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Perilesional injections of human platelet lysate versus platelet poor plasma for the treatment of diabetic foot ulcers: A double-blinded prospective clinical trial

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Abstract

Diabetic foot ulcer (DFU) is a major cause of morbidity, non-traumatic lower limb amputation in diabetic patients and a high-cost burden on the healthcare system. New therapeutic products are increasingly tested. Platelet-rich plasma (PRP) and human platelet lysate (hPL) are reported to be useful. This trial was conducted to test whether the healing effect of hPL in chronic DFU was due to plasma or platelet lysates in a prospective double-blind design. Autologous PRP was obtained from citrated blood, lysed, and used as drug 1 (active product). The platelet-poor plasma (PPP) was used as a drug 2 (placebo). Ten patients were enrolled in arm 1 and 9 in arm 2. The drugs were injected perilesionally every 2 weeks for a total of sixinjections. Adverse events were recorded until Week 14. The DFUs were scored per the Texas and Wegner systems. No patient showed any major adverse events. Some reported local pain post-injection. Wound healing was achieved in the hPL group in 9/10 of patients at a mean of 35.1 days. In the PPP group, no patient had healed by Day 84. The difference was statistically significant at $P < 0.00001$. We conclude that autologous hPL is safe and highly effective in healing chronic DFU and is superior to autologous PPP.

KEYWORDS

diabetes, foot ulcers, platelet lysate, regenerative medicine, wound healing

Key Messages

- the work describes a double-blind, placebo-controlled study of the healing of chronic diabetic foot ulcers using human platelet lysate.The aim is to show that the healing effect is ascribed to the content of lysed platelets and not to plasma. Ten patients were enrolled in the lysate group and ninein the plasma group
- wound healing was achieved in 9/10 patients in the lysate group, with a mean of 36 days. None of the nine patients in the plasma group healed

within 84 days. The result is statistically significant. We conclude that platelet lysate is highly effective in healing chronic diabetic foot ulcers

1 | INTRODUCTION

Diabetic foot ulcers (DFU) are commonly seen in diabetic patients, with lifetime prevalence reported to be from 15% to 25% and may reach up to 34%[1-3] DFU is a recurrent disease, and healing of an index wound may not mean a permanent remission if the appropriate preventive measures are not taken.[3] Approximately 40% to 65% of patients with DFUs experience a recurrence within 1 to 5 years.[1,4,5]

Moreover, DFU is a major cause of morbidity and mortality in diabetic patients. It is the major cause of non-traumatic lower limb amputation.[6,7] Five-year mortality for DFU and major amputations is estimated to be 30.5%, and 56.6%, respectively, compared with 5-year pooled mortality for all reported cancers, which is 31.0%.[8]

The increased hospitalisation and supportive care for DFU represent a considerable economic burden. The EURODIALE study looked at 821 patients with DFU in several EU countries and analysed the direct and indirect annual costs, estimating a total annual cost per patient of €10 091, mostly for hospital costs.[5]

In the United Kingdom, it was estimated that 20% of total spending on diabetes care is taken up by complications related to DFU[6,9-10] A study in the USA concluded that DFU imposes substantial additional costs on public and private payers.[10,11]

For the treatment of DFU, new therapeutic products are increasingly tested and used for the in situ administration of bioactive molecules alone or with wound dressings.[12] It has been reported that platelet-rich plasma (PRP) and human platelet lysate (hPL) could improve the healing of chronic wounds, including DFU.[9,13-18]

We have previously published two cases of DFU who were treated and healed by autologous peri-lesional hPL injections[18] as part of a larger trial, which we are reporting in this paper. Since autologous hPL uses a mixture of autologous plasma and autologous platelet lysates, the trial was set to test whether the healing effect was due to plasma or platelet lysates in a prospective double-blind design.

2 | PATIENTS AND METHODS

This is a prospective double-blind, placebo-controlled trial testing the use of an autologous hPL product with autologous platelet-poor plasma (PPP) used as a placebo. This study was approved by the Institutional Review Board (IRB) at the Cell Therapy Center, University of Jordan. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. Inclusion and exclusion criteria and patient selection were previously published by us[18] and are outlined below.

