European Review for Medical and Pharmacological Sciences 2024; 28: 3590-3597

Peripheral Blood Mononuclear Cells (PB-MNCs) for the treatment of chronic tissue dystrophy in a complex case of lower limb reconstruction

S. CARELLA[1], L. PATANÈ[1], M. CASTRECHINI[1], F. LO TORTO[1], G. MARRUZZO[1], U. REDI[1], M. GRECO[2], D. RIBUFFO[1], M.G. ONESTI[1]

[1]Department of Surgery "P. Valdoni", Plastic Surgery Unit, "Sapienza" University of Rome, Rome, Italy
[2]Plastic and Reconstructive Surgery Unit, Magna Graecia University of Catanzaro, Catanzaro, Italy

Abstract. – BACKGROUND: Recently, the infiltration of a subpopulation of cells represented by mononucleated cells extracted from peripheral blood [Peripheral Blood-Mononuclear Cells (PB-MNCs)] is becoming a useful technique for medical and surgical regenerative procedures. Due to the angiogenetic and regenerative properties of PB-MNCs, the infiltration of these cells is, in our opinion, a new option indicated in the treatment of pathologies characterized by tissue dystrophy, loss of vascularization, and non-healing wounds.

CASE PRESENTATION: A 25-year-old active smoker patient was diagnosed with Rhabdomyosarcoma of the anterior tibial muscle of his left leg and treated with neoadjuvant chemo- and radiotherapy (RT). After the tumor excision, the patient developed wound dehiscence with bone exposure and a perilesional radiation-induced chronic dermatitis characterized by skin dyschromia and hair thinning along the treated area. The patient underwent surgical debridement and reconstruction with autologous skin grafts and dermal substitutes, with poor outcomes due to graft failure. The patient was subsequently treated with surgical debridement and coverage with a reverse sural fascia-cutaneous flap. After 13 days, wound dehiscence was observed, and reconstruction of the dehiscent areas was performed with a split-thickness autologous skin graft with no success. After wound debridement, a new split-thickness skin graft was performed, and a concentrate of autologous PB-MNCs was injected in the flap and perilesional skin. After 14 days, graft take was reached, and improvements in perilesional tissue tropism were noted. At 2 months follow-up, the patient appeared completely healed.

CONCLUSIONS: In our opinion, the use of PB-MNCs to treat conditions characterized by tissue dystrophy, which require neoangiogenesis and cell regeneration, can be a useful and unconsidered technique that could be utilized to improve tissue tropism. Furthermore, prospective trials are necessary to validate our observations.

Key Words:
PB-MNCs, Regenerative surgery, Regenerative medicine, Sarcoma, Monocyte, Tissue dystrophy.

Introduction

Recently, the infiltration of a subpopulation of cells represented by mononucleated cells extracted from peripheral blood [Peripheral Blood-Mononuclear Cells (PB-MNCs)] is becoming a useful technique for medical and surgical regenerative procedures[1]. PB-MNCs derive from hematopoietic bone marrow cells, representing 3-8% of the white blood cells (WBC), and are resident in tissues as macrophages. Their role seems to be crucial in host defense, promotion, and resolution of inflammation, as well as in supporting cell proliferation and tissue repair processes[2]. Solid outcomes[1,3,4] show how human circulating monocytes are multipotent progenitors with high plasticity, which makes these cells capable of changing their phenotype in response to different environmental stimuli.

Several studies[5-7] demonstrated PB-MNCs' ability to induce angiogenesis and arteriogenesis, as occurs in tumor vascular supply, modulating the formation of tumoral blood and lymphatic vessels[5] as a consequence of local tissue ischemia.

Therefore, because of the angiogenetic and regenerative properties of PB-MNCs, the infiltration of these cells is, in our opinion, a new option indicated in the treatment of pathologies characterized by tissue dystrophy, loss of vascularization, and non-healing wounds.

Corresponding Author: Ugo Redi, MD; e-mail: ugo.redi@uniroma1.it

Here, we present a case of a chronic non-healing wound following the excision of a rhabdomyosarcoma of the leg. After conservative management with locally advanced dressings, the perilesional skin still appeared dyschromic, with vascular sufferance, and not fully healed. Considering the newest trends in regenerative surgery, we decided to adopt the autologous PB-MNC inoculation as a treatment option.

