Efficacy of platelet-rich plasma for the treatment of chronic wounds

Abstract
Aim: To assess the efficacy of platelet-rich plasma for the treatment of patients with chronic wounds of various aetiologies.

Methods. We analysed the treatment outcomes in 81 patients with chronic wounds of various aetiologies. For the treatment of 44 patients (experimental group), we used platelet-rich plasma flat clot therapy starting from phase II of the wound healing process. The frequency of dressing changes was once in 7 days, an interval that allowed patient transfer to outpatient care. For the treatment of 37 patients (control group), traditional topical agents were used.

Results. In three patients, owing to a large area of chronic wounds, 3-4 autodermoplastic closures of the wound were performed; 85.4% of patients achieved complete wound re-epithelialisation within 46.4 ± 4.3 days. In the control group, the autodermoplastics operation was performed in three patients, and only 11.8% of patients achieved wound re-epithelialisation within 3 months. The mean inpatient hospital duration was 11.0 ± 2.5 days in the experimental group and 23.1 ± 1.5 days in the control group. The mean cost of treatment was 785.25 Euros and 1649.02 Euros per patient in the experimental and control groups, respectively.

Conclusions. The treatment of patients with chronic wounds using platelet-rich plasma is safe, clinically beneficial and cost effective.

INTRODUCTION
The treatment of chronic wounds of different aetiologies is a topical issue of modern medicine. The acceleration of reparation processes and tissue regeneration entails not only clinical but also economical and social effects.

In the late 1990s, Robert E. Marx described the method of obtaining platelet-rich plasma (PRP) and its use as a gel in dentistry[1]. In 1994, Eduardo Antitua demonstrated that the PRP gel helps to accelerate bone regeneration and confirmed the presence of specific platelet-derived growth factor receptors (PDGFs) in the bone tissue[2]. In 2001, Russian scientists R.R. Akhmerov and R.F. Zarudiy developed the Plasmolifting™ technique that included the production and use of PRP injections in dentistry and dermatocosmetology[3,4].

PRP is a platelet suspension in plasma derived from human blood. The platelet concentration in PRP is 2- to 6-fold higher than that in total blood and may reach 1000 × 10⁹/L[5]. Platelets contain growth factors that are attracted locally to damaged progenitor cells to stimulate their proliferative activity and improve wound healing via autocrine and paracrine mechanisms. Platelet growth factors include PDGF, platelet-derived angiogenesis factor (PDAF), transforming growth factor-b (TGFb), insulin-like growth factor (IGF), platelet-derived endothelial cell growth factor (PD-ECGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), thrombospordin and osteonectin[6].

PDGF plays a particular role in tissue reparation and regeneration. PDGF was shown to stimulate the proliferative, secretory and migratory activity of mesenchymal cells[7] and is a co-factor for other growth factors — e.g., the angiogenic vascular endothelial growth factor (VEGF)[8]. Growth factors are released after human platelet activation. Once activated, the platelets release approximately 70% of their stored growth factors within the first 10 minutes. Complete release of platelet growth factors is accomplished within 1 hour. Therefore, it is recommended to activate platelets immediately before using PRP[9-11].
Apart from the growth factors, activated platelets release large amounts of substances that contribute to primary homeostasis, including serotonin, catecholamines, fibrinogen, fibronectin, factor V, factor 8 (von Willebrand factor), thromboxane A2 and calcium [9–13]. As a result, platelet aggregates (clots) are formed, causing platelet stabilisation by cross-linked fibrin and sticky glycoproteins. The fibrin matrix formed promotes normal cell infiltration with monocytes, fibroblasts and other cells that play an important role in wound healing.

The current use of PRP for the acceleration of bone repair and soft tissue growth has become a true breakthrough in dentistry, traumatology, sports medicine, cosmetology and surgery. PRP has already proven to be useful in tissue engineering and cellular therapy [9,10,14,15]. PRP is most widely used for the filling of large bone defects in maxillofacial surgery [11,16]. PRP can be used along with bone material (the patient’s own or bone substitute), applied to the implant site before the use of bone material, placed atop of the bone material or used as a biological membrane [6,11,16]. PRP has proven to be effective in accelerating soft-tissue healing and epithelialisation [6]. PRP is indicated for use in free connective tissue graft procedures, manipulations with mucoperiosteal flaps and soft tissue augmentation for cosmetic purposes in dentistry [17,18].

Following the implementation of PRP therapy in dentistry, the treatment began to be used in orthopaedics and traumatology. PRP technology is most widely used in the management of acute injury for the stimulation of osteogenesis in combination with osteosynthesis and in the treatment of arthrosis [19]. A method for the stimulation of neangiogenesis in the ischaemic tissues of the lower extremities using PRP has been developed [20].

