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Original Article

A case series of platelet rich plasma in chronic venous ulcers

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ABSTRACT

Introduction: Venous ulcers are the most common type of leg wounds (80%) and the main cause is chronic venous insufficiency. Autologous platelet-rich plasma (PRP) is a potential wound healing treatment due to its great variety of growth factors. The aim of this study was to describe in a case series the results of poor-leukocyte PRP (P-PRP) or saline for the treatment of chronic non-healing ulcers of the lower extremity.

Methods: Eight patients were treated according to the topical therapy: saline solution or P-PRP gel. All patients used double compression stocks and were assisted by a vascular practitioner for up to 12 months or until wound healing. The treatment was performed weekly with cleaning of the affected area, macroscopic evaluation (area measurement and photos) and P-PRP or saline application, and closure with Tegaderm®. Trial Registration: Retrospectively approved by Brazilian Clinical Trials, register number RBR-7zhgb3 (http://www.ensaiosclinicos.gov.br/rg/RBR-7zhgb3/).

Results: All patients showed signs of wound healing with a reduction in wound size and ulcer numbers, but more evident with P-PRP application.

Conclusions: The results suggested that P-PRP presented a better result when compared to saline solution in the healing process of long clinical course chronic venous ulcers, when associated to compressive stocks and topical care.

1. Introduction

Chronic wounds or ulcers are breaks in the skin that do not heal, or require a long time to heal, and frequently recur [1]. The costs associated with the long-term care of these chronic wounds are substantial and represent a worldwide health problem with high impacts at personal, professional, and social levels, with high costs in terms of human and material resources [2,3].

Non-healing ulcers may include many types of ulcers such as venous, arterial, diabetic, pressure and traumatic ulcers [3]. Amongst all of these, the venous ulcers are the most common form of chronic ulcers of the lower extremities, with a significant impact on quality of life and work productivity [4]. The prevalence varies between 1 and 2% in the population over 60 years of age presenting chronic venous insufficiency [5,6].

Wound healing is a coordinated dynamic tissue repair process, mediated by the interaction of molecular signals involving mediators such as growth factors, cytokines, and chemokines and cellular events [7]. The normal process of wound healing includes three phases: inflammation, tissue formation, and tissue remodeling. When the normal healing process is disrupted, a wound can become chronic in nature, leading to the arrest of the chronic inflammatory phase [8].

Chronic venous insufficiency is a determining factor for the appearance of chronic ulcers, which can be defined as a change in the functionality of the venous system, which may or may not be associated with obstruction of the venous flow. This venous dysfunction may affect the superficial venous system, deep or both, and even a congenital disorder [9].

Historically, the understanding of venous reflux disease focused upon the anatomical mechanisms of valvular incompetence. More recent investigations into the cellular and molecular aspects of

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venous insufficiency have shown that the disease is a complex multifactorial process reflecting both systemic abnormalities of connective tissue synthesis and cellular inflammatory reaction [10].

Other factors that commonly contribute to poor wound healing are: 1) local causes, such as wound infection, tissue hypoxia, repeated trauma, and presence of debris or necrotic tissue; 2) systemic diseases, such as diabetes mellitus, immunodeficiency, or malnutrition; and 3) certain medications, such as corticosteroids [11]. Conventional therapies such as dressings, surgical debridement, compression bandages and even skin grafting cannot provide satisfactory healing since these treatments are not able to provide necessary growth factors that can modulate the healing processes. The effectiveness of the current treatment of chronic wounds is estimated at 50%, implicating in the constant need to repeat treatments, making the process expensive [8].

Autologous platelet rich plasma (PRP) is a product derived from blood that is increasingly being widely used in clinical practice and, among other applications, has become an alternative approach to the dressings used to date for the treatment of chronic ulcers. The curative properties of PRP rely on the fact that platelets are a physiological reservoir of growth factors, which have an active role in tissue regeneration. It is well known that platelets contain a great variety of growth factors, with healing functions [12]. They are safe, simple, affordable and a less expensive procedure in the treatment of chronic ulcers with reportedly good results [6]. However, there are no clear gold standard protocols for PRP generation, and there are some limitations such as the poor characterization of the obtained products and the lack of regulation and standardization (Table 1).

The aim of this study was to evaluate the use of PRP for the treatment of chronic non-healing ulcers, using topical autologous P-PRP gel and compared to saline solution.

