Autologous platelet-rich plasma in the treatment of venous leg ulcers in primary care: a randomised controlled, pilot study

Objective: To examine the potential efficacy and safety of autologous platelet-rich plasma (PRP) in comparison with the conventional treatment (standard care, SoC) for the treatment of leg ulcers in patients with chronic venous insufficiency, in a primary health-care setting.
Method: A Phase I-II, open-label, parallel-group, multicentre, randomised pilot study was conducted. The outcome variables at baseline and at weeks five and nine included reduction in the ulcer area, Chronic Venous Insufficiency Quality of Life Questionnaire score, cost of the treatment for up to nine weeks and average weekly cure rate.
Results: A total of eight patients, each with at least a six-month history of venous leg ulcer (VLUs), were included in the study. A total of 12 ulcers were treated with either autologous PRP or standard SoC. Patients treated with PRP required wound care only once per week. In the SoC group, patients required intervention 2–3 times per week. A reduction in the mean ulcer size in the PRP group was 3.9cm^2 compared with the SoC group at 3.2cm^2, although the sample size was insufficient to reach statistical significance. Improvement in quality of life (QoL) score was observed in the patients in the PRP group.
Conclusion: This study offers proof-of-concept of the feasibility and safety of PRP treatment to inform larger clinical trials in patients with VLUs. Our preliminary results suggest that PRP delivers a safe and effective treatment for VLU care that can be implemented in primary health-care settings.
Declaration of interest: The authors have no competing interests to report.

platelet-rich plasma • primary care • randomised pilot study • standard care • venous leg ulcer

Autologous platelet-rich plasma (PRP) is used in a range of medical fields to enhance bone and soft-tissue healing. Autologous PRP is a plasma preparation obtained from peripheral blood centrifugation. The curative properties of PRP rely on the fact that upon activation, platelets release a large pool of growth factors and other cytokines that play active roles in several biological mechanisms involved in tissue repair.[1] The relative ease of preparation, applicability in the clinical setting, and favourable safety profile make PRP a promising therapeutic approach for devastating clinical conditions for which current treatments are insufficient, such as in chronic leg ulcer conditions.

In particular, chronic venous leg ulcers (VLUs) represent a common severe health problem. The prevalence of VLUs in the general population is 1–2%,[1–4] and this type of ulcer represents 75–80% of all VLUs. In Spain, between 250,000 and 300,000 people suffer from VLUs.[2]

Despite data showing the effectiveness of autologous PRP in VLUs,[5] the question of whether PRP can be implemented in VLU management in the primary health-care setting remains unexplored.

Aim

The goal of the study was to explore the feasibility, potential efficacy and safety of autologous PRP for treatment of VLUs in a primary health-care setting.

Methods

Study design

We conducted a Phase I-II, open-label, parallel-group, multicentre, randomised pilot study. Patients presenting with at least a six-month history of a VLU were informed of the study protocol and the treatment. The patients gave informed consent to receive the planned treatment and participate in the research, and were allocated randomly to the intervention group for treatment with autologous PRP or to the control group for treatment following the current recommendations for standard care (SoC). Participants were randomised using a computer in the Primary Care Research Unit of Bizkaia (PCRUB). After the informed consent form was signed, the doctors contacted the PCRUB to determine the assigned group.

The patients were assigned to two health centres in the Basque Health Service (Osakidetza) of the

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Ezkerraldea-Enkarterri health region from August 2014 to December 2015. All of the details appear in the published protocol[5] (ClinicalTrials.gov Identifier: NCT01817218).

Ethics approval

The current pilot study was reviewed and approved by the Cruces Hospital Clinical Research Ethics Committee (Ref: 48/570) on 24 April 2012.

Patients

Inclusion criteria for patients included being aged ≥40 years old, of either sex, having a VLU present for more than eight weeks without response to conventional treatment, having normal blood cell counts and haematocrit values, a VLU of ≤7.7cm in diameter, ankle brachial index (ABI) ≥0.8 and ≤1.5, independently mobile or with access to assistance from other persons for travel to the health centre. Exclusion criteria included pregnancy or breastfeeding, of fertile age and not using contraceptive methods, chronic use of immunosuppressants or antiretroviral drugs, clotting disorders, chronic infectious diseases, treatment with radiotherapy or chemotherapy, and active infection or fever at the beginning of the study.

Control group

SoC is the usual care delivered in our health services, and it is applied in accordance with the recommendations of the Basque Health Service (Osakidetza), i.e., two to three times a week to maintain a moist healing environment. We used a waterproof polyurethane dressing (Mepilex), which controls exudate. For ethical reasons, we did not withdraw blood unnecessarily, and thus, the control group that received conventional treatment with SoC was not blinded.[6]

PRP treatment

The patients in the intervention group received weekly wound care with autologous PRP. However, these patients were given a contact phone number to reach the nursing team and arrange an additional visit if they experienced any discomfort or pain.