2.1 | Inclusion criteria

1. Patients with type II diabetes mellitus (DM) between the ages of 18 and 70 with an ulcer of at least 6 weeks duration.
2. HbA1c of less than 13%.
3. Index foot ulcer located on the plantar, medial, or lateral aspect of the foot (including all toe surfaces), and the wound area (length*width) measurement between 0.5 cm^ 2 and 20 cm^ 2, inclusive.
4. Wounds located under a Charcot deformity have to be free of acute changes and went through appropriate structural consolidation.
5. University of Texas stage A Grade II or III ulcer.[19]
6. The protocol requires that post-debridement, the ulcer would be free of necrotic debris, foreign bodies, or sinus tracts.
7. Non-invasive vascular testing Ankle Brachial Index (ABI).
8. Otherwise medically free on physical examination (including a Semmes-Weinstein monofilament test for neuropathy).
9. Results of blood tests within the accepted range, including a full blood count, liver and kidney function tests, hepatitis screening, and blood chemistry, including HbA1c.
10. Signed, approved, and informed consent from each patient.

2.2 | Exclusion criteria

1. Patient currently enrolled or previously enrolled (within the last 30 days) in another investigative device or drug trial.
2. Ulcer area decreased by >50% during the 7-day screening period.
3. Ulcer is due to a non-diabetic aetiology or DFU not meeting inclusion criteria.
4. Evidence of gangrene in the ulcer or on any part of the foot.

5. Patient is currently receiving or has received radiation or chemotherapy within the last 3 months of randomization.
6. The patient has received growth factor therapy within 7 days of randomization.
7. Screening platelets count <100* 10^9/L, and or Hb less than <10 g/dL.
8. The patient is undergoing renal dialysis or has a known immune deficiency, known abnormal platelet activation disorder, liver disease, active cancer, eating/nutritional, hematologic, collagen vascular disease, rheumatic disease, or bleeding disorder.
9. History of peripheral vascular repair within 30 days of randomization.
10. Patient is known to have a physiological, developmental, physical, emotional, or social disorder or any other situation that may interfere with their compliance with the study requirements and/or the healing of the ulcer.
11. History of alcohol or drug abuse within the last year prior to randomization.
12. The patient has inadequate venous access for blood withdrawal.

Consenting patients were randomised in a double-blind manner into 2 groups: one group was given hPL, and the second one was given PPP as a placebo.

2.3 | hPL and PPP preparation

The method of preparation of hPL and PPP and their final concentration was as previously published.[18]

Briefly, 25 mL of blood was acquired from each participant by venous phlebotomy into citrated tubes. PRP was obtained by centrifuging the citrated blood at 900*g* for 10 min and taking the supernatant.

For the hPL arm, the platelet count in the PRP preparation was adjusted at 1000×10^9/l before further processing. PRP was further processed by two freeze/thaw cycles at −80°C and 37°C, respectively, followed by centrifugation at 3060*g* for 20 min, and filtration of the supernatant using 0.22 μL filter (BD, USA).

For the PPP arm, PRP was centrifuged at 3060*g* for 20 min, and the supernatant was collected and filtered using a 0.22 μL filter (BD, USA). A platelet count was used to confirm the lack of platelets in the final preparation.

2.4 | hPL and PPP injections

In group one, hPL was injected peri-lesional at ulcer margins every 2 weeks for a maximum total of six injections. In group two, PPP was used in the same manner every 2 weeks for a maximum total of six injections. Each injection was composed of 5 mL of the material to be injected, distributed in five 1-mL syringes. Adverse events were observed and recorded in a checklist form until Week 14.

The DFU was scored as per the Texas grading system and the Wegner scoring system. During each visit, the ulcer size was measured before the injection and recorded, then the surface area was calculated accordingly.

Wound healing was examined by a well-trained surgeon with experience in DFU. Photographic images were obtained at baseline, 4 weeks, 12 weeks, and 14 weeks or when the wound has healed, whichever came first.

2.5 | Outcome measures

The first outcome measure was treatment safety, determined by the number and severity of adverse events. Patients were monitored for 1 h post-treatment, then phone interviewed at 24 h and 1 week. They were physically examined every 2 weeks. Blood tests were performed monthly for up to 6 months as part of our safety follow-up policy.

The second outcome measure was efficacy, as measured by either a. complete healing at any given time point within 12 weeks b. partial healing measured by ulcer size reduction at 12 weeks. Treatment was considered a failure when neither a. nor b. above were reached at 12 weeks. In cases where ulcers fully healed before 12 weeks, the healing time was recorded.