2. Methods

2.1. Inclusion and exclusion criteria

The study was approved by 'The Ethics Committee' (CAAE: 18446013.1.0000.5404) of the participating institution and all donors signed the informed consent form. It was approved by Brazilian Clinical Trials, register number RBR-7zhgb3 (http://www.ensaiosclinicos.gov.br/rg/RBR-7zhgb3/).

Patients were eligible if they were at least 80 years old and presented non-healing venous lower limb ulcers located on the medial or lateral side of the leg. To confirm the diagnosis of venous insufficiency, all the patients made an ultrasound, evaluating venous and arterial system. The exclusion criteria included ulcers of another etiology, infected clinical signs at the moment of the initial treatment, tabagism, use of anticoagulant, immunosuppressive, or antibiotic therapy in the last 3 months, pregnancy, bleeding disorders, severe cardiovascular disease, peripheral arterial insufficiency, neoplasm, lupus, diabetes, uncontrolled systemic arterial hypertension (SAH), acquired immunodeficiency syndrome (AIDS), anemia, renal insufficiency (creatinine > 2 mg/dL), liver disease (OR > 2x upper limit, INR > 1.5), surgery or major trauma in the last 2 months, inclusion in other clinical studies in the last 30 days. Patients who presented three consecutive or six alternating absences were also excluded. The entire treatment and follow-up procedure of the study were explained by the investigator to the patients who met the inclusion criteria, who were included in the study only after signing the voluntary informed consent form.

Patients were examined by a vascular, who analyzed the history, ulcer etiology, arterial patency using ankle-brachial index (ABI), laboratorial parameters and indicated the right size of single elastic compression socks. History of patients included information regarding adverse events, concomitant medications, nutrition and weight-bearing status, and other aspects of care since the last visit.

2.2. Preparation of P-PRP

The blood collection was made into six tubes containing acid citrate dextrose solution in a ratio of 9:1 (blood:Acid citrate dextrose – ACD) for all patients. Once collected the sample was centrifuged at 300×*g* for 5 min to separate the red blood cells from the platelets and plasma. Then, the supernatant without the buffy coat (1 cm above the red blood cells) was collected and centrifuged again at 700×*g* for 17 min, as previously described [13]. After this second spin, 80% of the volume of supernatant was discarded and 20% of the volume was homogenized to produce the P-PRP. An aliquot of P-PRP was stored at −80 °C for platelet growth factors dosage.

The bottom layer of approximately 3.8 mL was taken and 0.2 mL of 10% calcium chloride was added to form the P-PRP gel. The patients who did not receive the P-PRP therapy also had a blood collection, which was discarded. For this therapy, all the patients had a blood collection weekly, and even with a lot of ulcers, all the wounds were treated with topical P-PRP or saline – depending on the group of evaluation. For patients with multiple ulcers we collected a higher quantity of tubes.

2.3. P-PRP characterization

The cell quantification was evaluated in a Hematologic Counter (Cell Dyn Emerald (Abbott)). In addition, it was measured the growth factors such as: Epidermal Growth factor (EGF), Platelet-Derived Growth Factor-AA (PDGF-AA), Insulin Like Growth Factor 1 (IGF-1) and Vascular endothelial growth factor (VEGF) were quantified by multiplex technology (R&D Systems) according to the manufacture's procedure.

2.4. Treatment procedure

After arterial insufficiency was excluded by ultrasound, the use of compression socks was introduced for all patients. For this, the patients were placed in dorsal decubitus position and the vascular put their index and middle fingers in the region of the ankle malleolus. When the pulsation was confirmed, the use of socks was approved, and the size was obtained through measurements of

Table 1
Reported studies for PRP use.

No. Patients	No. Applications and measurement	Method	Area cm^2	Ref
5	1 after ten days	L-PRP	200–300	Cieslik-Bielecka et al. 2018 [29]
40	1 for three days (mean of 6 sessions)	Platelet-rich plasma gel	<10	Moneib et al. 2018 [30]
58	weekly for 24 weeks	PRGF	13.69 ± 30	Escamilla Cardeñosa et al. 2017 [31]
24	1 subcutaneous injection and PRP gel applied topically for three days (24 weeks)	Platelet-rich plasma gel	0.5–10	Suthar et al. 2017 [3]

each leg. Patients were instructed to only take off their compression stockings at bedtime and to change them every six months.

The patients were treated with autologous P-PRP gel or saline solution (control) on the wound on the day of procedure, weekly, until wound closure or until one year after the first application. The ulcers were characterized according to the area in cm^2, and a macroscopic evaluation (photography) was made monthly. Because of the great heterogeneity regarding the number and the wound area, and time of non-healing ulcers, the patients were divided only according to sex and age.

