ABSTRACT

Introduction: Population ageing has led to an increase in the prevalence of many chronic diseases that occur in elderly patients including chronic wounds of various aetiologies, especially leg ulcers. The treatment of these wounds is lengthy and associated with health, economic and social problems. The aim of our study was to compare the outcomes of local injections of autologous growth factors with standard dressings for leg ulcer treatment.

Methods: The study included 25 patients with leg ulcers treated with autologous growth factors, and 15 patients treated with standard wet dressings only. The area and depth of ulcers were measured on days 0, 5, 28, 84 and 168, and statistically processed using the chi-square test, the Fischer exact test, the Wilcoxon two-sample test, the non-parametric paired Wilcoxon test and the Friedman analysis of variance (ANOVA) test at a significance level of 5%.

Results: Area and depth did not significantly differ between the two groups before initiation of the treatment ($p = 0.472$ and $p = 0.242$, respectively). During the study period, the average leg ulcer area decreased in both the study and control groups by 72% and 40%, respectively. The paired Wilcoxon test showed that this decrease was significant in the study group ($p < 0.001$), but not in the control group ($p = 0.075$).

Conclusion: Leg ulcers heal better when treated with autologous growth factor injections than when treated with standard dressings alone. A further study with a larger number of patients is needed to confirm the presented results. However, this method seems to be a promising way to treat ulcers of the lower extremities.

Keywords: Autologous growth factors; Chronic defects; Wound healing
Key Summary Points

Why was this study carried out?
The treatment of soft tissue trophic defects is difficult and is a current medical, social and economic problem.

We evaluated the therapeutic effects of instilling growth factors into lower leg defects and compared the results with patients treated with conventional therapy (wet dressings).

The study included 40 patients with leg defects and the length of follow-up was 168 days.

What was learned from the study?
Our study indicates that local injections of growth factors into leg ulcers leads to better healing than using standard therapy (wet dressings) alone.

INTRODUCTION

A wound is defined as damage to the integrity of the integument, mucosa or an organ [1, 2]. Wounds are classified as superficial, which are limited to the skin and subcutaneous tissues, and deep, which reach the fascia, muscles and neurovascular structures. Wounds can be caused by mechanical, thermic or chemical insults. Poor wound healing is often caused by chronic diseases, such as diabetes mellitus, peripheral artery disease and chronic venous insufficiency. Primary care of chronic non-healing and extensive wounds is the responsibility of surgical and dermatology disciplines. The goal of the treatment is for the wounds to heal with good functional and aesthetic results [3, 4].

Wound healing is a physiological process controlled by several regulatory mechanisms. This complex process is triggered immediately after ulcer formation and is completed only after the ulcer has healed completely [5, 6].

The first-line therapy for leg ulcers is topical dressings. Population ageing has led to an increase in the prevalence of many chronic conditions, including leg ulcers [7]. Treatment of leg ulcers is currently a lengthy and costly process associated with many complications [6, 8, 9]. The goal of our study was to investigate the efficacy of a new method of leg ulcer treatment that involves injections of autologous platelet-rich plasma (PRP) into the base of the ulcer lesions [10–12]. This new treatment was compared with a control group of patients who were treated only with standard wound care.

METHODS

Study Group

We included 25 patients treated using the PRP method and 15 patients treated with standard wet dressings. Inclusion criteria were: (1) leg ulcers of arterial, diabetic or post-traumatic aetiology, which had already been treated with conventional therapy for at least 5 months with no effect; (2) initial lesion size less than 10 × 10 cm; (3) patient age over 18 years; (4) glycaemia levels under 10 mmol/l and glycosylated haemoglobin levels less than 45 mmol/l; (5) no immunosuppressive therapy; (6) blood coagulation parameters within the normal limits; and (7) signed informed consent. Characteristics of the patient groups are described in Table 1.

Allocation to the PRP or control group was according to patient decision. Bandages were changed every three or four days. The study received approval from the Ethics Committee of the University Hospital of Pilsen, Czech Republic (approval number: 604217). The study was performed in accordance with the Helsinki Declaration of 1964 and its later amendments. All subjects provided informed consent to participate in the study.
Preparation of the platelet-rich plasma

Preparation of the platelet-rich plasma took place on an outpatient basis. Immediately after collection, 60-ml venous blood samples were put into a gravitational centrifuge (Zimmer Biomet, Warsaw, Indiana, USA) at 3200 rpm for 15 min in a special closed system. This separated the individual components of the blood samples (platelet-rich plasma, platelet-poor plasma and red blood cells). Using a special syringe [Artrex ACP Double Syringe (Artrex, Inc., Naples, Florida, USA)], 6 ml of platelet-rich plasma were collected. The platelet-rich plasma contains autologous growth factors, in particular platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β) and fibroblast growth factor [5, 7].