Case Report

Patient History and Clinical Presentation

A 25-year-old male patient came to our attention with a full-thickness wound with bone exposure (Figure 1a). In his clinical history, he reported a Rhabdomyosarcoma of his left leg treated with neo-adjuvant chemo- and radiotherapy and demolition surgery. The tumor was T2N0M0, stage IIIA, Grade G2, and so treated with chemotherapy composed of anthracycline and ifosfamide, followed by 50 grays (Gy) of radiation fractioned in 2 Gy per day. Especially because of the radiation therapy, the perilesional skin appeared dyschromic, sclerotic, and dyskeratotic with hair bulb loss. The patient underwent many reconstructive surgeries in other institutions, such as dermo-epidermal graft alone or combined with a dermal substitute, outcoming without healing improvements.

Patient and Wound Management

A cultural swab with an antibiogram was performed, and empirical antibiotic treatment with amoxicillin/clavulanic acid 1 g every 8 hours was set. In the meantime, treatment with Vacuum Assisted Closure (VAC) therapy was applied for 7 days before the reconstructive surgery in our institution. After treatment with VAC therapy (Figure 1b), granulation tissue was detected in the lateral portion of the wound, while bone exposure was still evident in the medial part.

The cultural swab resulted positive for *Pseudomonas aeruginosa*, and specific antibiotic therapy was set with an infusion of 2 g of Ceftazidime and 0.5 g of Avibactam every 8 hours/day for one week.

Therefore, reconstruction with a fascia-cutaneous sural flap was opted for (Figure 1c), considering the iatrogenic absence of the anterior tibial artery, probably injured during previous treatments, which excluded any microsurgical procedures. Contraindications were represented by the patient's poor compliance and smoking habits. The donor site was reconstructed using a dermal substitute and partial thickness autologous dermo-epidermal graft.

During the first post-operative days, the wound showed partial dehiscence of the suture placed between the flap (Figure 2a) and the peri-lesional skin, probably due to the tissue damage induced by radiotherapy. In fact, during the suturing time following the flap inset, the poor quality of the

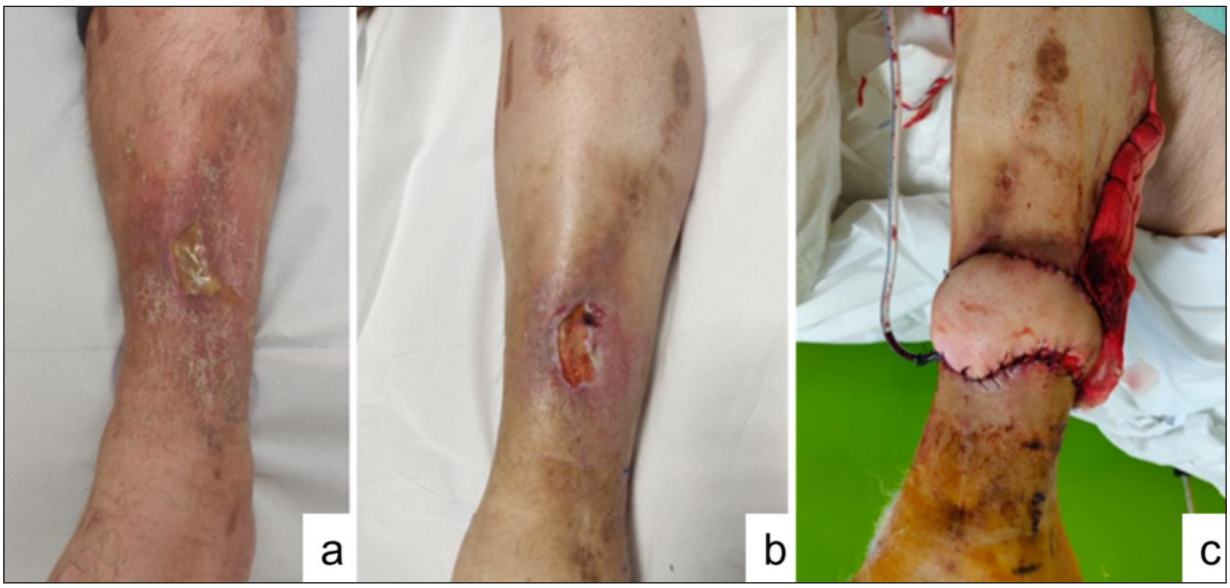

Figure 1. A full-thickness wound with bone exposure of the left leg before (**a**) and after (**b**) 7 days of NPWT. Wound reconstruction with a reverse fascia-cutaneous sural flap (**c**).

