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Autologous Platelet-Rich Plasma Reduces Healing Time of Chronic Venous Leg Ulcers: A Prospective Observational Study

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Abstract

The study investigated whether the application of dressings with autologous platelet-rich plasma (PRP) would reduce the healing time in patients with chronic venous leg ulcers. This is a prospective observational study that included 100 patients diagnosed with lower extremity venous insufficiency complicated by ulceration of a leg or foot, who had been after angioplasty of stenotic artery. Patients were divided into two groups of 50 each: treated with PRP (study group) and treated with conventional hydrocolloid dressings (control group). We followed the wound changes at Day 10, Day 20, and Day 30 of treatment and compared them with the baseline appearance at Day 0. We evaluated the appearance, area, and depths of wounds with ultrasound. The granulation process was examined histologically to document skin formation and wound tissue neovascularization. The findings were that treatment with PRP dressings resulted in a significant progressive reduction in ulcer size, irrespective of the ulcer's initial size, compared to treatment with conventional dressings. Further, the best effect of PRP was noticed in the category of largest wounds. After a month of treatment with PRP dressings, more than 50% of all ulcers were completely healed. The young epidermis appeared together with the granulation tissue, and the formation of dermis took shape after 20 days of treatment. We conclude that the use of PRP dressings is a safe, nonsurgical adjunctive procedure for treating chronic

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venous leg ulcers. The potential benefit of PRP dressings over conventional ulcer treatment requires further in-depth exploration.

Keywords
Chronic venous insufficiency · Granulation tissue · Healing time · Inflammation · Leg ulcers · Platelet-rich plasma · Skin formation · Wound management

1 Introduction

Chronic venous insufficiency is a pathologic process of the venous system in lower extremities characterized by incompetent venous valves and venous hypertension leading to edema, trophic skin changes, and venous ulcers. The prevalence of chronic venous insufficiency in the industrialized countries is enormously high, ranging from 20% to 64% (Wittens et al. 2015; Criqui et al. 2003). Venous leg ulcers are the most severe manifestation of venous insufficiency. The ulcers are slow to heal and have a high recurrence rate. Consequently, they pose a significant physical, emotional, and socioeconomic burden for patients, families, and the healthcare system. In tandem with a worldwide increase in longevity and obesity, the incidence of venous leg ulcers is growing (van Rij et al. 2008; Margolis et al. 2002). About 2.5–3.0 million Americans are affected by venous insufficiency and at least 600,000 suffer from chronic leg ulcers, resulting in an annual economic burden of up to $15 billion (Rice et al. 2014; Korn et al. 2002).

Numerous treatment modalities have been proposed for treating venous leg ulcers. Conventional methods include a topical treatment such as debridement, dressing, compression therapy, antimicrobials, and antiseptics. Systemic therapy includes the administration of antibiotics, pharmacological agents, and skin grafting. Alternative therapies are also used such as electromagnetic fields, hyperbaric oxygen therapy, intermittent pneumatic compression, lasers and infra-red light, negative pressure therapy, and venous leg surgery. None of these treatments are considered entirely satisfactory, and there are insufficient data to draw final recommendations for treatment of chronic venous leg ulcers (Athanerey et al. 2017).

Studies on the molecular mechanisms involved in tissue regeneration have led to the development of the new therapeutic methods for venous leg ulcers. Specifically, growth factors contained in platelet granules are conducive to tissue repair in chronic wounds because they act by regulating cellular proliferation, migration, and differentiation in addition to the synthesis of the extracellular matrix. Recent studies have revealed that growth factors modulate the healing of both chronic and acute wounds by interaction with cellular tissue receptors (Martinez et al. 2015).

The application of autologous platelet-rich plasma (PRP), which contains a greater concentration of growth factors than whole blood, has gained popularity in plastic and orthopedic surgery since it is effective and safe in terms of better survival of fatty tissue and bone grafts (Hsu et al. 2013; Sommeling et al. 2013). PRP contain a high concentration of growth factors, which are released from platelets, that contribute to the tissue regeneration process (Martinez-Zapata et al. 2016). The ability of PRP to induce migration of specific cell types could be harnessed to stimulate the healing process of surgical wounds and skin ulcerations (Roubelakis et al. 2014). Yet data on the efficacy of PRP in treatment of chronic venous leg ulcers are scarce. Thus, this study seeks to determine whether application of autologous PRP would reduce healing time in patients with such ulcers.