Therapy

On the first day of treatment solutions rich in autologous growth factors were directly injected into the bases of the leg ulcers. The ulcer base was injected in four quadrants with 5 ml of platelet-rich plasma. Each dose was injected at least 0.5 cm from the lesion edge. Patients received only one application of PRP during the duration of the study. After application the lesions were covered with the non-adherent anti-microbial foam dressing Mepilex Ag (Mölnlycke Health Care AB, Gothenburg, Sweden). The control group was treated using only Mepilex Ag. Dressings were changed at intervals of three to four days in both groups.

Monitoring the Effect of Treatment

The ulcer area and depth were measured on day 0, 5, 28, 84 and 168. Lesion depth was measured using a calibrated depth probe. The follow-up period was 168 days. Results were recorded in MS Excel (Microsoft Corporation, Redmond, Washington, USA).

Statistical Evaluation

Differences in the frequency of categorical variables of patients in both groups were compared using the chi-square test and the Fisher exact test. The Wilcoxon two-sample test was used to compare the distribution of the monitored parameters in both groups. Changes in parameters over time were analysed using the non-parametric paired Wilcoxon test and the

### Table 1 Demographic data and wound characteristics

|                  | Study group (N = 25) | Control group (N = 15) | P value |
|------------------|----------------------|------------------------|---------|
| Age (years)      | 59 (31–82)           | 62 (30–80)             |         |
| Sex              | 0.927                |                        |         |
| Males            | 7                    | 4                      |         |
| Females          | 18                   | 11                     |         |
| Diabetes         | 12                   | 7                      | 0.243   |
| Wound types      | 0.876                |                        |         |
| Venous           | 5                    | 3                      | 1.000   |
| Arterial         | 2                    | 1                      |         |
| Diabetic         | 6                    | 4                      | 0.850   |
| Mixed            | 12                   | 7                      | 0.934   |
| Wound dimensions |                      |                        |         |
| Surface area     | 11.2 cm²             | 8.2 cm²                | 0.242   |
| Depth            | 3.1 mm               | 2.7 mm                 | 0.472   |

### Table 2 Average lesion area over the duration of the study

|                  | Study group | Control group |
|------------------|-------------|---------------|
| Day 0            | 11.2 cm²    | 8.2 cm²       |
| Day 5            | 10.5 cm²    | 8.1 cm²       |
| Day 28           | 9.7 cm²     | 8.1 cm²       |
| Day 64           | 8.6 cm²     | 7.3 cm²       |
| Day 168          | 7.8 cm²     | 6.9 cm²       |
The statistical programs SAS (SAS Institute Inc., Cary, North Carolina, USA) and STATISTICA 9.0 (StatSoft Inc., Tulsa, Oklahoma, USA) were used to process the results.

RESULTS

The study group consisted of 25 patients (18 women and 7 men) treated with platelet-rich plasma injections. The mean age of these patients was 59 years (31–82 years). The control group comprised 15 patients (11 women and 4 men), with an average age of 62 years (30–80 years). Detailed characteristics are described in Table 1. Changes in the area and depth of lesions are shown in Tables 2 and 3. On day zero there was no significant difference between groups in either lesion area ($p = 0.472$) and depth ($p = 0.242$). At the end of the study, we observed a significantly higher number ($p = 0.045$) of completely healed lesions in the study group (11 of 25; 44%) compared with the control group (2 of 15; 13%) (Fig. 1). Patients treated with autologous growth factors had a 5.1-fold higher chance of complete healing.

Reductions in the size of the ulcers were seen in both groups on day 168. On day zero, the average lesion area in the PRP group was 11.2 cm$^2$, and in the control group the average lesion area was 8.1 cm$^2$. On day 168, the average lesion area decreased to 7.8 cm$^2$ in the PRP group and 6.9 cm$^2$ in the control group ($p = 0.050$). The average reduction in ulcer area in the PRP group was 3.34 cm$^2$, while in the control group, the decrease was only 1.2 cm$^2$ ($p = 0.002$). In terms of lesion depth, the PRP group had an average depth reduction of 1.32 mm, while the control group had a reduction of only 0.4 mm.