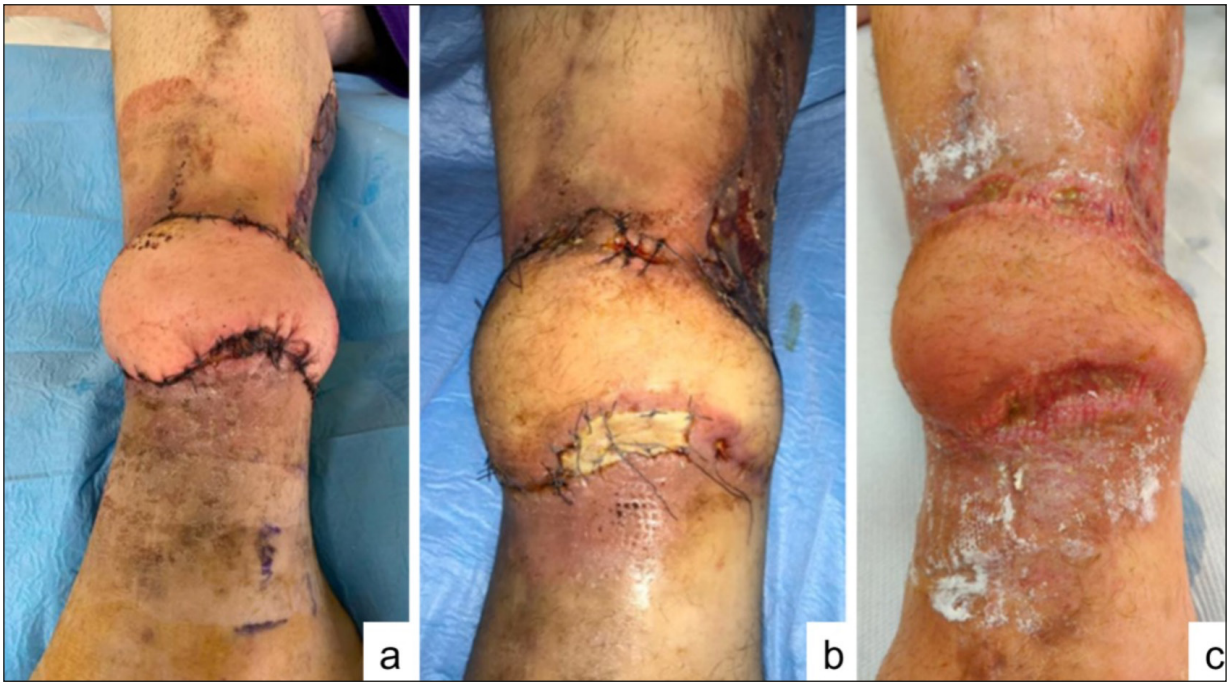

Figure 2. Partial suture dehiscence in the first postoperative days (**a**). At 8 days postoperatively, the sural flap is vital. The dehiscence in the lower part is treated with a dermo-epidermal graft and the upper part by primary reapproximation (**b**). Complete failure of this procedure is noted (**c**).

perilesional skin was noted, making difficult the primary suture of the wound. At 8 days, the flap appeared vital, and the dehiscence in the lower part of the wound appeared to be wider. The dermo-epidermal graft in the flap donor site underwent partial take (Figure 2a). At one month, a surgical revision of the wound was practiced with a dermo-epidermal skin graft, and VAC therapy was applied either on the donor site or the dehiscent area (Figure 2b).

At 2 months, non-engraftment of the dermo-epidermal skin graft in the lower area was noted, and a new dehiscence of the upper portion developed (Figure 2c). Afterward, VAC therapy was still carried out on the non-re-epithelialized areas. A new cultural swab was performed free from bacterial contamination.

At 3 months, one more surgical debridement was performed, and reconstruction with partial thickness and meshed dermo-epidermal graft was planned to close the dehisced area. At the same time of surgery, PB-MNCs were injected both in the flap and in the peri-lesional skin, and VAC therapy was applied over the skin graft (Figure 3). Dressing changes were done 2 times per week following VAC-therapy removal in postoperative day 14. Two months after the last surgery, the donor and recipient sites were completely healed, with an improvement of the peri-lesional skin tropism (Figure 3d).