3 | RESULTS

Patient screening and enrollment are shown in Figure 1 below, which details the patients' selection, enrollment, and the reasons for those patients who did not complete the study. A total of 40 patients with chronic DFU were screened for this study; 30 patients met the inclusion criteria and were enrolled. They were randomised into two arms: hPL (active product) and PPP (placebo) arms. Both arms received the assigned treatment on top of the standard of care. The standard of care for DFU at our institution is as per the WIFI classification system[20] and it consists of the following: wound bed preparation, debridement as appropriate, moist wound bed dressing, and secondary dressing by absorptive fibre or foam with an antimicrobial agent. As for the off load it is according to the case biomechanics, consisting of the total contact cast or removable cast. In total, 10 patients in the (active product)

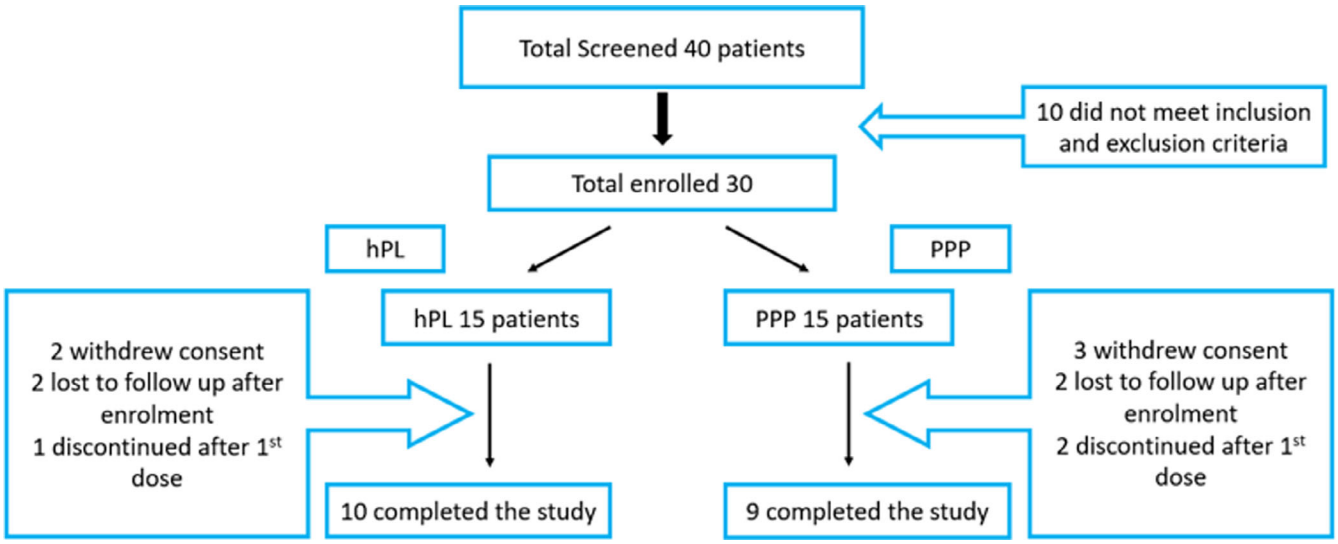

FIGURE 1 Patients screening and enrollment

TABLE 1 Patients characteristics and DFU status

	n[a]	Age (years) Mean/Median	Hba1c Mean	Duration of DFU (weeks)	Ulcer stage[b] (n)
hPL					
Males	6	62.5/62	8. 6	30	AI 1 AII 5
Females	4	60/62	8. 3	24	AI 1 AII 3
Total	10	61.4/62	8.48	27. 6	AI 2 AII 8
PPP					
Males	5	58. 2/61	8. 3	28	AI 1 AII 4
Females	4	59. 3/61.5	8	23.5	AI 1 AII 3
Total	9	58. 6/61	8.12	26	AI 2 AII 7

[a]n, number of patients.
[b]As per the University of Texas staging system.

arm and nine patients in the (placebo) arm completed the study, and their results were analysed.

In total, we enrolled six males and four females in the (active product) arm, with a mean age of 61.4 years, a mean ulcer duration of 27.6 weeks, and a mean ulcer size of 13.88 cm^2 (Range: 2-24.5 cm^2); five males and four females in the (placebo) arm, with a mean age of 58.6 years, a mean ulcer duration of 26 weeks, and a mean ulcer size of 11.8 cm^2 (Range: 1. 6-22. 2 cm^2). Other patients' epidemiology and characteristics are shown in Table 1.