2 Methods

2.1 Study Design

The prospective observational study included 100 patients diagnosed with lower extremity venous insufficiency complicated by ulceration

of a leg or foot, who had been after angioplasty of stenotic artery. The starting time of ulcers development was not considered an essential factor since a vast majority of ulcers were recurrent. Patients were subdivided into two groups: active treatment with PRP and a control group treated with a conventional method using hydrocolloid dressings (AQUACEL Ag Surgical dressing; ConvaTec Inc., Greensboro, NC); 50 patients each. The main inclusion criteria were ulcer size not exceeding 5 cm^2 lower limb ischemia clinically and radiologically evidenced with CT angiography, recent successful revascularization, and blood creatinine <1.0 mg/dL. Patients with ischemic ulcers were excluded.

We evaluated the appearance, size, and the depths of ulcer wounds with ultrasound. The granulation process was examined histologically. Duration of ulcers ranged from 6 to 16 months, with an average of 7.3 months. The area of ulceration was calculated by tracing its outline the ulcer size on carbon paper and then transferring it onto scaled grid paper. The size of a dressing fortified with PRP available in the healing kit (Regeneris Medical; North Attleboro, MA) determined the maximum size of the wound treated. Dry necrosis of the wound was present in both groups. All patients underwent a surgical debridement of the wound to remove nonviable tissue in order to decrease the bacterial load and stimulate epithelialization.

2.2 Study Procedures

PRP was obtained by drawing 18 mL of the patient's blood from a venipuncture. The blood was centrifuged to separate erythrocytes and leukocytes from plasma containing platelets. We added a branded reagent to the plasma, which activated the platelets to produce growth factors, applied PRP gel onto the ulcer wound cleaned from necrotic debris, and washed with physiological saline. The wound was covered with a hydrocolloid AQUACEL dressing, which was maintained for 10 days and then replaced with a fresh one. We repeated the whole procedure after 20 and 30 days of treatment. Patients were treated in like manner, except that the PRP-fortified dressing was omitted.

2.3 Data Collection and Analysis

The time to heal was taken as a primary outcome. We performed a power analysis to calculate the sample size with regard to the primary outcome, assuming α error of 5% and β error of 20%. The calculation indicated that 45 lesions in each group were necessary for the assessment. Another outcomes consisted of changes in size and depth of wounds, and the formation of granulation tissue, examined histologically, and ulcer epithelialization, examined with a 50 MHz ultrasound probe. In addition, we examined the formation of skin layer and wound vessels. Ulcers were stratified into four increasing categories depending on the initial area size, ranging from 1 to 5 cm^2. The outcomes were assessed at four different time points: baseline, and after 10, 20, and 30 days of treatment.

Continuous data were expressed as means ±SD and categorical data as numbers or percentages. Univariate analysis was performed using Student's *t*-test for continuous variables and Chi-square tests for categorical variables. One-way ANOVA was used for multiple comparisons. Multivariate analysis using linear and logistic regressions also were performed as required. A p-value <0.05 defined statistically significant changes. A commercial statistical SPSS v21 package was used for the analyses (IBM; Armonk, NY).

3 Results

We treated and analyzed a total of 100 venous ulcers in lower extremities in 100 patients. The PRP and control groups were grossly matched concerning age and gender (Table 1) as well as categories of ulcer wound size (Table 2).