Surgical Technique

The collection of mononuclear cells was performed in the operating room by sampling 120 ml of peripheral venous blood. A disposable kit was used as a closed-circuit system, which is in line with current international guidelines for the preparation of biological products (GMP). The blood drawn with a 120 ml syringe with the addition of 5 ml of heparin (or two 60 ml syringes, each with the addition of 2.25 ml of heparin) was injected into the kit bag. From here, the fluid passed through a selective gravity filter (Figures 3a and 3b). The negatively charged filter attracted the positive toti-nuclear cells (TNCs), while the residual cells (platelets and red blood cells, which are devoid of nuclei and negatively charged) bypassed the filter and were collected in a waste bag. The mononuclear cells were recovered by backwashing the filter with 10 cc of saline, which allowed the detachment of the cells from the filter walls.

Infiltrations were performed with a 1 ml syringe and 21-gauge needle every 1 cm^2 of the involved area at the subdermal level, in the amount of

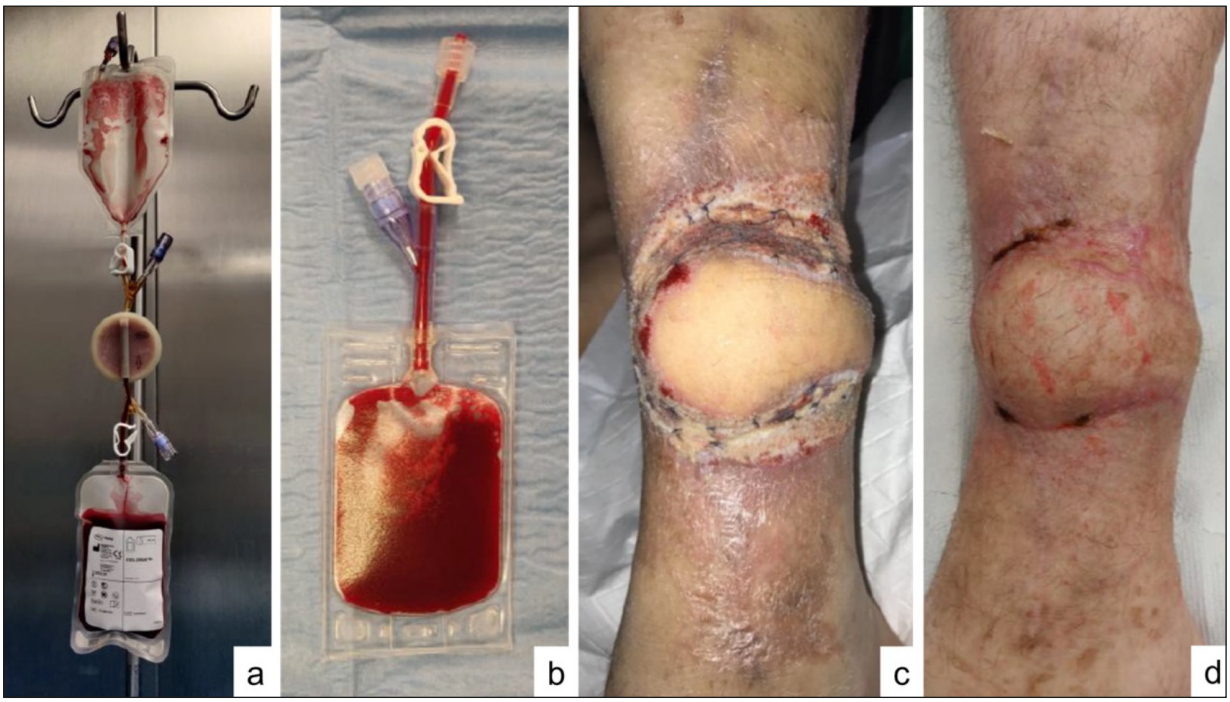

Figure 3. Filtration of 100 ml of peripheral blood, flew by gravity through the filter (**a**). Collection of the cell concentrate in a sterile bag (**b**). 10 syringes of PB-MNCs concentrate were then extracted from the bag and injected in multiple spots (**c**). Twenty days result following this procedure (**d**).

approximately 0.25 ml/cm^2 (Figure 3c). The infiltration was performed by the same operator. The entire procedure is intended to avoid granulocyte contamination, which is reported to impair results.

