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Hyperconcentrated Platelet-Rich Plasma (High-PRP) for the treatment of a non-healing ulcer of the lateral malleolus: a case report and literature review

M. Fernández Viña[1,2,3], L. Camozzi[1,3], M. I. Spitaleri[1,2], R. Fernández Viña[1,3], G. Reinchisi[1]

[1]Stem Cell Therapy Argentina, San Nicolás de los Arroyos, Argentina
[2]Facultad de Medicina y Cs. de la Salud, Universidad Abierta Interamericana, Rosario, Argentina
[3]Fundación Don Roberto Fernández Viña, San Nicolás, Argentina

Corresponding Author: Matias Cristian Fernández Viña, MD; e-mail: Matias.FernandezVina@UAI.edu.ar

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Abstract

Clinical Case: **A 77-year-old man with a history of hypertension (stage 2) and multiple phalanx amputations of the right hand due to Raynaud's disease, referred to our regenerative medicine Clinic for a cutaneous ulceration (of arterial origin) of the right lateral malleolus (size: 4x3 cm), that had previously been refractory to a 3-year conventional medical (topical) treatment prescribed by a dermatologist. The patient showed a complete healing of the ulcer after five injections of hyperconcentrated platelet-rich plasma (High-PRP).**

Materials and Methods: **For each application of High-PRP, the extraction of 51 ml of blood was carried out aseptically using the closed vacuum system with sterile tubes for humans (BD) with ACD Solution A. Centrifugation was performed for 48 minutes (*according to the Stem Cell Therapy Argentina® private protocol*) to obtain a hyperconcentrated Pure Plasma Platelet product (an average concentration of 1,900,000 platelets/μl (± 400,000).**

Results: **Overall, 5 injections of High-PRP (3 ml per each injection) were administered 30 days apart. Each 3 ml injection consisted of 10 consecutive injections (0.3 ml per each), administered by clockwise rotation, with a 3 ml luer lock syringe and a 30G needle, without local anesthesia. After 180 days, the ulcer was completed closed.**

Conclusions: **PRP could be considered as a safe and cost-effective treatment for cutaneous wounds, aimed to promote the healing process and shorten wound healing time. Therefore, the use of PRP can be considered as a valid option for the treatment of chronic wounds of different etiologies.**

Introduction

Chronic non-healing leg ulcers represent a major health issue globally, resulting in relevant financial, social and psychologic implications, and high costs in terms of human and material resources[1]. Diabetes mellitus remains a major cause of non-traumatic lower-extremity amputations (NLEAs) across the world, with approximately 85% of lower limb amputations being preceded by foot ulcers[2-4]. Conventional therapies such as dressings, surgical debridement and even skin grafting do not always lead to successful wound healing, since these treatments are not able to provide a sufficient amount of growth factors that are deemed to be critical for the healing process[5,6]. Autologous platelet-rich plasma (PRP) is an inexpensive method used for the treatment of non-healing ulcers, as it promotes the localized healing potential of growth factors[6]. Here, we present the case of a 77-year-old patient who showed the complete healing of a chronic ulcer of the right lateral malleolus after five injections of hyperconcentrated platelet-rich plasma (High-PRP).

Case report

A 77-year-old man with a history of hypertension (stage 2) and multiple distal phalanx amputations of the right hand due to Raynaud's disease, referred to our re-

generative medicine Clinic for a cutaneous ulceration (of arterial origin) of the right lateral malleolus (size: 4x3 cm). The ulcer had previously been refractory to a 3-year conventional medical (topical) treatment prescribed by a dermatologist. The ulcer was not infected when we did the first physical examination. At the admission, the patient reported a 5-year use of aspirin (100 mg per day) and enalapril (5 mg per day). There was no laboratory evidence that the ulcer was related to diabetes. Doppler ultrasonography of the lower extremities revealed the presence of occlusive lesions of the right superficial femoral artery. These findings were indicative of the presence of peripheral vascular disease causing an ulcer of arterial origin.

Materials and Methods

For each application of High-PRP, the extraction of 51 ml of blood was carried out aseptically using the closed vacuum system with sterile tubes for humans (BD) with ACD Solution A. Centrifugation was performed for 48 minutes (*according to the Stem Cell Therapy Argentina®, private protocol*) to obtain a hyperconcentrated pure plasma platelet product through a ThermoFisher Scientific refrigerated centrifuge. The gradient centrifugation was performed under good laboratory practices by a physician trained in Advanced Therapies and Regenerative Medicine, in order to obtain an average concentration of 1,900,000 platelets/µl (± 400,000) by using a ThermoFisher Scientific laminar flow and positive pressure High-Efficiency Particulate Air (HEPA)-filtered air system. Prior to the injection of PRP, a quality control of the process and the product was performed through a Wiener Lab Counter 19 hematology analyzer. Written informed consent was provided by the patient for permission to receive high-PRP and to publish this case report.