The wound was irrigated with normal saline solution and assessed for the presence of any form of infection and cleaned with chlorhexidine gluconate. This process was repeated weekly. In the case of the control patient, 3800 μL of physiological solution was added to the petri dish with 200 μL of 10% sterile calcium chloride, homogenized, wet in sterile gauze with the mixture and applied on the ulcer with gentle pressure. For topical P-PRP, 3800 μL of P-PRP was added in a petri dish with 200 μL of 10% sterile calcium chloride and after the gel formation, about seven minutes, the wound was cleaned as described above and the topical P-PRP gel was applied. In the case of large wounds, it was applied a higher quantity of P-PRP gel, around two or three petri dishes covering all the wound area.

P-PRP gel or saline solution was applied onto on all the wound(s) area, also was dressed for protection with the Tegaderm™ (3 M Medical Inc.) bandage and sterile gauze. The compression stock was used above the dressing.

2.5. Ulcer measurements

Care and management at each visit for treatment included wound cleansing and change of the dressing. Macroscopic evaluation was performed monthly. To evaluate wound contraction, a transparent plastic film was placed over the lesion and wound margins were traced at specific time-points [14,15]. After digitalization, the wound area was measured using Image J software (MD, USA). Wound area measurements and photographs were obtained once a month. Wound sizes were expressed as percentage of the initial wound area; in this way, the value in cm^2 on day 0 was considered 100% and on the other days measured in relation to day 0, as Previously published by Huber et al. 2019 [13], where: Day 0 (cm^2) = 100% and Day x (cm^2) = (x*100%)/Day 0 (cm^2). The data were evaluated by two different evaluators, a post-doc student and PhD student in a blind way, they received just the images and date of the evaluation, without knowledge about the treatment used.

2.6. Quality of life self-assessment questionnaire for venous diseases – CIVIQ20

A specific Chronic Venous Insufficiency quality of life Questionnaire CIVIQ20 was adapted to Portuguese and applied. This test evaluates the frequency and impact of the symptoms, sensations, types of discomfort in patients, which can make their daily lives more difficult. The intensity varied from one to five, one representing absence of discomfort, two an occasional discomfort, three a regular discomfort, four a frequent discomfort and five a constant discomfort. The CIVIQ20 was applied every 3 months completing 12 months of follow-up or until the ulcer healing.

2.7. Statistical analysis

A descriptive statistic of the numerical variables with values of median, minimum values and maximum. ANOVA was used to compare the variables between the applications for repeated measures. The data were transformed into ranks due to the absence of normal distribution. The level of significance was 5%. Analyses were performed with using GraphPad, version 6.

3. Results

3.1. Patients

In this study, sixteen patients with non-healing ulcers were selected; nine of them were excluded for several reasons: venous insufficiency (1), comorbidities (1), ulcer closure before starting the study (4), or refuse to participate in the study (3). Only eight patients with non-healing venous ulcer on similar anatomical areas (lower limbs) were included in the study.

All patients received the same treatment protocol that included daily use of double compression socks, and weekly cleansing, macroscopic evaluation, and analysis of ulcer infection. The only difference was the application of saline solution or autologous P-PRP gel. The size of the ulcers was assessed at baseline (visit 0), every month (visit no. 1, 2 etc.) or until the wound healed. Among the patients included, six were females (75%) and two were males (25%). Both groups had the same quantity of females and males, with a mean age of 56.5 years. In relation to Doppler ultrasound, one patient had perforator incompetence and seven patients had superficial vein incompetence. The time of non-healing ulcers presented by the patients' pre-treatment ranged from 2 to 53 years. The clinic characteristics of patients are presented in Table 2.

3.2. P-PRP characterization

The P-PRP was characterized according to the cell quantification (platelets) and growth factors concentration. The mean of platelet number in P-PRP was 1157×10^3 cells/μL (±195.20 cells), which represents 5.4 folds' baseline. The mean growth factors levels were 3775.23 pg/mL (±2507.96) for PDGF, >2050 pg/mL for VEGF, >2110 pg/mL for EGF and >46,610 pg/mL IGFBP1, showing a high concentration of all growth factors.