Discussion

The clinical outcomes reached by using PB-MNCs are supported by their angiogenetic and vasculogenetic properties. Vascular renovation is due to Circulating Endothelial Progenitor Cells (EPCs), a subpopulation of the secretive fraction of PB-MNCs in the peripheral blood that releases angiogenetic factors (VEGF-A, FGF, IGF-1)[8]. As it was demonstrated that EPCs are reduced in number and function in diabetic patients[9,10], PB-MNCs may represent a potentially useful treatment for patients with vascular and dystrophic illness.

Animal and Human Research

In animal models[11] of diabetes mellitus, transplantation of bone- or blood-derived EPCs has been demonstrated to improve the blood flow, neovascularization, and healing of diabetic ulcers in mice.

In some animal model studies[12] of critical ischemia of the limb with tissue injury, improvement of the ABI index (Ankle Brachial Index) was demonstrated as a reduction in the surface ulcer area and a reduction in pain after PB-MNCs implantation. Furthermore, it was found[13,14] that the whole injection of PB-MNCs plays a more important role than only an EPC fraction in the production of VEGF.

Recent studies[15-17] showed how local intramuscular transplantation PB-MNCs, and granulocyte colony-stimulating factor (G-CSF) had effective results in patients with no-option critical limb ischemia (CLI) who were not considered suitable to undergo surgical bypass or percutaneous transluminal angioplasty.

In addition to the examples above, it is fully clear that macrophages play important roles in the pathogenesis of autoimmune diseases, as in rheumatoid arthritis[18,19].

Wound Healing

Above all, scientific research continued to investigate the biological processes of neoangiogenesis and how these cells act by promoting the growth of new and functional vessels in damaged tissue. This is especially true because

neovascularization is significantly reduced in the absence of monocytes, and the increase in capillary density is proportional to monocyte accumulation[20,21].

Usually, monocytes are resident in the healthy tissue in their quiescent form, named "M0", in the dermis with fibroblasts. When a skin lesion develops in the reparative phase, monocytes "M0" are activated by specific cytokines and transformed into inflammatory macrophages (M1). This macrophage status maintains the inflammatory environment in the wound. As tissue repair proceeds, macrophages polarize into reparative macrophages (M2) due to the stimuli of interleukins, glucocorticoids, and TGFb1. Numerous studies[22-24] show that in the chronic stage of inflammation, M2 macrophage polarization - which is reported to be necessary to the healing process- interrupts. In fact, in vasculopathy and diabetic patients, as well as in patients with some inflammatory diseases and aging, acute inflammation becomes chronic due to macrophage M1 activation[22-24].

This phenotype is characterized by the persistence of the inflammatory state, which prevents physiological healing. Due to this fact, it has been shown[25,26] that by promoting the M2 phenotype through specific polarization of local M1 polarized macrophages, the inflammatory response can be redirected toward the healing phase. This was demonstrated[27] by implanting a cellular concentrate of monocytes into the target non-healing tissue, inducing macrophage polarization from M1 to M2.

Once polarized in the M2 subtype, macrophages recruit fibroblasts into the wound and promote their differentiation into myofibroblasts. Moreover, this cell population releases anti-inflammatory and pro-angiogenic factors, which facilitate the resolution of inflammation, recruit endothelial cells, and induce fibroblasts to deposit new extracellular matrix (ECM).

Our institution fully valued and applied the functional plasticity of human multipotential cell progenitors to support surgical outcomes and as first-line therapy in several pathologies. We widely applied cell-based therapy with cultured adipose-derived stromal cells (ADSCs) in the treatment of cutaneous manifestations in patients affected by systemic sclerosis (SSc), showing improvements in either mouth functional disability and physiological and pathological vulvar dystrophies[28-31].

However, a growing body of preclinical evidence[32-35] shows that growth factors, especially adipocytes and adipose-derived stromal cells, may have pro-tumorigenic potential. Despite that, no clear indication from clinical studies has demonstrated an increased risk of cancer recurrence upon adipose cell injections, and, on the whole, there is no evidence linking the same properties to monocyte injection[32-35].

Following our experience and well aware of all the above scientific evidence, we thought PB-MNC-based therapy would find its application in the treatment of chronic wounds since PB-MNCs could induce increased vascularization due to their angiogenic properties and tissue tropism improvement thanks to their eutrophic effect.

