenty ml of the patient's peripheral anticoagulated blood was passed through the filter for processing via gravity filtration. Mononuclear cells were trapped inside the filter, while the majority of plasma, platelets, and red blood cells were not retained. The enriched PBMNCs were then recovered by filter backflushing with10 ml of sterile saline solution and immediately used. For implantation, after appropriate surgical debridement of the wound bed, 10 ml of the obtained PBMNC suspension was injected along the perilesional area, at intervals of 1–2 cm and to a mean depth of 1.5–2 cm, using a 21G needle, under local anesthesia.

The procedure was managed by a vascular surgeon in an outpatient setting and sterile conditions, and was repeated three times at intervals of 15 days. The patient attended the outpatient department twice a week for 7 months for regular advanced dressings. All procedures were performed on days of scheduled HD sessions thus saving additional hospital visits and transportation costs. Following treatment, the granulation tissue improved gradually, progressively achieving complete wound healing (Fig. 2). The wound healing was possibly also favored by careful and frequent in-hospital dressings of the ulcer.

Since toe gangrene occurred, amputation of the toes was necessary, but limb rescue was successfully attained. Note that from the very first PBMNC implant, rest pain was reduced, as has been previously described [4, 5]. This allowed progressive reduction of drugs used for pain control (paracetamol and opioids), ultimately resulting in occasional use of paracetamol alone.

Here we describe what is, to our knowledge, the first case of implantation of autologous PBMNCs obtained with a new filtration system to treat an HD patient with no-option

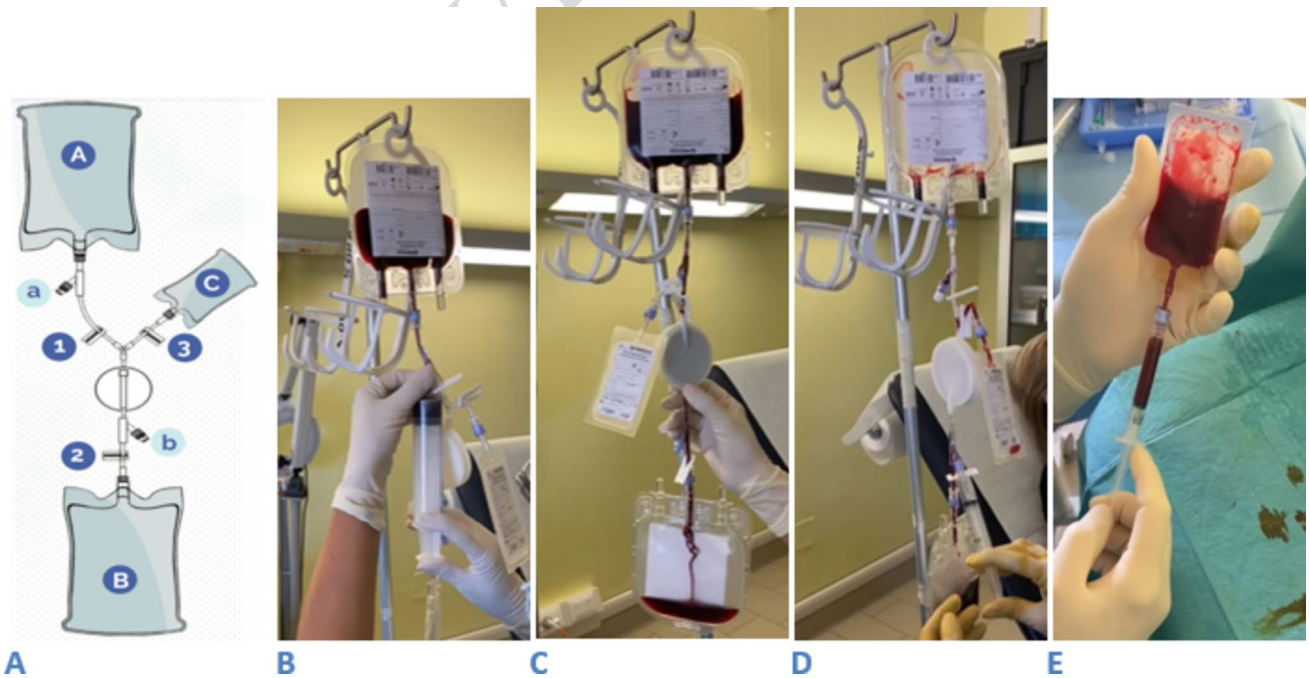

Fig. 1 PBMNC filtration technology. **A** Schematic of the MonoCytes system. **B** Withdrawal. **C** Processing. **D** and **E** Filter backwashing recovery