3.3. Area and number of ulcers

First, we considered the number of wounds in each patient and calculated the total ulcer area of the groups at the beginning of the study (T0), which was defined as 100%. Results showed a reduction of the mean total ulcer area in both groups with a statistically difference in the percentage of ulcer reduction area: 86% and 50% for P-PRP and saline, respectively ($p = 0.001$). The mean area was reduced from 10.78 cm^2 at visit 0 to 1.49 cm^2 at final visit for those treated with P-PRP, and from 21.19 cm^2 at visit 0 to 10.62 cm^2 in saline group (Fig. 1).

In relation to the number of ulcers, all the patients showed wound healing with reduction of number of wounds or complete healing. Three patients treated with P-PRP healed completely and the third patient had a reduction from nine to five ulcers. In the saline group three patients also healed and one had a reduction from three to one (Fig. 2).

Regarding quality of life, assessed by CIVIQ20 questionnaire, pain, difficulties to sleep, limitations for climbing stairs, kneeling and sitting, fatigue, shame, irritation and even difficulty to leave the house were common in both groups and did not show improvement during the follow-up.

3.4. Case series report

3.4.1. Patient 1

A 58-year old man with an extensive venous healing ulcer of the anterior face and malleolus of the left lower limb, without previous

Table 2
Clinical characteristics of the groups.

Group	Patient	Gender	Age	Ulcer number	Sum of initial total area (cm^2)	Time of non-healing (years)	Time of healing (months)
PRP	1	M	58	3	6.48	>10	12
	2	F	62	9	7.43	40	5 closed in 12 months
	3	F	60	4	4.30	20	6
	4	F	46	3	24.91	3	5
Saline solution	5	M	75	1	11.8	53	9
	6	F	32	1	0.88	2.5	6
	7	F	62	3	42.17	10	Reduced but did not closed
	8	F	57	1	4.84	24	5

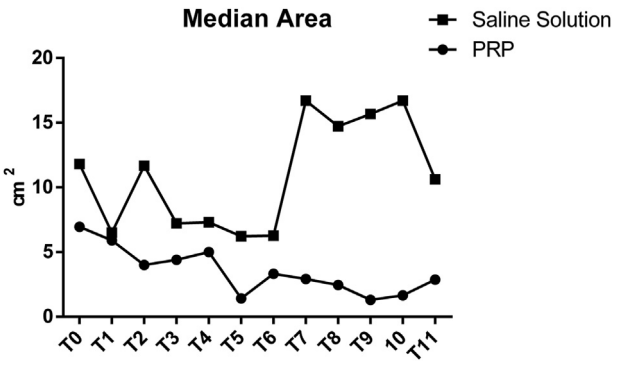

Fig. 1. Median area of wounds over time (12 months).

treatment for more than 10 years (Fig. 3). The doppler ultrasound showed involvement of the deep and superficial venous system with reflux in the left common femoral vein, segmental insufficiency in the sapheno-femoral junction of the great saphenous vein and insufficient perforation in the medial face of the left leg. Presence of collateral varices, according to the ectoscopy. He presented arterial hypertension. The ulcers initially measured 0.51, 3.80 and 2.17 cm^2 area (Fig. 3-left) and after 8 months the wounds began to close (Fig. 3-right). The median area of wounds during the treatment was 3.32 cm^2 (maximum 9.11 and minimum 1.49 cm^2).

3.4.2. *Patient 2*

A 62-year old woman with multiple ulcerated lesions in the anterior and lateral malleolar region of left lower limb, with

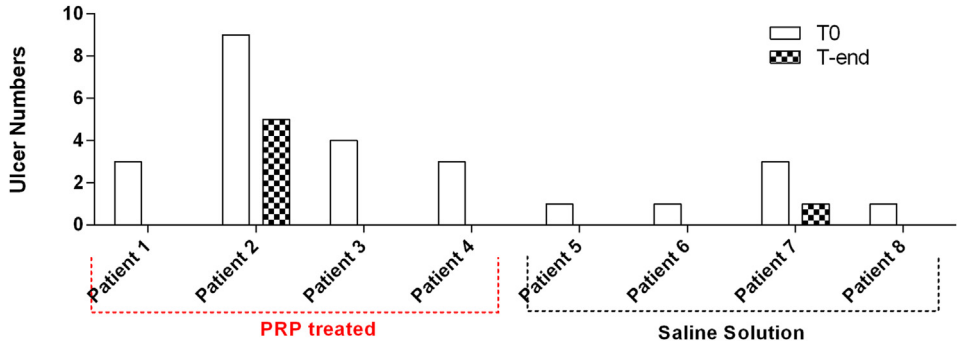

Fig. 2. Number of wounds at initial time (T = 0) and until final time (T-end).

