



## CONTAMINATED PROBLEMATIC SKIN WOUNDS IN DIABETIC PATIENTS TREATED WITH AUTOLOGOUS PLATELET - RICH PLASMA (PRP): A case series study.

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### ABSTRACT

**OBJECTIVE:** To study the effect of platelet-rich plasma (PRP) on contaminated problematic skin ulcers in patients with diabetes.

**MATERIAL AND METHODS:** A total of 6 patients had been treated within the period from 2012 to 2014; they had various types of problematic wounds and diabetes type 2. Patients' distribution by sex was as follows: 1 man and 5 women; mean age- 68 years. Ulcer types: acute (2 patients), hard-to-heal (2 patients) and chronic (2 patients) ulcers. The mean size of the skin and soft tissue defect was 9,5 cm<sup>2</sup>. Pathogenic microflora was isolated in 4 patients - *S. aureus* in three and *E. coli* in one. Based on a scheme developed by us, all cases were treated by administering platelet-rich plasma, derived by PRGF Endoret system. Follow-up period was within 4 – 6 months (4,5 on average). We used platelet rich plasma derived by PRGF Endoret system, applied on the wound bed on a weekly basis.

**RESULTS:** Application of PRP allowed successful closure of all wounds. There were no complications associated with treatment of PRP. Epithelialization of the wound took 15 weeks on average for all patients. One patient presented with hyperkeratosis. Initial score of followed wounds, based on the scales are as follows: Total wound score – 10 p. Total anatomic score – 8 p. Total score – 15 p. at the initial stage. At the end of the treatment period scores were as follows - 0 p., which means excellent results

**CONCLUSION:** We believe that the application of PRP may become optimal therapy in the treatment of contaminated problematic wounds in diabetic patients. PRP not only stimulates wound healing, but also has antimicrobial properties, which may contribute to the prevention of infections.

**Keywords:** problematic skin wounds, platelet rich plasma, functional scoring scales

Skin ulcers are defined as a loss of tissue affecting epidermis and dermis, and in some cases the process can reach up to the adipose tissue and muscle fascia [1]. Problematic skin wounds include wounds that do not heal for a period of at least 6 weeks. Date of injury, of diagnosis or the onset of initial treatment is considered the beginning of wound. Wounds resulting from big energy trauma where bone, tendon and metal prominences are observed also belong to this

group; patients with concomitant diseases which make reconstructive surgery impossible are also included. Problematic skin wounds include acute, hard-to-heal wounds and chronic wounds. Acute skin wounds located in the area of adjoining tendons, joints, the plantar surface of foot and bony prominence are also difficult to treat, especially in the event of a skin defect. Acute wounds are defined as disruptions in the integrity of the skin and sometimes the underlying tissues, where the healing process follows consecutive biological stages. Hard-to-heal wounds are considered those that do not heal after the fourth week of their appearance after being treated with standard methods for the particular pathology. Chronic is a wound that is not healing for a period of three months. Tests of the material obtained from chronic wounds show a significant reduction of the amount of growth factors as compared to acute wounds. A rapid metabolization of growth factors is observed in chronic wounds due to wound proteases, which may be of a bacterial or cellular origin. These problematic wounds cause patients severe emotional and physical stress. Besides the above mentioned reasons, reduction of growth factors in diabetic wounds results from the presence of fibrin sequestering around capillaries [2]. In recent years, there have been many attempts for the development of new therapeutic approaches and technologies in the field of regenerative medicine, tissue engineering and the use of autologous proteins and growth factors in order to support and accelerate tissue healing and regeneration. For this reason, additional biological stimulation of wound defect with platelet-rich plasma (PRP) is applied recently, where PRP carries the most important growth factors for skin and tissue regeneration thus this method could be used as an alternative to complicated surgical methods [3].

A contaminated ulcer is a wound that was possibly contaminated with bacteria but is not yet infected. Micro organisms colonize wounds, as this does not always cause obvious signs of inflammation. But it affects wound recovery and delays the healing process or leading to non-healing wounds. Atrophy or a decline in quality of granulation tissue, change of colour of this type of tissue to dark red or grey and an increase of wound secretion is observed in the event of expressed colonization [4].

Many factors contribute to chronic skin wounds inflammation. Facultative obligate anaerobes grow in wounds as a result of the combination of necrotic tissue and low oxygen levels. Human body seeks to increase the number

of polymorphonuclear leukocytes in and around the wound as a consequence of high microbial count. Cytotoxic enzymes, matrix metalloproteinases and free oxygen radicals are released as well [5, 6]. All this leads to a reduction of the extracellular matrix, inhibition of cell migration and prevention of the normal course of wound healing [7].

High concentration of platelets and growth factors is achieved after PRP preparation as the most significant ones are: platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor (TGF- $\beta$ ), basic fibroblastic growth factor (bFGF), epidermal growth factor (EGF), insulin like growth factor (IGF-1, IGF-2 and IGF-3), hepatocyte growth factor (HGF) [2].

Purpose: To study the effect of PRP on contaminated

problematic skin ulcers in patients with diabetes.