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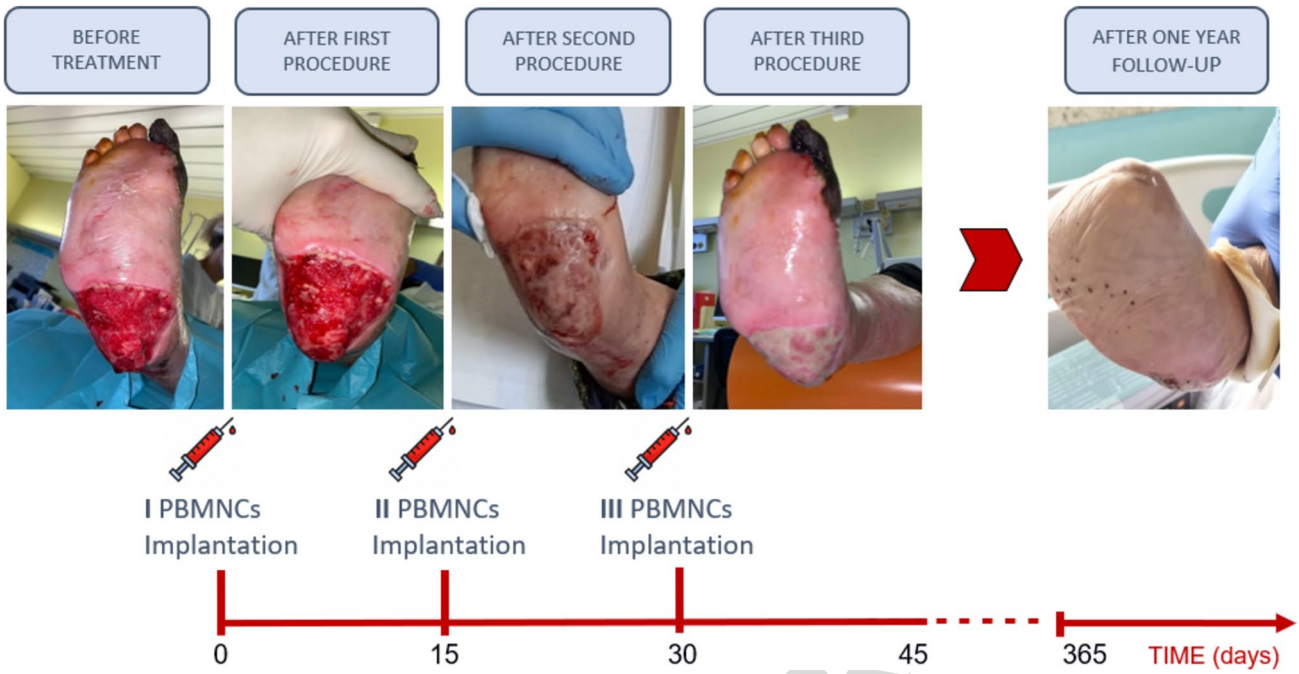

Fig. 2 Wound-healing process after each autologous PBMNC implantation procedure and at 1-year follow-up

critical limb ischemia in diabetic foot. Treatment was successful and prevented limb loss.

Peripheral blood mononuclear cells (lymphocytes, monocytes, and a small fraction of endothelial progenitor cells) have vascular regeneration properties that include three main mechanisms: angiogenesis, macrophage polarization, and paracrine stimulation [6]. Monocytes maintain their angiogenic potency in diabetic patients, even though endothelial progenitor cells are dysfunctional in diabetic vascular wounds because of hyperglycemia and oxidative stress, which explains how wound-implanted PBMNCs can effectively respond to damage by stimulating new vessel formation [7]. Moreover, PBMNCs switch the M1 macrophage phenotype (pro-inflammatory) to an anti-inflammatory phenotype M2 devoted to tissue repair [8]. It is noteworthy that in peripheral artery disease macrophages mostly present the M1 phenotype, perpetuating an inflammatory state and impairing new granulation tissue formation [9]. Finally, PBMNCs release pleiotropic paracrine and pro-angiogenic factors that stimulate tissue regeneration and enhance healing [10].

