RESEARCH OF MEDICAL FACTORS AND REASONS FOR HEALING FAILURE OF PROBLEMATIC SKIN WOUNDS TREATED WITH PLATELET-RICH PLASMA

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ABSTRACT

OBJECTIVE: To present our problematic skin wounds cases treated with PRP application of that has not responded to the treatment method.

MATERIAL AND METHODS: Out of a total of 154 hospitalized patients with complex skin wounds 83 have been treated with platelet-rich plasma, comprising the Experimental group (EG), and 71 patients with comparable wounds have been treated by utilizing traditional techniques for the respective pathology, comprising the Control Group (CG). Ratings introduced by Cancella AM are used for the evaluation of the respective wound. Statistical analyzes have been performed by using the software SPSS (Statistical Package for the Social Sciences). All cases were treated in a procedure, which includes activated platelet-rich plasma. The treatment algorithm we used is as follows: first, there is surgical treatment and debridement of the wound. Activated plasma is used to infiltrate the wound edges and the fibrin clot is used to fill the wound itself. A sterile dressing soaked with physiological saline is applied. The treatment is repeated on a weekly basis until the wound problem is resolved in full.

RESULTS: The results of our research indicate that 77 (92.78%) of patients enjoy a full recovery, while 6 (7.22%) of patients have no positive wound healing outcome (unhealed wound).

CONCLUSION: Theoretical and clinical studies prove that the PRP method is a reliable, rational, and modern option for PSW treatment. It should not be seen as the only one and universal.

Keywords: problematic skin wounds, platelet-rich plasma, healing failure,

Problematic skin wounds (PSW) are a serious challenge for everyday life. It is hardly a coincidence that they are referred to by several authors as the „silent epidemic“. PSW cover a wide range of injuries, including acute and potentially problematic (high-energy injuries and patients with intrinsic and extrinsic factors, affecting wound healing), hard-to-heal and chronic skin wounds (CSW). According to Lindholm et al., approximately 15% of acute skin wounds, treated by standard methods turn chronic, thus leading to

prolonged recovery time and all unwanted mental, emotional and social consequences related to chronic wound treatment [1].

The major reason for the difficult and delayed healing is the lack of growth factors (GF) in the wound bed due to various reasons, which has an adverse impact on normal tissue regeneration. Active platelets from platelet-rich plasma (PRP) deliver the necessary GF to the wound bed and peripheral wound edges. GF enhance the normal wound process and lead to PSW healing. Using PRP is a preferred treatment option for them with varying etiology and localisation, especially if conventional methods have been ineffective or when radical surgical treatment is impossible or counter indicative [2].

A number of concomitant diseases may be prerequisite for PSW. Chronic venous insufficiency (CVI) is the most common cause of chronic wounds on lower extremities, estimated to cover 60-80% of the cases. There are approximately 500000 patients in the USA suffering from such painful and bothering wounds. Although the prevalence peak is after the age of 60, this type of wound might as well occur in younger patients [3].

A major cause of chronic wounds, especially on lower extremities is diabetes mellitus. This disease is a worldwide public health problem and affects approximately 5% of the US population. According to 2010 statistics, over 6% of Europeans aged 20-79 have diabetes, amounting to about 33 million people, almost half of whom are under the age of 60. Diabetes mellitus and its complications are the third major cause of death in the United States, and about 60% of all non-traumatic amputations occur in diabetics. Diabetic foot complications are the most common reason for hospitalization of diabetic patients and their treatment often ends with amputation, and after such a procedure the possibility for developing a similar injury on the other foot within two years is 50%; patient's survival rate for the next three years is less than 50% [4].

Pressure sores (PS), also known as *decubital ulcers*, annually affect over 2,5 million people in the United States. A number of studies show differences between European countries: prevalence in Italy is 8.3 %, in Sweden, it is 22.9%. Risk factors: age over 65, impaired circulation, immobilization, malnutrition and incontinence [5, 6].

In this paper, we would like to present our PSW cases treated with PRP application of that has not responded to the treatment method [7].

MATERIAL AND METHOD

The research was carried out at the Clinic of Orthopedics and Traumatology, UMBAL Kanev Ruse, for a period of 84 months: from February 2009 to September 2016. Out of a total of 154 hospitalized patients with complex skin wounds, 83 have been treated with platelet-rich plasma, comprising the Experimental group (EG), and 71 patients with similar wounds have been treated by using traditional techniques for the respective pathology, comprising the Control Group (CG).

Scores introduced by Cancela AM are used for the assessment of the respective wound [3]. Each of these scores is used for assessing specific wound parameters: Total Wound Score (TWS); total anatomic score (TAS) of the wound and Total Score of Wound data (TSWD).

Statistical analyses have been performed by using the software SPSS (Statistical Package for the Social Sciences). During the research, the following three nonparametric tests have been used: Kolmogorov-Smirnov, Mann-Whitney and Wilcoxon to verify the significance of relations between features [8].

All cases have been treated in a process developed by us involving platelet-rich plasma acquired by the PRGF Endoret system. The treatment algorithm we used is as follows: first, there is surgical treatment and debridement of the wound. The first PRP application is on the second postoperative day. It is derived as follows: the required amount of blood is drawn in blood collection tubes containing sodium citrate. Tubes are centrifuged with a speed of 1800 rev./min. for 8 minutes. Using pipettes the plasma is drawn off and 10% CaCl₂ activator is added to it at a ratio of 1 ml. plasma to 50 microliters of activator. Within 5 min, the resulting mixture is active. After about 40 minutes, the fibrin clot is formed. In order to penetrate the wound edges, activated plasma is used and the fibrin clot is used to fill the wound

itself. A sterile dressing is added, saturated with physiological saline. The treatment is performed on a weekly basis until the wound problem is resolved in full.