Due to the vascular desert characterizing tissues affected by radiodermatitis, we decided to exploit the angiogenetic property of the PB-MNC in order to improve the peri-lesional skin tropism. The process of monocyte-macrophage differentiation is, therefore, potentially attractive when seeking therapeutic targets to amplify or modulate the inflammatory response. In our case, the reconstruction with the fascia-cutaneous sural flap led to all the mentioned conditions of metabolic stress, vascular insufficiency, and skin suffering due to radio- and chemotherapy and the smoking attitude of the patient. Considering the importance of a well-vascular supply to the flap, the local conditions limited the flap's integration in this case, as similarly happened with dermo-epidermal grafts.

About two months after the PB-MNCs injection, donor and recipient sites were completely healed, with an improvement in the peri-lesional skin tropism, demonstrating a synergic efficacy between the skin graft and the PB-MNCs local inoculation (Figure 4). Even if the follow-up is short, a current limitation of this study is that we could detect patient healing in a reasonable time after multiple failed treatment options.

Conclusions

In our opinion, the use of PB-MNCs to treat conditions characterized by tissue dystrophy, which require neoangiogenesis and cell regeneration, can be a useful and unconsidered technique that could be utilized to improve tissue tropism. Furthermore, prospective trials are necessary to validate our observations.

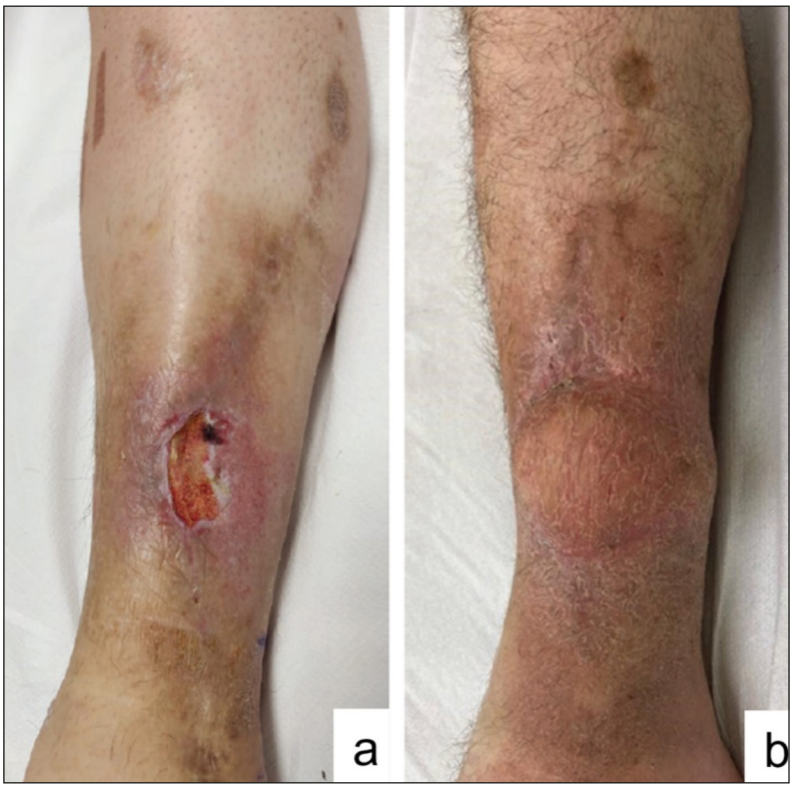

Figure 4. Pre- (**a**) and postoperative (**b**) status: about 2 months after PB-MNCs injection, donor and recipient sites were completely healed, with an improvement in the peri-lesional skin tropism, demonstrating a synergic efficacy between skin grafting and PB-MNCs local inoculation.

Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Approval
The study was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments. The ethics approval was waived due to the nature of the study.

Informed Consent
Informed consent was obtained from the patient.

Authors' Contributions
S. Carella: conception, design and critical revision; L. Patanè: conception, design, drafting of the article; M. Castrechini: drafting of the article; F. Lo Torto: critical revision; U. Redi: drafting of the article, interpretation of data; M. Greco: critical revision; D. Ribuffo: critical revision and validation; M.G. Onesti: conception, critical revision, validation; G. Maruzzo: interpretation of data.

Availability of Data and Materials
Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

Funding
None.