Qualified nurses performed the treatment with autologous PRP in the phlebotomy room located in the assigned outpatient clinic.

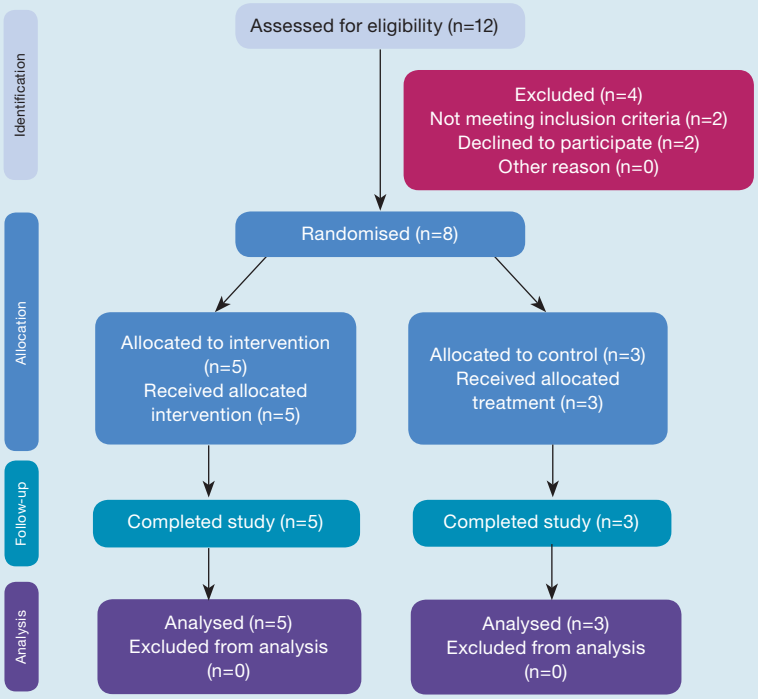
Before application of PRP, all wounds were visually inspected for any infection that might cause healing problems.

Treatment with autologous PRP involves five steps:

- Autologous blood collection
- Centrifugation
- Separation of PRP from red blood cells and leukocytes
- *Ex vivo* coagulation of the PRP to form a biological dressing that is applied to the ulcer bed
- Protection with a secondary dressing.

A volume of 9–30ml of blood (depending on the size of the patient's VLU) was withdrawn from the patient using a Vacutainer and sterile 4.5ml tubes containing 3.8% sodium citrate. Blood was centrifuged for eight minutes at 580g to separate the PRP. The PRP gel was formed by adding 50µl of $CaCl_2$ per ml of PRP in a glass container. The final concentration of $CaCl_2$ in PRP was 22mM. While the plasma was clotting, we prepared the wound by cleaning it with saline and performing mechanical debridement of non-viable tissue (sphacelus). After application of PRP gel in the wound bed, we covered it with a secondary dressing (either foam polyurethane or hydrofiber) dressing was used to protect the autologous gel and improve patient comfort.[6]

Fig 1. Patient selection process for inclusion in study



Intervention and control group

Both groups received compression therapy depending on the tolerance of the patient.[7] A tubular bandage (Tubilast SC) was specially designed for vascular problems to facilitate return circulation through a gradual and decreasing compression from the foot to the compression stockings, with the ability to apply 10–40mmHg of pressure.

Outcome measures

Average weekly treatments: this main variable of the study was calculated by counting the number of weekly visits in which the patient received wound treatment (involving a change of the primary dressing).

VLU area: the surface area of the VLU was expressed in centimetres squared, and measured at baseline and at five and nine weeks using ImageJ software (created by Research Services Branch of the National Institute of

Table 1. Baseline characteristics

	Intervention PRP	Control SoC
Patient number	n=5	n=3
Gender (women)	3 (60%)	0 (0%)
Age, years (SD)	76.6 (8.7)	69.7 (11.1)
Outcome, days (SD)	641.2 (695.2)	283.0 (312.1)
CIVIQ score (SD)	60.8 (17.8)	41.7 (17.1)
Previous injuries (yes)	4 (80%)	3 (100%)
Diabetes (yes)	1 (20%)	1 (33.3%)
Ulcer number	n=7	n=5
Ulcer area, cm^2 (SD)	7.1 (9.1)	8.9 (6.8)
Pain, VAS (SD)	1.9 (2.0)	9.6 (0.9)