Table 1 Demographic data of patients with leg ulcers due to chronic venous insufficiency treated with platelet-rich plasma (PRP) dressings and with conventional hydrocolloid dressings (control group)

	PRP group ($n = 50$)	Control group ($n = 50$)
Gender (F/M) (n)	16/34	11/39
Age range F/M (year)	55–86/53–89	57–79/54–79
Cigarette smokers (n)	19	17
Arterial hypertension (n)	26	21
Coronary heart disease (n)	5	7
History of myocardial infarction (n)	2	1
Atherosclerosis of lower extremities (n)	24	39
Renal failure (n)	15	17
Age of diabetes mellitus onset (year)	7–21	9–18
Insulin therapy (n)	47	49
Oral hypoglycemic drugs (n)	3	1

The PRP-treated group demonstrated a significantly better healing on Day 10 of treatment in the categories of the smallest and largest wounds compared to the control group ($p < 0.01$). The wound area was significantly smaller in the PRP-treated group on Day 20 and Day 30, compared to the control group, in all size categories ($p < 0.01$ for all). We found the best effect on wound healing of PRP-fortified dressing in the largest wound size category (Table 2). The area of ulcers appreciably decreased in all individual patients already at Day 10 of treatment with PRP dressings, the decrease became greater at Day 20, and the ulcers were completely healed in 28 (56%) out the 50 patients at Day 30 (Table 3).

The initial phase of healing, i.e., granulation, was observed as early as 10 days after PRP application (Fig. 1a). It involved the ingrowth of granulation tissue and a reduction in wound exudate. We also noticed less fibrin at the bottom of a wound and reduced swelling of the wound edges. The formation of dermis accelerated in the second phase of healing after 20 days, along with enhance neovascularization (Fig. 1b). The third phase of healing, observed after 30 days' treatment, consisted of the formation of epidermis over a layer of the freshly formed dermis (Fig. 1c). Granulation tissue contained a rich microvasculature (Fig. 2a), with clearly enhanced blood supply to tissue (Fig. 2b). There were no observable complications during the study period. Occasional minor injuries to the wound did not delay healing. Eventually, all the ulcer wounds healed, and the average duration of therapy was 35 days. No additional wound treatment was required.

4 Discussion

In this study, patients with venous leg ulcers who received treatment with the addition of PRP exhibited a significantly shorter time to healing. Overall, we achieved excellent results by applying a standardized PRP protocol for treatment of chronic venous leg ulcers, which had been unresponsive to conventional treatment modalities. All the PRP-targeted patients had a complete wound healing within 5 weeks, with an improvement in all healing parameters. The procedure was safe as it did not result in any deterioration in wound status. Venous ulcers are a severe complication of chronic venous insufficiency. They are characterized by chronic inflammation and are resistant to local therapies (O'Meara et al. 2000). An appropriate surgical procedure to reduce venous hypertension at the ulcer site and local debridement to remove necrotic tissues are essential for treatment success (Smith 2006). A better understanding of the pathophysiology of chronic venous leg ulcers healing should help define the therapy of choice and the optimal care planning.

A role of various growth factors in wound healing has long since been acknowledged. However, growth factors, one by one, have been shown to fail to respond to multiple needs of

Table 2 Categories of wound area of leg ulcers due to chronic venous insufficiency in patients treated with platelet-rich plasma (PRP) dressings and in the control group treated with conventional hydrocolloid dressings at successive time points of healing

Measurement time point	Group	Category of wound area (cm^2)	Mean area ± SD (cm^2)	*n*
Baseline	PRP	1.0–2.0	1.7 ± 0.2	6
		2.1–3.0	2.5 ± 0.4	6
		3.1–4.0	3.7 ± 0.3	13
		4.1–5.0	4.5 ± 0.3	25
		Overall	3.7 ± 1.0	50
	Control	1.0–2.0	1.9 ± 0.2	7
		2.1–3.0	2.8 ± 0.1	5
		3.1–4.0	3.5 ± 0.3	15
		4.1–5.0	4.7 ± 0.3	23
		Overall	3.7 ± 1.1	50
Day 10	PRP	1.0–2.0	1.0 ± 0.2	6
		2.1–3.0	1.7 ± 0.4	6
		3.1–4.0	2.7 ± 0.3	13
		4.1–5.0	3.4 ± 0.4	25
		Overall	2.7 ± 1.0	50
	Control	1.0–2.0	1.3 ± 0.2	7
		2.1–3.0	2.0 ± 0.2	5
		3.1–4.0	2.5 ± 0.3	15
		4.1–5.0	3.8 ± 0.3	23
		Overall	2,9 ± 1.0	50
Day 20	PRP	1.0–2.0	0.5 ± 0.3	6
		2.1–3.0	1.0 ± 0.4	6
		3.1–4.0	1.7 ± 0.5	13
		4.1–5.0	1.8 ± 0.3	25
		Overall	1.5 ± 0.6	50
	Control	1.0–2.0	0.9 ± 0.2	7
		2.1–3.0	1.7 ± 0.1	5
		3.1–4.0	1.9 ± 0.2	15
		4.1–5.0	2.9 ± 0.2	23
		Overall	2.2 ± 0.8	50
Day 30	PRP	1.0–2.0	0.1 ± 0.2	6
		2.1–3.0	0.3 ± 0.4	6
		3.1–4.0	0.9 ± 0.4	13
		4.1–5.0	1.1 ± 0.2	25
		Overall	0.8 ± 0.4	50
	Control	1.0–2.0	0.8 ± 0.2	7
		2.1–3.0	1.3 ± 0.3	5
		3.1–4.0	1.7 ± 0.2	15
		4.1–5.0	2.1 ± 0.2	23
		Overall	1.7 ± 0.5	50