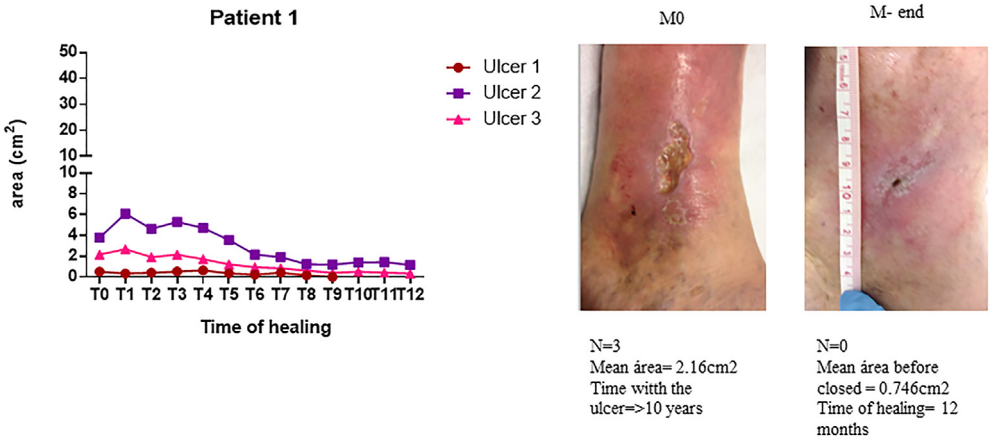

Fig. 3. Patient 1 before and after PRP treatment. Patient's wound progress, area in cm^2 versus time (left) and wound progress at initial time and after 12 months (Right).

exuberant dermatofibrosis and skin eczema. She had had previous surgery for varicose veins at 30 years of age, and wounds for almost 40 years. After a healing period of 8 years the ulcers recurred, with several unsuccessful treatments. Doppler ultrasound showed a compromised superficial venous system, large saphenous vein with reflux and segmental dilation enters the sapheno-femoral junction and the middle third of the thigh and in the knee segment; parietal saphenous vein with reflux and segmental dilatation in the ankle segment. The ulcers initially measured 1.55, 1.31, 1.27, 1.20, 0.89, 0.58, 0.35, 0.21 and 0.07 cm^2 and despite having closed after six months, re-opened in other areas. The nine ulcers observed at the initial treatment were reduced to four after 11 months (figure not shown). The median area of wounds during the treatment was 2.56 cm^2 (maximum 7.43 and minimum 0.67 cm^2).

3.4.3. Patient 3

A 60-year old woman with a non-healing ulcer on the right lower limb for 20 years, referred to multiple wounds that healed and opened at other locations during that time. She had had a bilateral variceal surgery at 17 years of age, worsening after gestation and presented arterial hypertension. Before treatment, she had a dorsal and malleolar wound in the right foot with hyperpigmentation, dermatosclerosis and varicose veins. Doppler ultrasound showed involvement of the superficial venous system, with insufficiency and dilatation over the entire extension of the left common femoral vein and incompetent sapheno-femoral junction. Presences of collateral varices detected on the medial side of the thigh and leg. The ulcers area initially measured 2.53, 1.11, 0.50 and 0.16 cm^2 and healed after 6 months (Fig. 4). The median area of wounds during the treatment was 0.13 cm^2 (maximum 4.30 and minimum 0.04 cm^2).

3.4.4. Patient 4

Patient 4 was a 46-year old woman with a non-healing ulcer on the right lower limb for 3 years. Before treatment, she presented a malleolar wound on the right foot. Doppler ultrasound showed an insufficiency and a dilation of the sapheno-femoral junction on the distal third of the thigh. The ulcer areas initially measured 17.54, 6.14 and 1.23 cm^2 and healed after 5 months (Figure not shown). The patient withdrew from treatment and presented recurrent ulcers infections during the study. The median area of wounds during the treatment was 15.00 cm^2 (maximum 26.65 and minimum 0.43 cm^2).

3.4.5. Patient 5

Patient 5 was a 75-year old man with a non-healing ulcer on the right lower limb for 53 years with previous treatments, with healing and recurrence. Doppler ultrasound showed a compromised superficial venous system, large saphenous vein with segmental reflux and incompetent saphenopopliteal junction. Initially, he presented ulcerated lesions on the lateral side, with exuberant varicose veins, dermatofibrosis and hyperchromic stains. Comorbidities were hypothyroidism and hypercholesterolemia. The ulcer initially measured 11.80 cm^2 and healed after 9 months (Fig. 5). The median area of wounds during the treatment was 7.22 cm^2 (maximum 16.70 and minimum 0.48 cm^2).