Results

Overall, 5 injections of High-PRP (3 ml per each injection) were administered 30 days apart. Each 3 ml injection consisted of 10 consecutive injections (0.3 ml per each), administered by clockwise rotation, with a 3 ml luer lock syringe and a 30G needle, without local anesthesia. After 180 days, the ulcer was completed closed (Figure 1). Table 1 lists the different platelet doses used per each injection and the median dose employed during the follow-up.

Discussion

Chronic leg ulcers represent a significant financial burden on the health care systems worldwide[1]. Chronic cutaneous ulcers have different etiologies. The majority of leg ulcers are secondary to chronic venous insufficiency. Other highly prevalent lesions include arterial ulcers, pressure ulcers, neuropathic ulcers (mainly secondary to diabetes), and Martorell hypertensive ischemic leg ulcers[2,7].

Chronic pressure ulcers affect patient health, emotional state, and quality of life, causing considerable morbidity and mortality. Conventional treatment of these wounds with topical antibiotics and pharmacological growth factors can lead to beneficial results after a long-term period, due to the chronic inflammatory state and the senescence of local reparative cellular reprogramming, which result in high costs for healthcare systems globally[1,5,8].

The biochemical microenvironment promoting the chronic nature of these lesions is characterized by an abnormally prolonged inflammatory phase, due to an increase in proinflammatory cytokines and a high metalloproteinase activity. Hypoxia and repeated infections induce the expression of a number of proteolytic enzymes, resulting in a consequent deficiency of growth factors and fibrin, which impairs the wound healing process. Levels of matrix metalloproteinases (MMPs), specifically MMP2 and MMP9, are increased in chronic wounds[9]. Moreover, tissue inhibitor of metalloproteinase-1 (TIMP-1) is decreased in nonhealing chronic wounds, resulting in increased levels of collagenolytic activity[10]. Excessive expression and activation of some MMPs in chronic cutaneous wounds correlates with delayed wound healing and severity of ulcers[11,12].

Platelet-rich plasma (PRP) is an autologous therapeutic technology that is gaining interest in regenerative medicine due to its potential to stimulate and accelerate tissue repair and regeneration[13-15]. Platelets embedded within blood clots have a central role in the physiological process of wound healing as regulators of inflammation, angiogenesis, cell migration and proliferation[15,16]. Platelets play a crucial role in wound healing process due to their fundamental role in hemostasis and production of cytokines and growth factors[11]. Specifically, PRP is defined as an autologous biological product derived from the patient's blood; after multiple centrifugation processes, a plasma fraction is obtained with