## MATERIALS AND METHODS

**Patient Selection** - A total of 6 patients had been treated within the period from 2012 to 2014; they had various types of problematic wounds and diabetes. These series of patients have diabetes mellitus type 2 and blood sugar approximately 6 mmol/l sustained with insulin. Patients' distribution by sex was as follows: 1 man and 5 women; mean age- 68 years. Ulcer types: acute (2 patients), hard-to-heal (2 patients) and chronic (2 patients) ulcers. The mean size of the skin and soft tissue defect was 9,5 cm<sup>2</sup>. Pathogenic microflora was isolated in 4 patients - *S. aureus* in three and *A. Coli* in one. Patients' data is summarized in Table 1.

**Table 1.**

Sex/ years	m 67 y	f 82 y	f 73 y	f 72 y	f 62 y	f 50 y
decubitus wound		+	+			
diabetes	+	+	+	+	+	+
Skin necrosis without metal osteosynthesis	+				+	+
Skin necrosis with metal osteosynthesis				+		
size cm <sup>2</sup>	8	10	7	6	12	16
depth	II	IV	III	I	V	IV
anatomical region	1	1	2	3	4	4
contamination	*		*		x	*
M. Parkinson			+			

Legend: *Depth of the wound*: I - bone, II - tendon, III - muscle, IV - fascia, V - fat (subcutaneous tissue); *Anatomical region*: 1 - Achilles, 2 - foot, 3 - calcaneus, 4 - lateral. Malleolus; *Contamination*: \* - *S. aureus*, x - *E. coli*.

### Preparation of Activated Platelet-Rich Plasma

(PRP)- The treatment algorithm we used is as follows: first there is surgical treatment and debridement of the wound and followed by culture and antibiogram. The first PRP application is on the second postoperative day. It is derived as follows: the necessary amount of blood is drawn in blood collection tubes containing sodium citrate. Tubes are centrifuged with speed of 1800 rev./min. for 8 minutes. Using pipettes the plasma is drawn off and 10% CaCl<sub>2</sub> activator is added to it at a ratio of 1 ml. plasma to 50 microliters of activator. The resulting mixture is active in 5 min. Fibrin clot is formed after approximately 40 minutes.

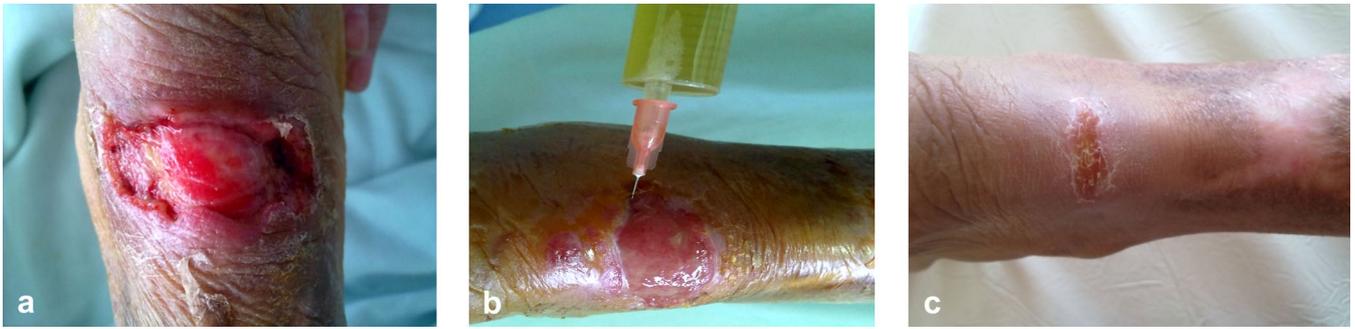
**Wound Care Using PRP**- Activated plasma is used to infiltrate the wound edges and the fibrin clot is used to fill the wound itself. Sterile dressing is applied. The procedure is repeated on a weekly basis until the wound problem is resolved in full, after taking off the sterile dressing the wound is cleaned with physiological saline. Each visit is documented photographically and the size of the wounds is measured by a ruler in centimetres. No antibiotic treatment is administered to those patients. Results have been evaluated based on the following functional scales, proposed by Cancela et al. [8].

## RESULTS

Application of PRP allowed successful closure of all wounds. There were no complications associated with treatment of PRP. Epithelialization of the wound took 15 weeks on average for all patients. One patient presented with hyperkeratosis. Initial score of followed wounds, based on the scales are as follows: Total wound score – 10 p. Total anatomic score – 8 p. Total score – 15 p. at the initial stage. At the end of the treatment period scores were as follows - 0 p., which means excellent results, i.e full recovery of wounds for all our patients, regardless of the type of wound.

### Case reports

Woman, aged 82, with diabetes mellitus type 2, with decubitus wound on the Achilles tendon, affecting the fascia, wound size about 10 square cm, resulting from a dorsal plaster splint for the conservative treatment of a fracture of the patella. It was impossible to close the wound edges. After surgical treatment of the wound (debridement) followed application of platelet rich plasma. The first two photos-initial stages, the third photo 15-week recovery of skin and hyperkeratosis. a) initial stage, b) 7th week, c) 15 th week (fig. 1).