ORCID ID
Ugo Redi: 0000-0003-0136-4263
Luca Patanè: 0000-0003-4811-5248

References

1) Seta N, Kuwana M. Human circulating monocytes as multipotential progenitors. Keio J Med 2007; 56: 41-47.
2) Lavin Y, Merad M. Macrophages: gatekeepers of tissue integrity. Cancer Immunol Res 2013; 1: 201-209.
3) Stout RD, Suttles J. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J Leukoc Biol 2004; 76: 509-513.
4) Coillard A, Segura E. In vivo Differentiation of Human Monocytes. Front Immunol 2019; 10: 1907.
5) Delprat V, Michiels C. A bi-directional dialog between vascular cells and monocytes/macrophages regulates tumor progression. Cancer Metastasis Rev 2021; 40: 477-500.
6) Höckel M, Schlenger K, Doctrow S, Kissel T, Vaupel P. Therapeutic angiogenesis. Arch Surg 1993; 128: 423-429.
7) Falero-Diaz G, Barboza CA, Pires F, Fanchin M, Ling J, Zigmond ZM, Griswold AJ, Martinez L, Vazquez-Padron RI, Velazquez OC, Lassance-Soares RM. Ischemic-Trained Monocytes Improve Arteriogenesis in a Mouse Model of Hindlimb Ischemia. Arterioscler Thromb Vasc Biol 2022; 42: 175-188.
8) Fadini GP, Miorin M, Facco M, Bonamico S, Baesso I, Grego F, Menegolo M, de Kreutzenberg SV, Tiengo A, Agostini C, Avogaro A. Circu-

lating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. J Am Coll Cardiol 2005; 45: 1449-1457.

9) Loomans CJ, de Koning EJ, Staal FJ, Rookmaaker MB, Verseyden C, de Boer HC ,Verhaar MC, Braam B, Rabelink TJ, van Zonneveld AJ. Endothelial progenitor cell dysfunction: a novel concept in the pathogenesis of vascular complications of type 1 diabetes. Diabetes 2004; 53: 195-199.

10) Tepper OM, Galiano RD, Capla JM, Kalka C, Gagne PJ, Jacobowitz GR, Levine JP, Gurtner GC. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002; 106: 2781-2786.

11) Schatteman GC, Hanlon HD, Jiao C, Dodds SG, Christy BA. Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. J Clin Invest 2000; 106: 571-578.

12) Minamino T, Toko H, Tateno K, Nagai T, Komuro I. Peripheral-blood or bone-marrow mononuclear cells for therapeutic angiogenesis? Lancet 2002; 360: 2083-2084.

13) Dubsky M, Jirkovska A, Bem R, Fejfarova V, Pagacova L, Sixta B, Varga M, Langkramer S, Sykova E, Jude EB. 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. Diabetes Metab Res Rev 2013; 29: 369-376.

14) Han JW, Sin MY, Yoon YS. Cell therapy for diabetic neuropathy using adult stem or progenitor cells. Diabetes Metab J 2013; 37: 91-105.

15) Sermsathanasawadi N, Pruekprasert K, Chruewkamlow N, Kittisares K, Warinpong T, Chinsakchai K, Wongwanit C, Ruangsetakit C, Mutirangura P. Peripheral blood mononuclear cell transplantation to treat no-option critical limb ischaemia: effectiveness and safety. J Wound Care 2021; 30: 562-567.

16) Panunzi A, Madotto F, Sangalli E, Riccio F, Sganzaroli AB, Galenda P, Bertulessi A, Barmina MF, Ludovico O, Fortunato O, Setacci F, Airoldi F, Tavano D, Giurato L, Meloni M, Uccioli L, Bruno A, Spinetti G, Caravaggi CMF. 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 2022; 21: 196.

17) Khodayari S, Khodayari H, Ebrahimi-Barough S, Khanmohammadi M, Islam MS, Vesovic M, Goodarzi A, Mahmoodzadeh H, Nayernia K, Aghdami N, Ai J. Stem Cell Therapy in Limb Ischemia: State-of-Art, Perspective, and Possible Impacts of Endometrial-Derived Stem Cells. Front Cell Dev Biol 2022; 10: 834754.

18) Alivernini S, MacDonald L, Elmesmari A, Finlay S, Tolusso B, Gigante MR, Petricca L, Di Mario C, Bui L, Perniola S, Attar M, Gessi M, Fedele AL, Chilaka S, Somma D, Sansom SN, Filer A, McSharry C, Millar NL, Kirschner K, Nerviani A, Lewis MJ, Pitzalis C, Clark AR, Ferraccioli G, Udalova I, Buckley CD, Gremese E, McInnes IB, Otto TD, Kurowska-Stolarska M. Distinct synovial tissue macrophage subsets regulate inflammation and remission in rheumatoid arthritis. Nat Med 2020; 26: 1295-1306.