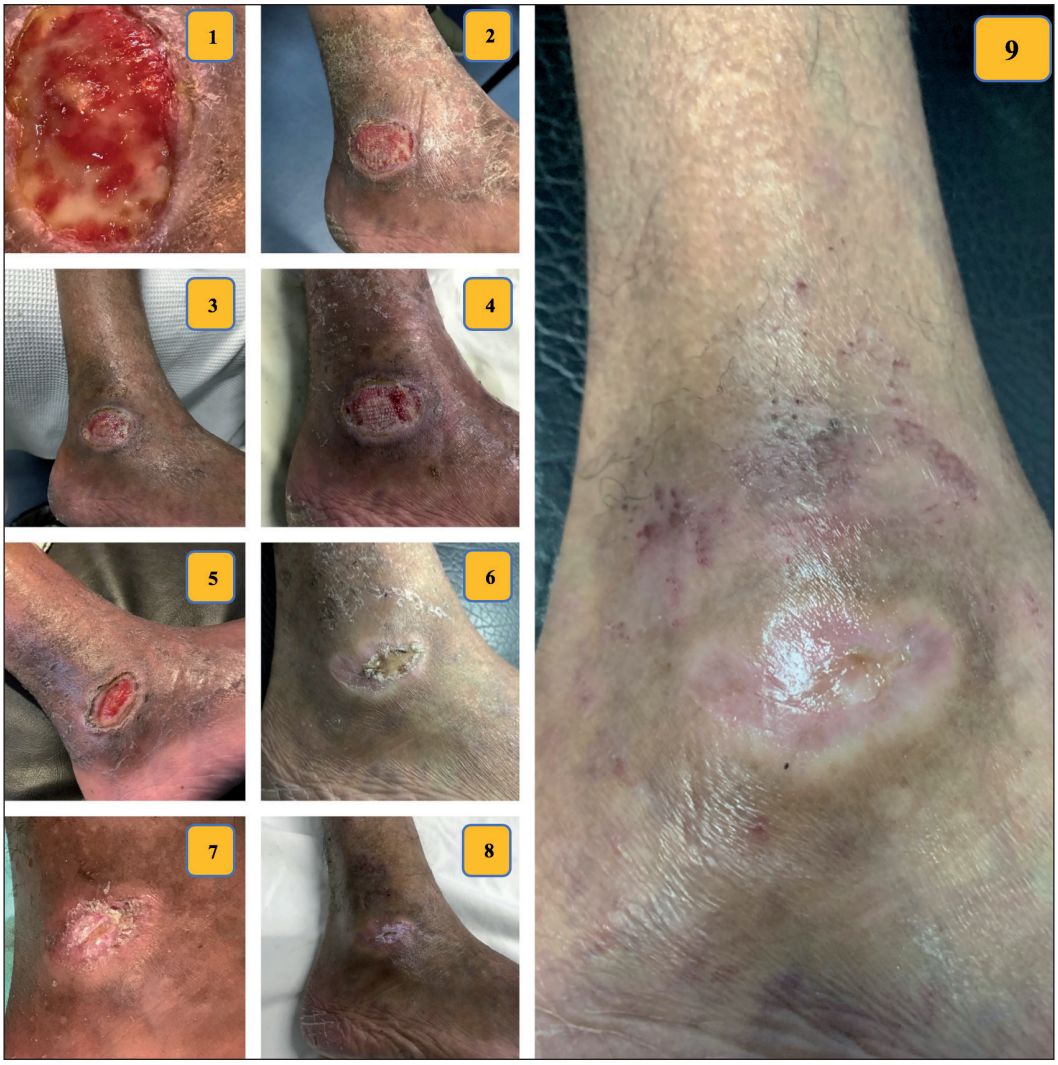

Figure 1. Gradual healing of the ulcer of the right lateral malleolus after five High-PRP injections. 1. Baseline (T0): **1st High-PRP injection.** 2. 10th day: Follow-up visit. 3. 30th day: **2nd High-PRP injection.** 4. 60th day: **Follow-up visit and 3rd High-PRP injection**. 5. 90th day: **4th High-PRP injection.** 6. 120th day: **Follow-up visit and 5th High-PRP injection**. 7. 127th day: Follow-up visit. 8. 140th day: Follow-up visit. 9. 180th days: Follow-up visit.

a platelet concentration higher than that in circulating blood[15,17,18]. There are several growth factors which are known to be involved in the wound healing process, such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factors (IGF-1 and IGF-2), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), and keratinocyte growth factor (KGF)[5,9,18-21].

PRP has a wide range of clinical healing applications in several diseases and conditions, such as musculoskeletal and connective tissue diseases[22,23], nerve injury[24,25], diabetic foot ulcers[26], ocular epithelial defects[27], and oral and dental diseases[28,29].

Growth factors released from platelets and involved in wound healing process belong to different families, namely[17,21]:

- Transforming growth factors (TGFs)
  - TGF-α: promotes proliferation and chemotaxis of keratinocytes and fibroblasts
  - TGF-β1 and TGF-β2: promote angiogenesis, upregulate collagen production, inhibit collagen degradation, and stimulate chemotaxis of inflammatory cells, such as monocytes and fibroblasts
- Vascular endothelial growth factor (VEGF): stimulates angiogenesis in response to tissue hypoxia

Table 1. Different platelet doses used per each injection and median dose employed during the follow-up.

Injection	Platelets (103/μl) (basal blood)	mL - Total platelet dose
1st injection	1324 (247.000)	3 - 3.972.000
2nd injection	1719 (255.000)	3 - 5.157.000
3rd injection	1489 (252.000)	3 - 4.467.000
4th injection	1390 (210.000)	3 - 4.170.000.
5th injection	1299 (228.000)	3 - 3.897.000

Median platelet dose: 4.332.600/3 ml.

- Platelet-derived growth factor (PDGF): enhances migration of macrophages and fibroblasts, and promotes collagen and proteoglycan synthesis
- Epidermal growth factor (EGF): stimulates collagenase secretion by fibroblasts to degrade the extracellular matrix during the remodeling phase, and promotes keratinocyte and fibroblast proliferation
- Fibroblast growth factor (FGF): promotes angiogenesis, granulation tissue formation, and epithelialization via endothelial cell, fibroblast, and keratinocyte migration, respectively[17,21].