Autologous cell therapy has been reported to have the potential to modify the natural history of no-option critical limb ischemia, in terms of major amputation and overall survival rates [4, 11]. Several randomized clinical trials have demonstrated that PBMNC treatment is safe and effective for vascular regeneration in patients with critical limb ischemia and diabetic foot ulcer [6]. Though these studies have some limitations (heterogeneity, small sample size, and short follow-up), their results are promising for the treatment of no option-critical limb ischemia patients.

Few studies have examined cell therapy in ESKD patients with critically ischemic limbs [3]. Some studies identified dialytic therapy as a negative predictor for the efficacy of PBMNC treatment [12, 13]. However, Hoshino et al. [14] noticed that PBMNC treatment in seven diabetic patients on HD with severe intractable peripheral artery disease was associated with an improvement in pain scores and quality of life at 24 weeks, without major adverse events. Angiographic findings and ulcer size improved in 3 out of 7 and 3 out of 4 patients, respectively. In our patient, the PBMNC extraction procedure was different from that employed in most previous studies, since PBMNCs were harvested by apheresis after pre-treatment with subcutaneous granulocyte colony-stimulating factor [14].

The selective filtration-based device that we used to obtain the PBMNC concentrate offers several potential advantages. It is a single-step, closed-loop system, which implies minimization of contamination risks, especially if compared with apheresis which requires several manipulation phases. Moreover, it is an easily reproducible and fast procedure, with quicker cell processing; the blood is filtered by gravity, avoiding acts that could alter or stress cells (e.g., centrifugation), and the core of the system is a membrane that can select cells by dimensional filtration, thus eliminating inflammatory cells. Two recent studies in no option-critical limb ischemia patients with diabetic foot ulcers showed a positive clinical outcome (reduction of the amputation rate,

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improved wound healing) at 1- [15] or 2- [5] year follow-up upon treatment with PBMNCs obtained through filtration systems.

The overall cost of the approach here proposed may cause concern in times of restricted health care resources. However, the cost should be compared with the much higher cost of a major amputation, including inpatient days, operating room needs, post-surgical management in intensive care, possible complications, supportive care during convalescence, and rehabilitation. Furthermore, and first of all, we should consider the social and psychological impact that would result from a major amputation. Patients on HD suffer from severe symptoms and psychological burden that can have a profound impact on quality of life, and that could worsen after a stressful event like an amputation.

The case herein presented reports a successful example of real-life management of such challenging patients. This approach, that needs further larger-scale validation, may be considered for HD patients suffering from peripheral artery disease/critical limb ischemia, particularly those who are ineligible for revascularization.

Author contributions All authors have been personally and actively involved in substantial work leading to the paper. All authors read and approved the final manuscript.

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Data availability Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Ethical approval was not sought in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.

Consent to participate Informed consent was obtained from the participant included in the study.

Consent to publish The participant has consented to the submission of the case report to the journal.