Woman, aged 62, with diabetes mellitus type 2, with a lateral malleolus fracture and metal osteosynthesis. After removal of the synthesis, skin necrosis on the wound edges presented on the 20 postoperative days. *E. coli* was isolated from the wound. Wound debridement was implemented and a skin defect with area of 12 sq. cm occurred. Treatment with PRP was started on the second postoperative day. Final score on the 12th week - full epithelization of wound. a) initial stage, b) necrectomy and fibrin clot, c) 12th week (fig. 2).



Woman, aged 73, with diabetes mellitus type 2, chronic decubitus wound on the foot, with Parkinson's disease; *S. aureus* was isolated from the wound. Because of the concomitant diseases she could not use aids, and continued stepping on her foot, thus hindering treatment. Wound debridement was implemented and a skin defect with area 7 sq cm occurred. Treatment with PRP was started on the second postoperative day. Final score on 16th week - full epithelization of wound. a) initial stage, b) 8th week, c) 16th week (fig. 3).



## DISCUSSION

The third major cause of death in the United States is diabetes mellitus and its complications, and about 60% of all non-traumatic amputations are performed to patients with diabetes. Wound prevalence in these patients is from 4% to 10% [9, 10]. Complications of diabetic foot are the most common cause for hospitalization of patients with diabetes, often resulting in amputation. This is an expensive procedure with cost amounting to about 40,000 dollars for a wound on a lower limb. In addition, it has social and emotional components resulting in disabled young patients in productive age. It has been proved that the longer there is a wound the greater the amputation probability [11, 12].

In addition to growth factors there are proteins with an antibacterial and fungicidal action stored in platelets. These proteins could prevent development and protect the body from infection. Various studies have emphasized the importance of platelets as a source of antimicrobial peptides such as platelet factor-4, RANTES, connective tissue activating peptide 3, platelet basic protein, thymosin  $\beta$ -4, fibrinopeptide A and B, human beta defensin-3 [13, 14].

The activity of those peptides against bacteria is more pronounced as compared to their activity against fungi. In a study, Bielecki et al. report that the growth of *S. aureus* and *Escherichia coli* is suppressed by PPR gel, and no such

effect is observed with *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* [15]. There is no correlation between the leukocyte and platelet concentrations and the antimicrobial activity. In another study, Moojen et al. use platelet concentration similar to that of the previous study, but with twice the leukocyte concentration [16]. Plasma antibacterial activity depends on the platelet and leukocyte concentration.

In a study of neuropathic ulcerations on diabetic feet Margolis et al. report a greater efficiency of the use of PRP as compared to conventional therapies, with a more evident effect in more severe wounds [17]. In another study Driver et al. report the results of using PRP in treatment of ulcerations on diabetic feet. A total of 40 ulcerations are reported and a significant improvement is observed in patients treated with PRP, in comparison to control group treated with saline solution. The number of completely cured ulcerations has increased (81.3 and 42.1%, respectively), regardless of the treatment time (average difference of 28 days) [8]. In a study Anitua et al. report that antimicrobial effect of PRGF against four staphylococcus strains is the strongest during the first hours after application. They underline that using plasma should be focused mostly on prevention than on infection treatment [18].

The review of our material of 6 patients found that the application of PRP faster influenced acute cases, with fewer applications (9-10) and a median time for complete wound healing: 3 months. There is larger number of applications (13-21) when treating chronic wounds and a longer median time for wound healing: 5 months. This shows that the methodology is more efficient when treating acute cases. In our series of patients we did not see any side effects and complications, which is most likely due to the fact that PRP is autologous substance. By its application biological material is added to wounds thus accelerating phases of wound healing, resulting in extraordinary high rate of complete

healing - 100% in our group of patients. When treating our patients we did not use antibiotics in any of the cases despite presence of pathogenic flora in 4 of them. We believe that this proves the antimicrobial effect of PRP. Our idea is to provide an alternative to the other methods of treatment of such wounds, such as skin flaps, medications- acebrine ointment, silver ions, dressings - Acticoat 7 absorbent, ALGISITE M, ALLEVYN, etc., mechanic stimulation – vacuum aspiration, gene therapy, electrical stimulation, cell-based therapy (epithelial stem cells), hyperbaric oxygenation. We wanted to present our cases of diabetes mellitus patients, we had treated with PRP application. Using platelet-rich plasma is preferred treatment opportunity for patients with problematic skin wounds of various etiology and localization, especially when other conventional methods have not been effective or radical surgical treatment is counter indicative. Platelet-rich plasma not only reduces treatment duration and cost, but also the number of dressings, hospitalization time (most patients could be followed-up and treated with platelet-rich plasma in outpatient practice, dressings are changed at intervals of 6-8 days) and improves patients' quality of life. In particular, all patients demonstrated reduced sensitivity to pain in the wound after starting this treatment.

## CONCLUSIONS

We believe that the application of PRP may become optimal therapy in the treatment of contaminated problematic wounds in diabetic patients. PRP not only stimulates wound healing, but also has antimicrobial properties, which may contribute to the prevention of infections.

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