nonhealing tissues. For that reason, interventions such as PRP therapies are currently under investigation in order to target a broad set of factors involved in the different stages of healing, which are often interrupted by a range of comorbidities. PRP carries molecules that play a crucial role in many wound healing phases such as hemostasis, inflammation, cell migration and proliferation, extracellular matrix production, and tissue remodeling (Steed 1995).

Table 3 Changes off baseline (Day 0) in the wound area of leg ulcers due to chronic venous insufficiency at successive time points of healing in individual patients treated with platelet-rich plasma (PRP) dressings

	Day 0	Day 10	Day 20	Day 30
Patient	Wound area (cm^2)			
1	3.6	2.8	1.7	0.6
2	2.8	2.1	1.2	Healed
3	3.9	3.0	2.3	0.4
4	2.3	2.0	1.4	Healed
5	4.8	4.0	3.3	1.1
6	3.3	2.6	1.9	Healed
7	1.9	1.3	0.5	Healed
8	3.7	3.0	1.2	Healed
9	4.0	3.5	2.7	0.6
10	3.8	3.5	2.4	1.0
11	4.1	3.1	2.0	0.3
12	2.5	1.8	0.8	Healed
13	3.1	2.6	1.2	0.2
14	1.9	0.8	0.1	Healed
15	2.6	1.6	0.5	Healed
16	4.8	3.9	2.1	1.2
17	4.2	3.1	2.0	0.8
18	1.5	0.8	0.2	Healed
19	2.2	1.0	0.4	Healed
20	3.1	2.6	1.6	0.3
21	1.9	1.0	0.4	Healed
22	2.6	1.4	0.5	Healed
23	4.5	3.6	2.2	1.0
24	3.9	2.8	1.0	0.3
25	2.7	1.4	0.5	Healed
26	3.0	2.1	1.0	0.3
27	2.4	1.3	0.5	Healed
28	2.6	1.6	0.5	Healed
29	1.5	0.6	0.2	Healed
30	5.0	3.9	2.3	1.1
31	2.3	1.3	0.3	Healed
32	2.6	1.4	0.6	Healed
33	2.1	1.3	0.4	Healed
34	2.8	1.5	0.6	Healed
35	2.0	1.3	0.4	Healed
36	4.6	3.6	2.7	1.2
37	3.5	2.3	1.1	0.3
38	2.1	1.3	0.5	Healed
39	1.8	0.9	0.2	Healed
40	2.3	1.0	0.3	Healed
41	2.0	0.9	0.4	Healed
42	4.5	3.6	2.0	1.0
43	3.3	2.1	1.0	0.2
44	1.7	1.0	0.2	Healed
45	4.9	3.8	2.1	1.1
46	2.3	1.1	0.2	Healed
47	3.0	1.9	1.0	0.4

(continued)

Table 3 (continued)

Patient	Day 0	Day 10	Day 20	Day 30
	Wound area (cm^2)			
48	2.2	1.0	0.3	Healed
49	4.6	3.8	2.3	1.1
50	3.2	2.4	1.3	0.5