3.4.6. Patient 6

Patient 6 was a 32-year old woman with ulcerated lesions with no previous treatment of the left lower limb. Presence of varicose veins for 11 years. Hyperchromic stains and scar of medial malleolar ulcer. Doppler ultrasound showed compromised superficial venous system, with large saphenous vein with dilatation and segmental reflux of the saphenous–femoral junction to the knee. The ulcer area initially measured 0.88 cm^2 and after 6 months the ulcer was 0.33 cm^2, which afterwards completely healed (Figure not shown). The median area of wounds during the treatment was 0.33 cm^2 (maximum 0.88 and minimum 0.10 cm^2).

3.4.7. Patient 7

Patient 7 was a 62-year old woman with ulcerated lesions on the right lower limb. Presence of varicose veins for 10 years, healed once 4 years ago. She presented repeating infections during the treatment. Doppler ultrasound showed a compromised superficial venous system, with segmental reflux of the saphenous–femoral junction. Caliber collaterals, located on the medial side of the thigh draining to the saphenous–femoral junction The ulcer initially measured 67.23 cm^2 and after 12 months the area was 10.62 cm^2 (Fig. 6). The median area of wounds during the treatment was 15.28 cm^2 (maximum 67.23 and minimum 10.62 cm^2).

3.4.8. Patient 8

Patient 8 was a 57-year old woman with a non-healing ulcer on the right lower limb for 24 years. Before treatment, she had a malleolar wound on the right foot. Doppler ultrasound showed an insufficiency and a dilation of the sapheno-femoral junction, and presence of collateral varices. The ulcer initially measured 4.84 cm^2 and reduced after 5 months, the patient however, withdrew from treatment (Figure not shown). The median area of wounds during the treatment was 0.69 cm^2 (maximum 4.84 and minimum 0.19 cm^2).

4. Discussion

Conventional methods for treatment of chronic wounds such as mechanical debridement, occlusive dressings, and local antibiotics in case of infection, often lack effectiveness. Autologous PRP is an alternative method [16], helping to repair tissue in various oral and maxillofacial surgical procedures [17]. PRP has gained interest in

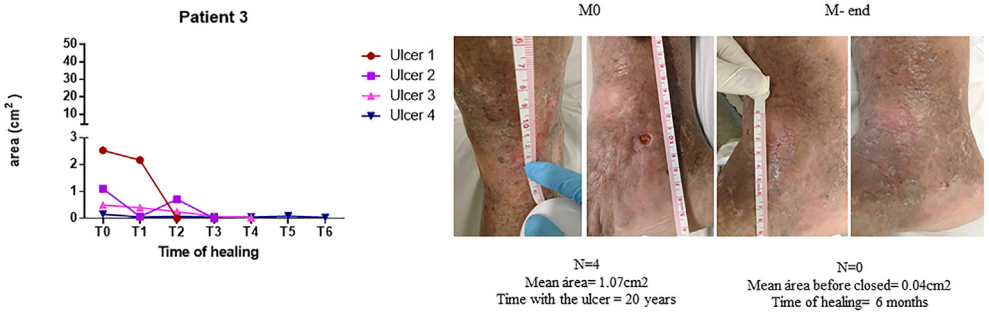

Fig. 4. Patient 3 before and after PRP treatment. Patient's wound progress, area in cm^2 *versus* time (left) and wound progress at initial time and after 5 months (Right).