3.1 | Treatment outcome and statistics

Haematology and chemistry blood tests done monthly showed no changes in both group. There were no significant adverse events observed either by physical examination or through patients' self-reported questionnaires. Minor adverse events were documented following injections, including local pain, swelling, infection, bleeding, redness, and hotness. The total number of occurrences of these events throughout the full course of treatment was documented and charted in Table 2.

Nine patients in the hPL group achieved full healing at or before the determined ending time point (12 weeks). One patient only showed partial healing (around 80% reduction in ulcer surface area) at Week 12. The treatment was considered a failure for that patient for the purposes of study analysis, but the patient was given two additional doses on a humanitarian basis. The ulcer showed full healing on day 110. Details are shown in Table 3A.

None of the patients in the PPP group showed full healing at 12 weeks. Some patients showed ulcer size reduction, as detailed in Table 3B.

Using analysis of variance (ANOVA) one-way test, the healing efficiency calculated based on ulcer surface area was 0.98 (SD ±0.0632) in the hPL arm and 0.0422 (SD ±0.0529) in the PPP arm. The difference between hPL and PPP arms was found to be statistically highly significant in favour of hPL arm with $P < 0.00001$.

Figure 2A shows representative photographs of DFU treated by autologous human platelet lysate (hPL) (Figure 2A), and representative photographs of DFU treated by autologous platelet-poor plasma (PPP) (Figure 2B) at different time points as shown.

TABLE 2 Total number of adverse events encountered during the whole course of treatment

Group	Injection site pain	Local swelling	Local infection	Local bleeding	Redness and hotness
hPL	36	10	6	5	9
PPP	28	7	4	1	6

TABLE 3 Details of the number of injections and treatment outcomes. (A) hPL group. (B) PPP group

Patient number	Gender	Days needed for full healing	Number of injections for full healing	Healing percentage after hPL therapy
1	M	78 days	6 Injections	100%
2	F	110 days	8 Injections	Partially healed at week 12 (80%)
3	M	21 days	2 Injections	100%
4	M	56 days	4 Injections	100%
5	F	30 days	3 Injections	100%
6	M	48 days	4 Injections	100%
7	F	18 days	2 Injections	100%
8	M	17 days	2 Injections	100%
9	F	31 days	3 Injections	100%
10	M	32 days	3 Injections	100%

Patient number	Gender	Days of follow-up looking for healing	Number of injections given	% of DFU size reduction at week 12
1	F	84	6	10%
2	M	84	6	0%
3	M	84	6	5%
4	F	84	6	15%
5	F	84	6	0%
6	M	84	6	3%
7	F	84	6	5%
8	M	84	6	0%
9	M	84	6	0%

4 | DISCUSSION

DFU are a challenging problem in diabetes care since they are commonly seen, with a lifetime prevalence reported to be from 15% to 25% and may reach up to 34%.[3] In addition to their poor healing, DFU are known to recur, are costly to the health care budget, and do not have an effective, universally approved therapy.[1,10]

New therapeutic products are being tested for in situ administration of bioactive molecules alone or with wound dressings.[12] Platelet-based products, either in the form of PRP or hPL, have been tested with variable degrees of success.[9,13-18] In these papers, the contribution of platelet-poor plasma (PPP) to DFU healing was not defined, and the question remains whether PPP may have contributed to the healing.

In this study, we aimed to explore the value of hPL versus PPP in a double-blind prospective trial comparing the efficacy of perilesional injection of autologous hPL versus autologous PPP in chronic diabetic foot ulcers. As such, PPP was used as the control "placebo" arm since it has the same colour as hPL and has the same volume but lacks the "active" component of platelet lysates.

As can be seen from our results, the hPL has a superior therapeutic effect, and nine out of 10 patients healed within a short time, with a mean of 36.1 days from the start of treatment. We did not study the recurrence rate in these patients. We did not have any cases of worsening infection or uncontrolled infection, or amputation. We did not record any serious adverse events.