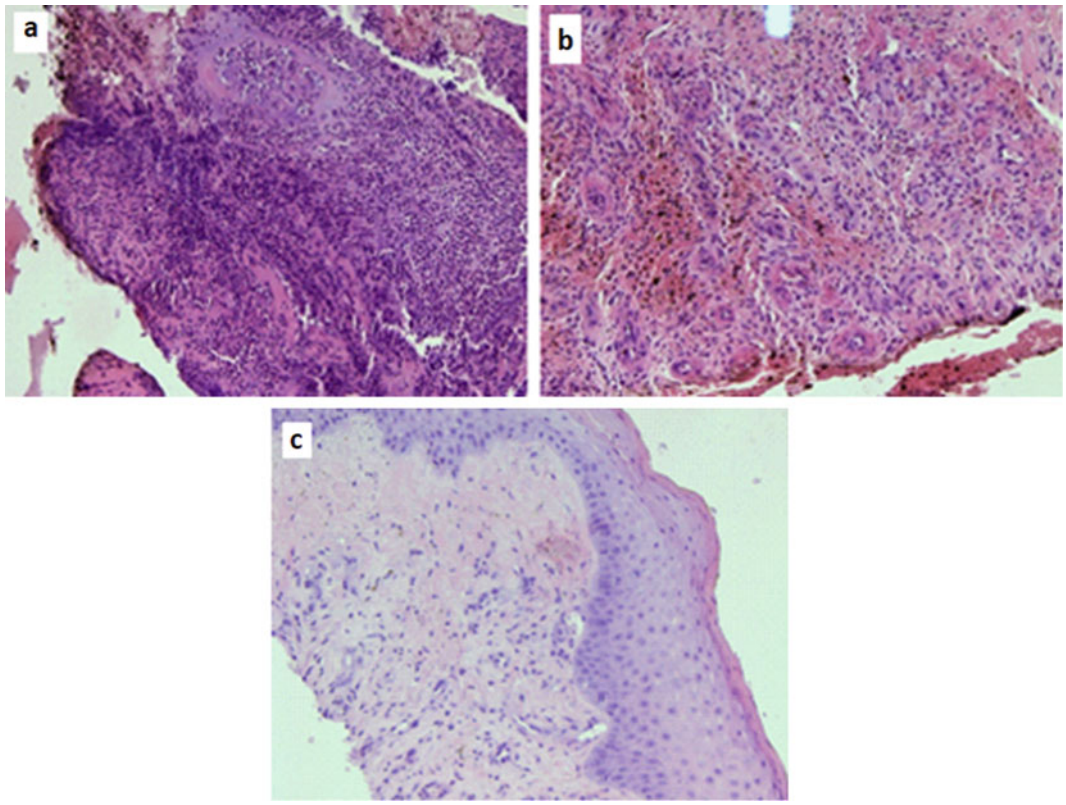

Fig. 1 Sequential phases of healing of a leg ulcer in a patient with chronic venous insufficiency after application of autologous platelet-rich plasma (PRP) dressings: (**a**) initial phase of healing – granulation tissue at Day 10; (**b**) continuing formation of dermis in well-perfused granulation tissue at Day 20; (**c**) right-formed layers of dermis and epidermis at Day 30

The results reported herein are consistent with several other recently published studies. Babaei et al. (2017) have noticed the formation of healthy granulation tissue and early complete closure of every wound in 150 patients with diabetic foot ulcers after topical application of PRP. Likewise, Suthar et al. (2017) have noticed that treatment of nonhealing ulcers of different etiologies with subcutaneous autologous PRP injections, combined with topical application of PRP gel, leads to a significant reduction in wound size as a result of suppression of cytokine release. In addition, there is a reduction in pain and inflammation at the injury site. Comparable results have been obtained after topical use of autologous PRP in secondary wounds due to necrotizing soft tissue infections (Hersant et al. 2017). Cieslik-Bielecka et al. (2018) have shown that PRP eradicates microorganisms from, and induces neovascularization in, chronic ulcers of the crural region