19) Lin Y, Huang M, Wang S, You X, Zhang L, Chen Y. PAQR11 modulates monocyte-to-macrophage differentiation and pathogenesis of rheumatoid arthritis. Immunology 2021; 163: 60-73.

20) Huang P, Li S, Han M, Xiao Z, Yang R, Han ZC. Autologous transplantation of granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells improves critical limb ischemia in diabetes. Diabetes Care 2005; 28: 2155-2160.

21) De Angelis B, Gentile P, Orlandi F, Bocchini I, Di Pasquali C, Agovino A, Gizzi C, Patrizi F, Scioli MG, Orlandi A, Cervelli V. Limb rescue: a new autologous-peripheral blood mononuclear cells technology in critical limb ischemia and chronic ulcers. Tissue Eng Part C Methods 2015; 21: 423-435.

22) Rodero MP, Khosrotehrani K. Skin wound healing modulation by macrophages. Int J Clin Exp Pathol 2010; 3: 643-653.

23) Miao M, Niu Y, Xie T, Yuan B, Qing C, Lu S. Diabetes-impaired wound healing and altered macrophage activation: a possible pathophysiologic correlation. Wound Repair Regen 2012; 20: 203-213.

24) Sindrilaru A, Peters T, Wieschalka S, Baican C, Baican A, Peter H, Hainzl A, Schatz S, Qi Y, Schlecht A, Weiss JM, Wlaschek M, Sunderkötter C, Scharffetter-Kochanek K. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. J Clin Invest 2011; 121: 985-997.

25) Kim H, Kim JJ, Yoon YS. Emerging therapy for diabetic neuropathy: cell therapy targeting vessels and nerves. Endocr Metab Immune Disord Drug Targets 2012; 12: 168-178.

26) Han JW, Sin MY, Yoon YS. Cell therapy for diabetic neuropathy using adult stem or progenitor cells. Diabetes Metab J 2013; 37: 91-105.

27) Willenborg S, Eming SA. Macrophages - sensors and effectors coordinating skin damage and repair. J Dtsch Dermatol Ges 2014; 12: 214-223.

28) Scuderi N, Ceccarelli S, Onesti MG, Fioramonti P, Guidi C, Romano F, Frati L, Angeloni A, Marchese C. Human adipose-derived stromal cells for cell-based therapies in the treatment of systemic sclerosis. Cell Transplant 2013; 22: 779-795.

29) Onesti MG, Fioramonti P, Carella S, Fino P, Marchese C, Scuderi N. Improvement of Mouth Functional Disability in Systemic Sclerosis Patients over One Year in a Trial of Fat Transplantation versus Adipose-Derived Stromal Cells. Stem Cells Int 2016; 2016: 2416192.

30) Giuseppina Onesti M, Carella S, Ceccarelli S, Marchese C, Scuderi N. The Use of Human Adipose-Derived Stem Cells in the Treatment of Physiological and Pathological Vulvar Dystrophies. Stem Cells Int 2016; 2016: 2561461.

31) Rosa I, Romano E, Fioretto BS, Matucci-Cerinic M, Manetti M. Adipose-derived stem cells: Pathophysiologic implications vs therapeutic potential in systemic sclerosis. World J Stem Cells 202113: 30-48.

32) Liu X, Zhao G, Huo X, Wang Y, Tigyi G, Zhu BM, Yue J, Zhang W. Adipose-Derived Stem Cells Facilitate Ovarian Tumor Growth and Metastasis by Promoting Epithelial to Mesenchymal Transition Through Activating the TGF-β Pathway. Front Oncol 2021; 11: 756011.

33) Piccotti F, Rybinska I, Scoccia E, Morasso C, Ricciardi A, Signati L, Triulzi T, Corsi F, Truffi M. Lipofilling in Breast Oncological Surgery: A Safe Opportunity or Risk for Cancer Recurrence? Int J Mol Sci 2021; 22: 3737.

34) Zhang Y, Daquinag A, Traktuev DO, Amaya-Manzanares F, Simmons PJ, March KL, Pasqualini R, Arap W, Kolonin MG. White adipose tissue cells are recruited by experimental tumors and promote cancer progression in mouse models. Cancer Res 2009; 69: 5259-5266.

35) Devalaraja S, Haldar M. Intratumoral Monocyte Transfer to Examine Monocyte Differentiation in the Tumor Microenvironment. STAR Protoc 2020; 1: 100188.