This biological basis supports the feasibility of therapeutic strategies based on the use of high doses of platelet concentrates to promote wound healing, such as High-PRP.

PRP is categorized by the Food and Drug administration (FDA) as a minimally-manipulated tissue, as well as an autologous blood product[30]. One of the advantages of these preparations is that they are easily obtained from the patient's blood after a single or multiple centrifugations; thus, PRP can be deemed as a safe, easy to use and cost-effective blood product[31,32]. At present, major challenges that need to be addressed are the lack of clear gold standard procedures for PRP generation, little characterization performed on the obtained products, as well as lack of regulation and standardization[33]. In our center of regenerative medicine in Argentina, we employ a precise and standardized protocol developed to obtain a reproducible dose of platelets in PRP concentrates.

Within the existing different methods for PRP preparation, there is discrepancy in technical details, such as the inclusion of leukocytes or erythrocytes, along with differences in speed and duration of the centrifugation process[34]. In our study, we did not include leucocytes or erythrocytes, that could be tested in the five injections. The only component of our PRP was represented by platelets concentrated in a volume of 3 ml plasma and consisting of higher doses of platelets compared to those present in the basal blood.

With regard to PRP preparation methods, Anitua et al[35] outline the importance of excluding leukocytes, because they can alter the function of some growth factors and interfere with their anti-inflammatory actions. The Harvest Technologies corporation claims that these details are not important and that their PRP contains some erythrocytes[36]. Moreover, other authors prefer the inclusion of leukocytes for the treatment of chronic ulcers or tendon injuries, due to the antimicrobial properties of leukocytes[37,38].

PRP can be generally considered as a safe approach for treatment of chronic wounds. In fact, no adverse events, such as increased risk of infection or hypersensitivity reactions, have been reported in clinical trials, although further randomized controlled trials are needed due to the low quality of evidence[39].

In this case report, excellent outcomes were achieved after PRP application. No adverse events or complications have occurred thus far following five PRP injections. Previous conventional treatments, such as wound dressings and surgical debridement, were ineffective in treating this refractory wound. Indeed, skin and extracellular matrix regeneration can be impaired in such instances, resulting in skin erosion and wound deterioration; also, primary fresh wounds fail to heal, progressing towards a chronic state. Thus, important growth factors and cytokines (such as those contained in high-dose pure PRP concentrates) are required to initiate and promote the healing process.

Crovetti et al[40] published a prospective non-blinded study investigating the efficacy of platelet gel (PG) in the healing of cutaneous chronic wounds. The wounds of the patients enrolled in this study (n=24) were of different origin and etiologies. The therapeutic protocol consisted of a once-weekly application of platelet gel (PG) of either autologous or homologous origin. At the time of the study publication, nine patients showed complete wound healing[40].

Driver et al[41] conducted the first prospective, randomized, controlled multicenter trial in the United States investigating the safety and efficacy of autologous PRP gel for the treatment of non-healing diabetic foot ulcers. Participants included 72 patients with type 1 and type 2 diabetes (aged 18 to 95 years) from 14 investigation sites; participants had an ulcer of at least 4-week duration[41]. In this study, authors investigated the efficacy of autologous PRP gel compared to control (saline gel). Patients were randomized into two groups: i) standard of care with PRP gel, or ii) control (saline), and were evaluated biweekly for 12 weeks or until healing. Thirteen out of nineteen (68.4%) of the PRP gel and nine out of twenty-one (42.9%) of the control wounds healed. Wounds in the PRP group healed after a mean time of 42.9 days (SD, 18.3) *vs.* 47.4 days (SD, 22.0) in the control group. In our case report, the patient showed complete wound healing 180 days following the first PRP injection (Figure 1). It would be interesting to compare our results to those obtained by Driver et al[41] using PRP gel.

Conclusions

Regenerative therapies represent valid strategies, alternative to conventional treatment, to accelerate wound healing and improve quality of life of patients with chronic non-healing wounds. In this regard, PRP could be considered as a safe and cost-effective treatment for cutaneous wounds, aimed to promote the healing process and shorten wound healing time. In particular, the use of PRP represents a valid option for the treatment of chronic wounds of different etiologies. The variability of response to PRP application in terms of wound healing can be attributed to several factors that can influence the outcomes of this treatment (e.g., wound etiology, presence of diabetes, type of wound). Other possible variables able to explain the variability in PRP characteristics across the studies include: i) PRP preparation methods; ii) growth factor content of autologous PRP varying between participants, even in patients with similar blood platelet counts; iii) time interval between consecutive PRP applications.

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Conflict of Interest:

The authors have no conflict of interest to disclose with regard to this article.

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