Currently, several papers have been published on PRP use for the management of chronic venous stasis leg ulcers developed against the backdrop of chronic arterial or chronic venous insufficiency. The results of these studies allowed the conclusion that the use of PRP in the combination treatment of stasis ulcers provides a wide range of local therapeutic effects, improves treatment results, enables considerable reduction of the length of treatment and increases patient quality of life that is also of economic importance [20–25].

**METHODS**

We analysed the treatment results of 81 patients with chronic wounds of different aetiology – namely, venous leg ulcers (VLUs): 8 patients; ulcers of mixed aetiology (UMEs): 20 patients; leg ulcers with underlying diabetic foot syndrome (DFS): 20 patients; post-traumatic and postoperative ulcerated scars and trophic ulcers (STUs): 19 patients; and decubital ulcers (DUs): 14 patients. The exclusion criteria were as follows: suspicion of ulcer malignisation, presence of systemic vasculitis, oncologic condition or haematologic disorder, or low adherence of patients to their treatment.

Following the bacteriological testing, all of the patients were administered treatment against the main disease and topical treatment aimed at bacterial clearance and decontamination of the wound. The microbial landscape at baseline is presented in Table 1.

All of the patients were divided randomly into two groups at admission. Patients who did not meet the inclusion and exclusion criteria were excluded from the study.

Forty-four patients in phase II of wound healing (i.e., the study group that included 17 men and 27 women with an average age of 56.0 ± 3.1 years and VLU (3 patients), UME (8 patients), DFS (12 patients), STU (14 patients) or DU (7 patients), with an average wound area of 90.2 ± 14.1 cm²) received PRP flat clot applications. Different methods for PRP preparation have been described (e.g., double- or single-spin centrifuge harvesting). However, a common algorithm exists for PRP preparation that comprises three stages: blood collection, PRP isolation from whole blood using centrifugation and activation of platelets contained in the PRP [20]. We used the method described by Eduardo Anitua and BTI equipment (Spain) to obtain PRP in the form of a gel (flat clot) [23]. The volume of blood drawn depends on the size of the wound and is approximately half of the wound area in cm². For example, with a wound area of approximately 100 cm² and

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Study group n=44</th>
<th>Reference group n=37</th>
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<tr>
<td>St. aureus</td>
<td>MSSA 6 (13.6%)</td>
<td>6 (16.2%)</td>
</tr>
<tr>
<td>MRSA</td>
<td>3 (6.8%)</td>
<td></td>
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<tr>
<td>Enterococcus spp.</td>
<td>4 (23.5%)</td>
<td>1 (2.7%)</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td>8 (18.2%)</td>
<td>1 (2.7%)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>–</td>
<td>2 (5.4%)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2 (4.5%)</td>
<td>2 (5.4%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4 (9.1%)</td>
<td>5 (13.5%)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>2 (4.5%)</td>
<td>7 (18.9%)</td>
</tr>
<tr>
<td>Microbial association</td>
<td>9 (20.5%)</td>
<td>8 (21.6%)</td>
</tr>
<tr>
<td>Microbial growth not revealed</td>
<td>6 (13.6%)</td>
<td>5 (13.5%)</td>
</tr>
<tr>
<td>Average bacterial load in the wound</td>
<td>106</td>
<td>106</td>
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a PRP clot thickness of about 1-2 mm, 45-50 ml of whole blood would be required. The volume of PRP obtained on average is 20-25% of the original volume per individual, depending on the rheological properties of the blood of each patient. The time from the moment of blood collection to wound dressing was 20-30 minutes. The PRP flat clot was covered with an atraumatic mesh dressing and a secondary dressing on top of the former. The dressing frequency was once in 7 days, allowing patient transition from inpatient to outpatient care.

Thirty-seven patients in phase II of wound-healing (the reference group that included 17 men and 20 women with an average age of 69.5 ± 2.2 years with VLU (5 patients), UME (12 patients), DFS (8 patients), STU (5 patients) or DU (7 patients), with an average wound area of 79.6 ± 12.3 cm²) received traditional topical therapy such as povidone-iodine gel, Olasol spray, Actovegin gel and modern interactive wound dressing materials.

Planimetry of the wounds of all of the patients was performed every seventh day from the time the patient was enrolled in the study. After discharge from the hospital, all of the patients were followed up in an outpatient setting with a frequency of visits of once a week.

RESULTS
The method that we used enabled us to obtain plasma with a platelet content of approximately 338% higher than that of whole blood. Previously, we studied the effect of PRP on the proliferative activity of human cells in vitro using M-22 human fibroblast cell culture (the study was carried out jointly with the Laboratory of Cell transplantation and Immunotyping of the Sklifosovsky Scientific Research Institute of Emergency Medicine), and we have confirmed the effectiveness of this method in terms of the PRP stimulation of regeneration processes[27-29].