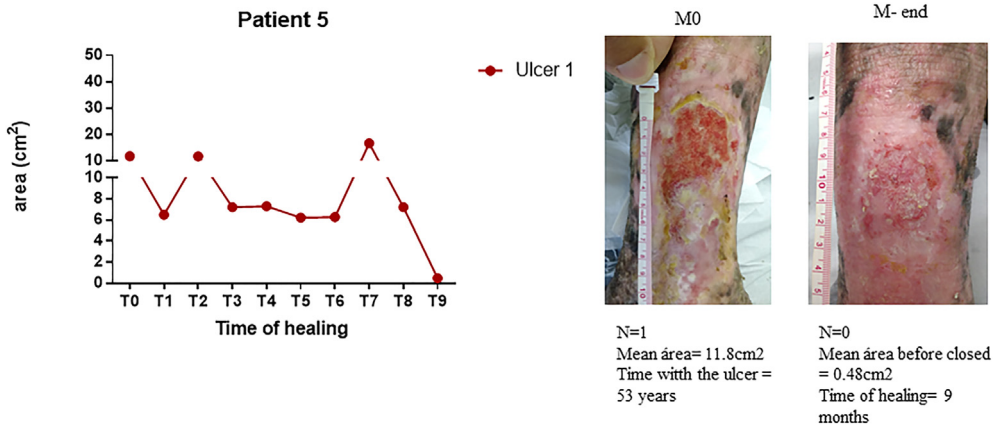

Fig. 5. Patient 5 before and after saline solution treatment. Patient's wound progress, area in cm^2 versus time (left) and wound progress at initial time and after 9 months (Right).

the clinical and diverse research field [18] and has been extensively used in regenerative medicine over the last two decades [16].

PRP provides abundant platelets concentrated into a small volume of plasma [19]. The growth factors released from the α-granules of the activated platelets, along with plasma proteins namely fibrinogen converted to fibrin, fibronectin and vitronectin play a pivotal role in the modulation of tissue repair and regeneration [20] and modulate the wound microenvironment to create a better chance of healing. Despite various and extensive applications, the efficacy of PRP is being called into question because of the lack of large randomized controlled trials, and lack of consensus regarding the PRP preparation processes. Regarding wound repair, the miscellaneous of ulcer etiology is another point to be considered [16].

We performed the characterization of P-PRP including platelet quantification and growth factors concentration. We demonstrated an adequate number of cells with more than 3 folds baseline. Indeed, we showed the presence of PDGF, VEGF, EGF and IGFBP1 in high levels of concentration. These findings allowed to affirm that the P-PRP used in those patients were good quality products.

We believe that there is a difficulty in the comparison of results, given that the method of obtaining PRP in addition to the number of lesions treated and the follow-up period were substantially different (Table 1). In addition, we used P-PRP and the method of application in our study demonstrated to be useful to relieve the pain presented by most patients with venous ulcers and to reduce the number and the area of the ulcers.

The complete healing observed in six patients was achieved after administration of both treatments (three in P-PRP and three in saline group). The total healing was observed between 5 and 12 months during treatment. The reduction in wound size was observed in all patients, though more significant in the P-PRP group (86% *vs.* 50% of closing area), and also with a decrease in the number of ulcers (78% *vs.* 50%) (Fig. 1). Both groups showed improvements in the wounds, probably related to the control of local infection and the use of compression socks. Even both groups used compression socks, the use of P-PRP presented a more favorable effect, with faster and better results when compared to the use of saline solution. Compression therapy remains the gold standard treatment for chronic venous insufficiency. Whilst compression therapy does not lower extremity venous pressure, it can reduce interstitial pooling. This in turn may improve tissue perfusion and provide an anti-inflammatory role [21]. Our results highlight that the use of compression therapy plays a key role in the healing process of venous ulcers, improving the pathophysiological process involved in the genesis of the disease [21].

Previous studies described large area and long clinical course time of ulcers as unfavorable prognostic factors. Meaume et al. [22] found lesions of venous origin of more than three months' clinical course and area affected of more than 10 cm^2 as non-favorable factors [22]. Phillips et al. [23] described a significant correlation between initial area of the ulcer and duration, considering that lesions smaller than 5 cm^2 and at least one-year of clinical course responded better to the treatment [23]. Moffat et al. [24] performed