The statistically significant results of this pilot study demonstrated that hPL, when used as a peri-lesional injection, has a favourable effect on the rate of healing of

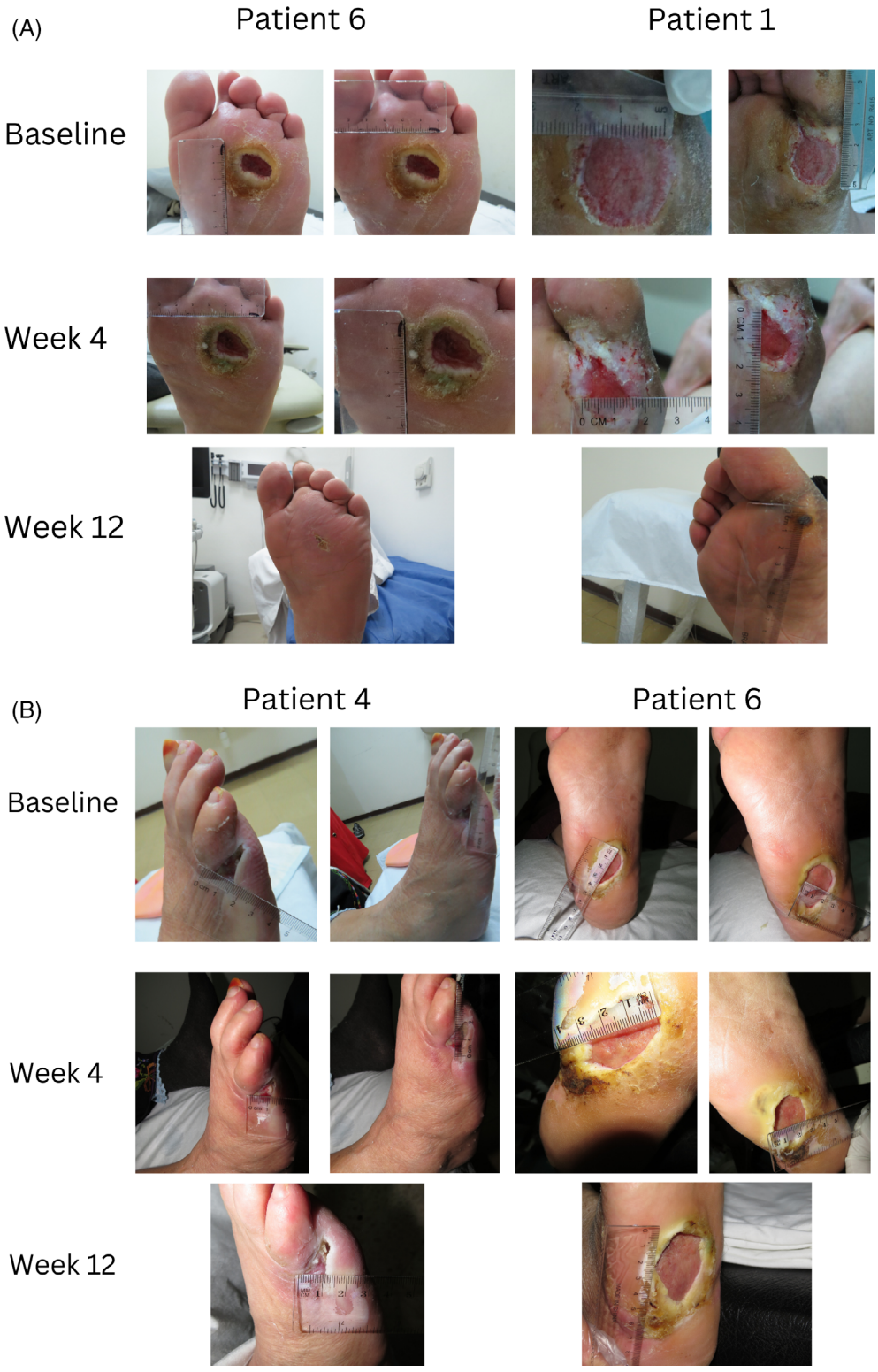

FIGURE 2 Representative photographs of DFU treated by autologous human platelet lysate (hPL) at different time points. Representative photographs of DFU treated by autologous platelet poor plasma (hPL) at different time points

chronic DFU. The multiple regulatory proteins retained in hPL through the described preparation process seem to play a significant role in modulating the healing cascade to a semi-acute state. Further specification of these factors is required before such definitive claims can be made. This is consistent with previous work done with PRP,[13-15,17] and limited work with hPL.[18] Topical PRP products have been reported to produce good therapeutic effects on DFU.[21]

We are the first to report on perilesional hPL injections in DFU in a double-blind, controlled study. Though our patient population is small, we believe it contributes

to the concept because of the double-blind design and comparison with plasma.

Furthermore, it seems that this modality is safe. We think the PPP has little, if any, therapeutic effect on DFU. Our work should stimulate a larger study to confirm these findings.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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