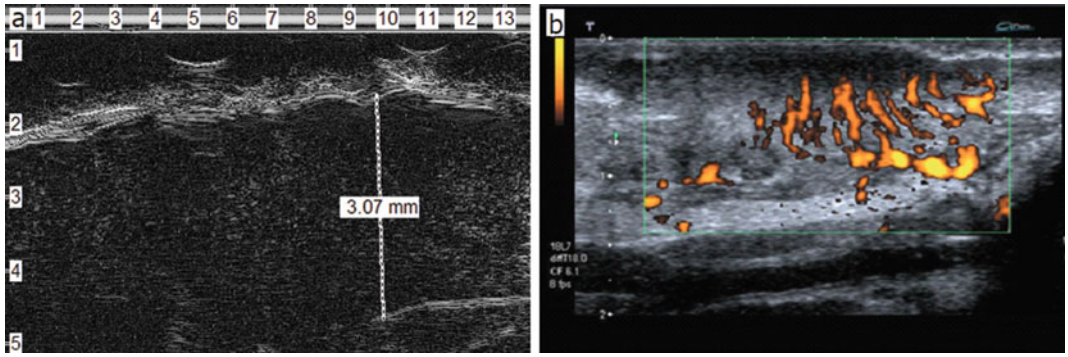

Fig. 2 Day 30 of healing of a leg ulcer in a patient with chronic venous insufficiency: (**a**) right-formed layer of dermis, indicated by a vertical line in mm; (**b**) neovascularization – increased blood flow in the granulation tissue

in AIDS patients. In another study, Man et al. (2001) have shown a quantitative improvement in human skin wound healing after topically treating cutaneous flaps with autologous PRP.

In this study, we adopted the TIME strategy for local wound treatment. The strategy consists of removal of necrotic tissues (T – tissue debridement), control of wound infection and inflammation (I – inflammatory and infection control), maintaining the wound moisturized (M – moisture balance), and wound stimulation (E – stimulation of epidermalization) (Leaper et al. 2012). The TIME strategy produces good results in recently formed wounds. Chronic wounds of more than 2 months in duration require, in addition, the use of growth factors. When using these factors, we observed not only progress in healing but also changes in the characteristics of ulcers. Wound secretion became intense and light, resembling plasma. The ulcer floor, initially flat and covered with necrotic tissues and fibrin, became rough and filled with patches of granulation tissue. Swelling and inflammation decreased significantly.

The use of autologous PRP as a biological dressing significantly increased the wound healing rate and resulted in a complete healing of chronic leg ulcers in all patients suffering from venous insufficiency. The first phase of healing consisting of granulation appeared as fast as 10 days after PRP application, along with a reduction in exudate and a layer of fibrin at the wound floor, and in swelling of wound edges. These changes might likely be due to the action of antibacterial and chemotactic cytokines whose content increases in the wound as reported by Rosner et al. (2001). Prostaglandins formed and released from platelets, potent vasodilators, may accelerate the formation of renewed layers of dermis; the process would be facilitated by a dense network of newly formed microvessels, supplying the granulation tissue with blood as depicted in Fig. 2b. We found in this study that ulcers caused by venous insufficiency in the leg of all area size healed faster when PRP-fortified dressings were applied to the wound. Further, the best effect of PRP was noticed in the largest category of wound. The findings of this study were that with the use of autologous PRP, wounds up to 3.7 cm^2 healed entirely within a month, and more massive wounds healed within 40 days of treatment onset.

A limitation of this study was a relatively short follow-up period, which makes it impossible to draw firm conclusions as to the long-term results of ulcer wound healing with the aid of autologous platelet-rich plasma. Nonetheless, we believe we have shown that platelet-rich plasma is highly effective in shortening and improving the healing process. The autologous platelet-rich plasma emerges as a safe, nonsurgical adjunct procedure for treating chronic venous leg ulcers. Additional studies performed in larger groups of patients and with extended follow-up periods are required to confirm these findings.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was reviewed and approved by the Bioethics Committee of the Medical University of Warsaw in Poland (permit AKBE/127/15).

Informed Consent Written informed consent was obtained from all individual participants included in the study.

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