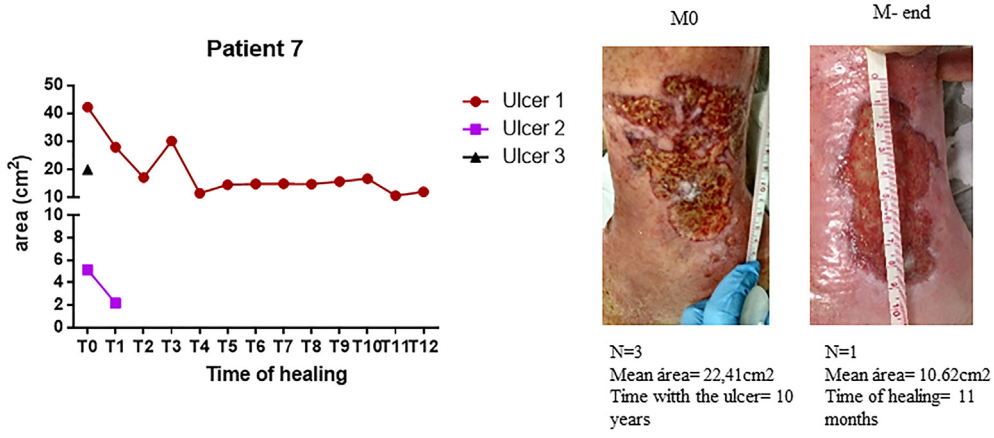

Fig. 6. Patient 7 before and after saline treatment. Patient's wound progress, area in cm^2 *versus* time (left) and wound progress at initial time and after 11 months (Right).

a study to evaluate the efficacy of the pressure bandage in venous ulcers and found a worse prognosis in patients in which the size of the lesion was greater than 10 cm^2 and in those cases in which the average clinical course time was greater than six months [24]. Margolis et al. [25] found that a large area of the venous ulcer along with a long clinical course time are indicators of a poor result regarding healing [25].

We included patients with a high heterogeneity in size (4.3–24.9 cm^2 for P-PRP group and 0.88–42.0 cm^2 for saline group) and in the average of time of non-healing (3 to more than 10 years for P-PRP group, and 2.5–53 years for saline) in both groups (Table 2). Furthermore, the P-PRP group presented 3 to 9 ulcers, and the control one to 3 ulcers. Despite the limitation of the low casuistic, the comparison of the groups allowed us to demonstrate a significant reduction of the mean total ulcer area in P-PRP group when compared to saline ($p = 0.001$). However, it is important to highlight that even patients with long time non-healing ulcers presented improvement characterized by decrease of the size/area and reduction in ulcer number.

Even with these positive results, using the validate CIVIQ20 questionnaire to assess the quality of life, we did not demonstrate an improvement during the follow-up. Maybe the sequelae of those patients, with so many years with these limitations can in part explain these findings.

Infection is one of the most common and important complications in the care of venous ulcers with a negative impact on the clinical course of lesions, which seriously compromises patient well-being. An important finding of our study was that only two of the ulcers in our groups revealed signs of infection. In this context, there are studies which have revealed the antibacterial activity of the P-PRP compared to some pathogens which reside in chronic wounds [26,21]. It is important to note, that the type of PRP is one of the points of literature discussion – regarding the presence of leukocytes, including the buffy-coat layer in the PRP preparation, named as PRP leukocyte rich (L-PRP); or the use of a PRP in the absence of this buffy-coat layer, named as P-PRP. Although the P-PRP used in our study is a preparation without leukocytes, recent *in vitro* studies have found similar antimicrobial activity both in the preparations of PRP that contained leukocytes and those that did not [22,26]. In addition, the use of P-PRP, improve the homogeneity of the product and reduced donor-to-donor variability. Anitua et al. related the importance of the absence of neutrophils, due to the secretion of proteases and elastase that could be destructive for growth factors and the increase of reactive oxygen species which is deleterious for cell survive [27]. Although there is some debate about the use of P-PRP or L-PRP in diverse treatments, these are some reasons that we choose the use of P-PRP in this study.

Finally, coinciding with other studies, we found no adverse effects related to application of P-PRP.

The strength of our study is patient's selection, which included only ulcers of venous etiology, with a long time of clinical course. Additionally, we included ulcers of large areas, with a long follow-up (12 months). Our study included weekly care, with replacement of the P-PRP gel or saline solution and cleaning of ulcer area. In addition, we characterized P-PRP through platelet number and steps of production, and quantified growth factors, demonstrating a good final product with adequate cell and growth factors levels.

Also, we cannot discard that a different route of P-PRP application like subcutaneous around the ulcers, as showed by some authors [3,28] could impact in the results. We did not use this method of application because of the scaffold property of PRP used as topical application (gel) and due to the pain for the patient during the application.

As study limitations we have the small number of patients and the heterogeneity of the groups regarding ulcer size and number of ulcers in each group. Indeed, histopathologic assessment obtained by skin biopsy, before and after the treatment with PRP, would be interesting, however proved to be a challenge on friable skin and the samples could not be collected.

5. Conclusions

P-PRP showed a better result when compared to saline solution in the healing process of chronic long clinical course venous ulcers, when associated to compressive stocks and topical care. This was demonstrated by a significant decrease in the area of the ulcers.

Declaration of competing interest

The authors declare that there is no conflict of interest.

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