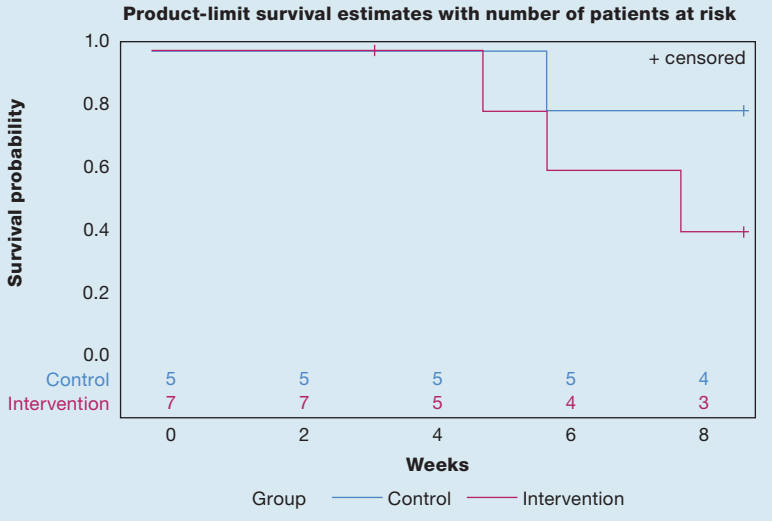
PRP—platelet-rich plasma; SoC—standard care; SD—standard deviation; CIVIQ—Chronic Lower Limb Venous Insufficiency Questionnaire; VAS—visual analogue scale

Mental Health in the US) to analyse photographs of the wounds taken at these time points. Two blinded measures were performed for each VLU, and a mean of both areas was used.

Chronic Lower Limb Venous Insufficiency Questionnaire (CIVIQ) score: this specific scale for assessing the quality of life (QoL) of patients with chronic venous insufficiency considers four dimensions—physical, psychological, social and pain. The CIVIQ score from the questionnaire yields a number between 20 (corresponding to the lowest QoL) and 100 (best QoL). The questionnaire was administered and a nurse recorded the score at the baseline treatment session, week five and at week nine.

Safety: we assessed any adverse effects recorded by the patients and in the weekly interview performed by the nurse, who inquired as to pain and assessed the degree of infection and the type of exudate, as well as any adverse effects associated with the treatment. This information was compared with the results in the control group. Each patient, in both groups, used a diary to self-report adverse effects during the week and was given a telephone number to contact the nurse if needed.

Fig 2. Kaplan-Meier survival curves of platelet-rich plasma treatment (intervention group) compared with standard care (control group)



Costs: the follow-up in our study protocol occurred at nine weeks, and we could not reach patients after this period. Thus, the costs due to non-healing VLUs after nine weeks could not be included in this estimation. To compare the costs in both groups, we used a cost-minimisation approach. The evaluation criteria for the cost per VLU included treatment time, cost per nurse and nursing auxiliary, number of treatments, dressings, and the costs of the materials necessary to prepare PRP.

Statistical analysis

The categorical variables are presented as frequencies, percentages and continuous variables using measures of central tendency and dispersion, mean and standard deviation (SD).

The efficacy analysis was performed on an intention-to-treat (ITT) basis by comparing the percentage reduction in VLU area observed in each of the groups after five and nine weeks of treatment, with respect to the baseline. Using linear regression analysis, we estimated the effect attributable to the intervention using the difference between the changes occurring in the two groups, with adjustment by baseline measurements. Differences between percentages of healed VLUs were compared using the chi-squared test. Kaplan-Meier curves and Cox proportional hazard regression analysis were used to analyse complete VLU closure. A p-value of <0.05 was considered statistically significant. All analyses were performed using SAS version 9.2 statistical software.

Results

During the study period, 12 patients presented with VLUs, but only eight patients (with a total of 12 VLUs) were recruited; two patients did not meet the inclusion criteria and two declined to participate in the study (Fig 1).

Baseline data from patients allocated to the control and intervention groups is shown in Table 1. The control group included three patients with five VLUs. The intervention group included five patients, with seven VLUs meeting the criteria. In the intervention group the VLU was present for 641±695.2 days without response to conventional treatment versus 283.0±312.1 in the control group. All patients in the intervention group received a single treatment per week, whereas patients in the control group received VLU care on average 2.5 times per week.

One patient in the intervention group and another patient in the control group showed signs of infection and were treated with antibiotics for one week. Another patient in the control group reported a perilesional itch sensation that resolved spontaneously after two weeks. There were no intergroup differences with respect to adverse events.

Kaplan-Meier survival curves (Fig 2) showed no significantly increased VLU closure with PRP treatment (log-rank p=0.2194). The Cox proportional hazard regression showed that VLUs treated with PRP were 4.31 times more likely to close than VLUs treated with SoC (hazard p=0.3692). At the end of treatment, average CIVIQ scores were 77 points for the intervention group and 50.8 for the control group. The basal adjusted difference between the intervention and control group was 10.99 points (95%CI –50.5 to 72.5). Table 2 shows the costs of each treatment up to nine weeks.