We demonstrated, using pair tests, that the study group had more frequent reductions in lesion area over time. When comparing the lesion size on day 0 and day 168, better results were achieved in the group treated with PRP. Using the parametric (repeated) ANOVA test, we

| Table 3 Average lesion depth over the duration of the study |
|-------------------------------------------------------------|
|                | Study group | Control group |
| Day 0          | 3.1 mm      | 2.7 mm        |
| Day 5          | 3.1 mm      | 2.8 mm        |
| Day 28         | 2.7 mm      | 2.9 mm        |
| Day 64         | 2.2 mm      | 2.2 mm        |
| Day 168        | 1.8 mm      | 2.2 mm        |

![Fig. 1](image1.png) Percentages of healed and unhealed lesions in the two groups

![Fig. 2](image2.png) Changes in lesion depth
demonstrated a statistically significant reduction in lesion area in the study group compared with the control group ($p = 0.046$). We analysed changes in depth of the lesions with paired Wilcoxon tests. There was a significant decrease in the study group ($p = 0.002$) and a non-significant decrease in the control group ($p = 0.390$) (Fig. 2).

We also compared the results with our previous study from 2013, when we applied growth factors locally by applying them on a nanofiber carrier [5]. Here we demonstrated better overall cure rates in patients by injecting lesions with PRP (Fig. 3). However, we failed to demonstrate a statistically significant difference relative to time, lesion depth and area.

**DISCUSSION**

Chronic wounds have a strong negative impact on quality of life and are often very difficult to heal. Better understanding of the physiological mechanisms behind wound healing, developing new dressing and compliance with the rules of antisepsis have led to new procedures in chronic wound care [1, 2, 13]. Many of these are based on PDGF and TGF-β. A positive effect of these agents on cell proliferation, chemotaxis, differentiation and synthesis of the extracellular matrix has been demonstrated [3, 4]. PGDF is an important anabolic factor in bone metabolism. It stimulates proliferation and chemotaxis of osteoblasts. TGF-β is found in high levels in bone matrix and promotes wound healing. When applied topically, it has a stimulating effect on osteoblast growth, fibroblasts, blood capillary formation, extracellular matrix remodelling and leads to formation of granulation tissue [3].

The primary goal in leg ulcer treatment is rapid healing with a functional and aesthetically acceptable scar. Standard chronic wound treatment should be simple and ideally short term, but many secondary problems can occur, such as infections or necrosis, especially in the elderly, leading to a decrease in quality of life [9].

Ulcers should be maintained in conditions that guarantee physiological healing. This usually requires a moist environment, which is necessary for rapid cell growth and supports angiogenesis and fibrinolysis [14, 15]. Proper healing also requires that lesions be kept clean, with the dressing providing an adequate barrier between the external environment and the ulcer.

Wound dressings can be classified as being inactive, interactive or bioactive [16, 17]. Inactive dressings have a high absorption capacity. They are mostly made of cotton, synthetic fibres or multiple layers of these materials. To maintain a moist environment with inactive dressings, it is necessary to soak them in saline and then cover them with a waterproof film. A disadvantage of these dressings is that when they are changed, they may traumatise the underlying newly formed granulation, which can be uncomfortable or even painful for the patient.

Interactive dressings are made of special materials. They favour optimal conditions for wound healing and are used for different stages of healing. Dressing changes are much less painful, and it is possible to let the dressings
stay on the wounds for several days. Currently, there are about 300 different types of dressings available from several different companies. A Cochrane review analysed 42 randomised controlled trials on wound healing material [16, 18]. None of these studies provided evidence that better healing was achieved with hydrocolloids, alginates, foam dressings or hydrogels. Nevertheless, in our study, we used the same anti-microbial foam dressings in both patient groups to eliminate a potential bias between the groups.

The most modern method of treating chronic lesions is to use bioactive dressings. These dressings directly adhere to the wound and usually are directly involved in the healing processes. After healing, they often remain in the wound and are incorporated into the final scar [19, 20].

Even though we tried the PRP method in a small number of patients, we believe that the results of our pilot study demonstrate the benefits of a one-time local injection of autologous growth factors into the base of lesions (Figs. 4 and 5). We achieved similarly favourable results in our previous study through the superficial application of autologous growth factors to the lesion using a nanofiber carrier [5]. However, problems with standardizing the surface application of growth factors prompted us to prepare a study that would demonstrate the effectiveness of growth factors using a better-standardized method that involved local injection. We suggest that this method can be used to treat primary and secondary wounds; we also see an opportunity for the use of this method in the treatment of soft tissue injuries and burns.

The limitations of our study are associated with the small number of patients. A multicentric study with a larger number of patients could confirm our results.

**CONCLUSION**

The study demonstrated a significantly higher rate of healing of leg ulcers after applying local injections of autologous growth factors into the base of lesions when compared with classic ‘wet’ dressings.
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Compliance with Ethics Guidelines. The study received approval from Ethics Committee of the University hospital of Pilsen, Czech Republic No. 1604217. The study was performed in accordance with the Helsinki Declaration of 1964, and its later amendments. All subjects provided informed consent to participate in the study.

Data Availability. All data are available from the first author.

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