At the Purulent Surgical Department of City Clinical Hospital no. 13, we conducted a randomised controlled study to evaluate the efficacy of PRP use for the treatment of chronic non-healing wounds of different aetiology. Considering the presence of wound defects mainly in the soft tissues, we used PRP in the form of a flat, gel-like clot. Results were evaluated over a 3-month period.

In the study group, the average number of applications of PRP in one patient was 6.0 ± 0.6. In three patients, the large size of the wound defect after 3-4 applications made the transplant free split the skin flap over the wound; complete epithelialisation of chronic wounds was achieved in 35 patients (85.4%) in 46.4 ± 4.3 days. The method was not effective in five patients (1 VLU, 1 UME, and 3 STUs); full epithelialisation was achieved in one patient within a period of more than 90 days. In the comparison group, skin grafting was achieved in three patients, and epithelialisation of the wound up to 3 months was attained in only four patients (11.8%). Treatment failure – i.e., lack of epithelialisation of the wound within a period of 90 days – was observed in 23 patients (62.2%) in the comparison group: 2 VLUs, 9 UMEs, 6 DFSs and 6 STUs.

The average duration of hospitalisation was 11.0 ± 2.5 days in the study group and 23.1 ± 1.5 days in the comparison group. Importantly, the average duration of hospital treatment was 7.5 days in patients with a high content of platelets with grains in the plasma (300-600,000/ml) and 12.7 days in patients with secondary (150-290,000/ml) and low (100-130,000/ml) content of platelets on average – i.e., the efficiency of treatment with autologous PRP depends on the content of the functionally suitable platelets. The average cost of treatment for one treated patient differed by more than two fold and amounted to (at the rates of 2012) 785.25 Euros and 1649.02 Euros in the experimental and control groups, respectively. The study results are presented in Table 2.

CASE REPORT
Patient P., an 18-year-old male, presented to the outpatient department with a postoperative ulcerated scar and trophic ulcer in the interscapular region. In 2006, he had undergone surgical endocorrection of scoliosis using a single-plate endocorrector that was removed in 2011 due to the occurrence of multiple purulent fistulas. The wound defect was corrected by plastic repair that later resulted in scar formation and trophic ulceration. Upon primary examination, the wound defect had dimensions of $63 \times 54$ mm, and the wound bed was filled with sluggish granulations. No microorganisms were detected in the wound before the initiation of treatment with PRP. The PRP applications

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group</th>
<th>Reference group</th>
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<tbody>
<tr>
<td>Average number of topical applications of PRP per patient</td>
<td>6.0 ± 0.6</td>
<td>–</td>
</tr>
<tr>
<td>Complete wound re-epithelialization within 90 days</td>
<td>35 patients (85.4%)</td>
<td>4 patients (11.8%)</td>
</tr>
<tr>
<td>Mean time to complete re-epithelialization, days</td>
<td>46.4 ± 2.3</td>
<td>&gt; 60</td>
</tr>
<tr>
<td>Average inpatient hospital stay, days</td>
<td>11.0 ± 2.5</td>
<td>23.1 ± 1.5</td>
</tr>
<tr>
<td>Average cost to treat one patient, EUR</td>
<td>785.25</td>
<td>1649.02</td>
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and wound dressings were carried out with 7-day intervals. During wound dressing, the PRP clot was covered with atraumatic Atrauman Ag silver-impregnated wound dressing and gauze pads. The duration of treatment was 21 days and included three dressings using PRP. Treatment resulted in complete re-epithelialisation of the ulcer by day 28 of the treatment (Fig. 1).

DISCUSSION

PRP therapy is the preferable treatment option in patients with chronic non-healing wounds of different aetiology and localisation, particularly when other more conventional therapies lack evidence of effectiveness or when radical surgical treatment is not possible or contraindicated.

The use of PRP not only reduces the duration and cost of treatment but also decreases the number of dressings, shortens the inpatient hospital stay (because most of the patients can be followed up on an outpatient basis with intervals between dressing changes of 6-8 days) and improves the patients’ quality of life. Notably, all of the patients demonstrated reduced sensitivity to pain after the PRP treatment had started.

The advantages of using an autologous PRP include no risk of disease transmission, introduction of growth factors and cytokines directly into the wound, restoration of metabolic processes, neoangiogenesis, improvement of cellular metabolism and activation of local immunity.[30-32]

References


22. Obolenskiy V. PRP in treating patients with chronic wounds. // Bone Marrow Transplantation. – Vol. 47. – Suppl. 1. – April 2012. – P. 5308 - 5309.