Table 2. Cost-minimisation approach of platelet-rich plasma compared with standard care (€)

	Mean	Standard deviation	95% CI	p-value
PRP	163.0	65.9	[81.2 - 244.8]	
SoC	147.3	29.7	[110.4- 184.2]	0.640

PRP—platelet-rich plasma; SoC—standard care; 95% CI=95% confidence interval

Table 3. Reduction of ulcer in the intervention group compared with the control group

Outcome measure	Intervention (PRP)	Control (SoC)	p-value
Reduction in ulcer area at 9 weeks (cm^2)	3.9	3.2	0.6818
Reduction percentage in ulcer area at 9 weeks	82.8%	40.8%	0.2756
Reduction in ulcer area at 5 weeks (cm^2)	0.9	0.8	0.9432
Reduction percentage in ulcer area at 5 weeks	39.6%	22.9%	0.5482
Percentage ulcers healed at 9 weeks	60.0%	20.0%	0.5238
Percentage ulcers with a further reduction of 75% at 9 weeks	80.0%	20.0%	0.2063
Percentage ulcers with a further reduction of 50% at 9 weeks	80.0%	60.0%	1
Percentage ulcers with a greater reduction of 75% at 5 weeks	28.6%	20.0%	1
Percentage ulcers with a greater reduction of 50% at 5 weeks	57.1%	20.0%	0.2929
Pain reduction (VAS) at 9 weeks	6.6	–0.01	0.3126
Average weekly treatments	1	2.5	<0.001
Average treatment time (minutes)	46.6	21.7	<0.001

PRP—platelet-rich plasma; SoC—standard care; VAS—visual analogue scale

Discussion

VLUs constitute an important public health problem. The high prevalence and associated costs create a high financial burden for health services and are detrimental to health quality of the affected population.[8]

The safety of PRP treatment has been shown in multiple clinical conditions, including chronic ulcers in the lower limbs with multiple aetiologies.[9–13]

The findings of this study shows significant differences in the average weekly treatments between the PRP intervention group and the control group, but the large amount of variance due to the small sample size hinders significant findings in size reduction (Table 3).

This study did not find any significant difference in the number and severity of adverse events. Previous studies report lower numbers of adverse events in patients treated with PRP.[14–16]

In the authors' experience, PRP application reduced local pain and improved the patient's QoL as assessed by the mean scores from the CIVIQ. This finding is in agreement with Salazar el al.,[8] who reported pain reduction and enhanced QoL using the SF-12 score.

Previous studies involving case series with low numbers of subjects (17–19 patients) showed that PRP accelerated ulcer healing.[16,17] Yilmaz et al. showed complete healing in 94.7% of the ulcers, in a mean time of 4.82 ± 2.16 weeks.[16] Sarvanjnamurthy et al. showed reductions of 94.7%±11.12% in the area and 95.6%±10.19% in the ulcer volume after 5.1±3.1 weeks.[17]

VLUs treated by conventional SoC (in accordance with the recommendations of our institutional guidelines) take at least 10–12 weeks to heal, incurring significant financial costs to both the health system and to patients, as well as impairment to the QoL of patients. The hypothesis of this study was that the autologous PRP-based approach might be able to reduce the costs associated with the management of this type of health problem.[18] VLUs in the intervention group involved a single treatment per week and healed more rapidly, compared with VLUs treated in the control group. However, PRP treatment involved higher costs than conventional treatment, although not significantly more. Indeed, the Spanish Medicines Agency (AEMPs) has ruled PRP as an experimental drug, and in the context of primary care, the AEMPs required two nurses to perform PRP preparation and ulcer management (one nurse working in the sterile field and the other supplying the required ulcer-care material). In addition, the time spent in applying PRP was longer than the time required to perform conventional treatments.

Daily treatments are associated with high risk of infection. Previous studies showed that PRP is a safe, simple and effective procedure in treating chronic VLUs.[5]

Limitations

In this study, organisational changes hindered clinical trial completion. Thus, the main limitation was that a sufficiently large sample size was not obtained.

Conclusions

The high proportion of the population served in primary health-care means that this setting offers opportunities for delivering social and health research, and for establishing preventative measures that can be extrapolated to the entire community. In any intervention measure or research, the closer to the day-to-day reality, the better the application will perform in clinical practice. This statement is particularly true in the case of ulcers as most of these types of wound are managed in primary health-care. Further research, with a larger sample size, should be carried out in this setting to look at the long-term cost implications of delivering VLU treatment. JWC

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