References

1. Criqui MH, Matsushita K, Aboyans V, Hess CN, Hicks CW, Kwan TW, McDermott MM, Misra S, Ujueta F et al (2021) Lower extremity peripheral artery disease: contemporary epidemiology, management gaps, and future directions: a scientific statement from the American Heart Association. Circulation 144(9):e171–e191. https://doi.org/10.1161/CIR.0000000000001005
2. Morbach S, Furchert H, Gröblinghoff U, Hoffmeier H, Kersten K, Klauke GT (2012) Long-term prognosis of diabetic foot patients and their limbs: amputation and death over the course of a decade. Diabetes Care 35(10):2021–2027. https://doi.org/10.2337/dc12-0200
3. Gilhotra RA, Rodrigues BT, Vangaveti VN, Malabu UH (2016) Prevalence and risk factors of lower limb amputation in patients with end-stage renal failure on dialysis: a systematic review. Int J Nephrol. https://doi.org/10.1155/2016/4870749
4. Rigato M, Monami M, Fadini GP (2017) Autologous cell therapy for peripheral arterial disease systematic review and meta-analysis of randomized, nonrandomized, and noncontrolled studies. Circ Res 120:1326–1340. https://doi.org/10.1161/CIRCRESAHA.116.309045
5. Scatena A, Petruzzi P, Maioli F, Lucaroni F, Ambrosone C, Ventoruzzo G, Liistro F, Tacconi D, Di Filippi M, Attempati N, Palombi L, Ercolini L, Bolognese L (2021) Autologous peripheral blood mononuclear cells for limb salvage in diabetic foot patients with no-option critical limb ischemia. J Clin Med 10(10):2213. https://doi.org/10.3390/jcm10102213
6. Zamboni M, Pedriali M, Ferretto L, Scian S, Ghirardini F, Bozza R, Martini R, Irsara S (2022) PBMNCs treatment in critical limb ischemia and candidate biomarkers of efficacy. Diagnostics 12(5):1137. https://doi.org/10.3390/diagnostics12051137
7. Kaushik K, Das A (2019) Endothelial progenitor cell therapy for chronic wound tissue regeneration. Cytotherapy 21(11):1137–1150. https://doi.org/10.1016/j.jcyt.2019.09.002
8. Kloc M, Ghobrial RM, Wosik J, Lewicka A, Lewicki S, Kubiak JZ (2019) Macrophage functions in wound healing. J Tissue Eng Regen Med 13:99–109. https://doi.org/10.1002/term.2772
9. Qing C (2017) The molecular biology in wound healing and non-healing wound. Chin J Traumatol 20(4):189–193. https://doi.org/10.1016/j.cjtee.2017.06.001
10. Beer L, Mildner M, Gyöngyösi M, Ankersmit HJ (2016) Peripheral blood mononuclear cell secretome for tissue repair. Apoptosis 21:1336–1353. https://doi.org/10.1007/s10495-016-1292-8
11. Beltrán-Camacho L, Rojas-Torres M, Durán-Ruiz MC (2021) Current status of angiogenic cell therapy and related strategies applied in critical limb ischemia. Int J Mol Sci 2:2335. https://doi.org/10.3390/ijms22052335
12. Kawamura A, Horie T, Tsuda I, Abe Y, Yamada M, Egawa H, Iida J-I, Sakata H, Onodera K, Tamaki T, Furui H, Kukita K, Meguro J-I, Yonekawa M, Tanaka S (2006) Clinical study of therapeutic angiogenesis by autologous peripheral blood stem cell (PBSC) transplantation in 92 patients with critically ischemic limbs. J Artif Organs 9:226–233. https://doi.org/10.1007/s10047-006-0351-2
13. Horie T, Onodera R, Akamastu M, Ichikawa Y, Hoshino J, Kaneko E, Iwashita C, Ishida A, Tsukamoto T, Teramukai S, Fukushima M, Kawamura A, Japan Study Group of Peripheral Vascular Regeneration Cell Therapy (JPRCT) (2010) Long-term clinical outcomes for patients with lower limb ischemia implanted with G-CSF-mobilized autologous peripheral blood mononuclear cells. Atherosclerosis 208:461–466. https://doi.org/10.1016/j.atherosclerosis.2009.07.050
14. Hoshino J, Ubara Y, Hara S, Sogawa Y, Suwabe T, Higa Y, Nakanishi S, Sawa N, Katori H, Takemoto F, Fujimoto Y, Ohta E, Ohara K, Takaichi K (2007) Quality of life improvement and long-term effects of peripheral blood mononuclear cell transplantation for severe arteriosclerosis obliterans in diabetic patients on dialysis. Circ J 71(8):1193–1198. https://doi.org/10.1253/circj.71.1193
15. Panunzi A, Madotto F, Sangalli E, Riccio F, Sganzaroli AB (2022) Results of a prospective observational study of autologous peripheral blood mononuclear cell therapy for no option critical limb threatening ischemia and severe diabetic foot ulcers. Cardiovasc Diabetol 21(1):196. https://doi.org/10.1186/s12933-022-01629-y