RESULTS

The findings of our research show that 92.78% of patients enjoy a complete recovery, while 7.22% of patients have no positive wound healing outcome (unhealed wound).

In our series 6 (7.22%) out of 83 (92.78%) patients have not responded to PRP treatment of their PSW. The duration of weekly PRP application has been 12 weeks. Prior to PRP administration each of these patients has been treated by the standard medical method and due to failure to heal has been referred to PRP application. Those 7.22% of patients who have not responded positively in terms of wound healing are the subject of this scientific research. The research focuses on the reasons and factors preventing recovery.

Wound parameters assessment of unsuccessfully treated patients did not discover reduction of quantity indexes TAS, TSW and TSWD towards 0 or near 0 with a 0.05 deviation, which would mean full wound healing. Its values remained near the initial ones. After reporting such unfavourable results, patients have been referred to alternative methods of PSW treatment.

Table 1 shows a breakdown of unsuccessfully treated patients by applying PRP, who are only 7.22% of the total number of patients included in the research (6/83). Patient No. 4 has been referred to a vascular surgeon and the treatment has led to below knee amputation (fig. 1). The rest of the patients have been referred to a plastic surgeon. Patients No 2 and No 3 (fig. 2 and fig.3) underwent free skin plasty with a positive outcome. At the beginning of treatment, Piperacillin was intravenously administered to patient # 2 for 7 days due to the presence of an infectious agent *P. aeruginosa*. There is no information about the rest of the patients and their follow-up treatment. All patients have diabetes mellitus, and patient No 4 has poor control over their diabetes.

Table 1. Breakdown of unsuccessfully treated patients by applying PRP

No.	sex	age	etiology	kind of wound	agent	concomitant disease	size mm ²	undermining mm	wound location	depth mm
1	m	63	Achilles rupture	1	-	diabetes	1600	10	2	5
2	f	75	traumatic wound	2	<i>P.aeruginosa</i>	chronic venous insufficiency	6000	10	4	10
3	f	77	traumatic wound	2	-	diabetes chronic venous insufficiency	8000	10	4	5
4	m	79	decubitus wound	2	-	diabetes arterial insufficiency	10000	5	1,3	10
5	m	70	decubitus wound	3	-	-	1000	1	3	5

6	f	71	decubitus wound	3	E.colli	-	600	1	2	5
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Legend: Anatomical region: 1 - ankle, 2 - Achilles, 3 - foot, 4 - calf.

Kind of wound: acute skin wound-1; chronic skin wound-2; hard-to-heal skin wound-3.

Fig. 1. Patient No.4 a) onset, b-c) wound development, d) 12 weeks of treatment, the wound has no signs of healing.

Fig. 2. Patient No.3 a) onset, b-c) wound development, d) 12 weeks of treatment, the wound has no signs of healing, significantly enlarged size.

Fig. 3. Patient No.2 a) onset, b-c) wound development, d) 12 weeks of treatment, the wound has no signs of healing, significantly enlarged size.

DISCUSSION

PRP has been used for over 20 years for wound healing enhancement. The healing process of hard-to-heal wounds and CSW is impaired due to various factors. As a result, there is lack of GF in the wound and the stages of wound recovery are impaired. Autologous PRP is composed of cytokines, GF, chemokines and fibrin scaffolds obtained from patient's blood. Molecular and cellular induction of a normal wound healing process similar to that observed with platelet activation is achieved by adding GF from PRP to the wound [3].

A literature review revealed abundant evidence regarding the positive role of PRP in wound healing. There are few studies showing that the use of various platelet-rich products does not support the recovery of CSW [9, 10, 11].

Cited research papers show the insufficient effect from PRP application for tissue regeneration and wound healing. Recently there have been numerous scientific reports showing full wound healing with the use of various PRP products. Yet new well-designed studies are necessary to prove the benefits of using PRP.

There are various factors that can affect wound healing. They are divided into two basic groups: intrinsic and extrinsic factors. Advanced age is an intrinsic factor that can contribute to the development of PSW. The skin of the elderly is easily injured, with no adequate stress response in terms of gene regulation of stress-related proteins [12]. Concomitant diseases are more common in elderly patients: diabetes mellitus, arterial and CVI, systemic diseases, etc. The normal process of tissue regeneration is affected also by some medications (non-steroidal anti-inflammatory

drugs or steroids), impaired mobility (causing pressure sores), various changes in cellular and molecular characteristics of the skin of the elderly. In addition, the clinically impaired healing of their wounds is associated with delayed cell proliferation, changes in extracellular matrix production and composition, and altered response to GF action. These factors, alone or in combination, can contribute to increasing the frequency and duration of the existence of wounds [13].

Most cells function normally in an oxygen-rich environment and it is advisable to preserve such an environment. Various diseases leading to tissue ischemia can contribute to the development of CSW, for example: chronic fibrosis, atherosclerosis, edema, sickle cell disease, arterial insufficiency and related diseases. It is difficult to treat wounds in immunosuppressed patients as well. Skin with reduced sensitivity is difficult to heal and such patients cannot protect themselves from injury. These intrinsic factors are difficult to be included in the therapy assignment process but must be taken into account [14].

Along with intrinsic factors, extrinsic factors also affect wound healing. Numerous medications can affect the healing process by inhibiting platelet aggregation (aspirin), by suppressing the immune system (corticosteroids) and radiation and chemotherapy, which sometimes suppress the immunity and sometimes lead to cellular destruction. Inadequate nutrition leading to protein, vitamins and iron deficiency should not be underestimated. Last but not least is emotional stress, which can also have a negative impact on wound healing, possibly by increasing blood pressure and cortisol levels, suppressing immunity, and reducing the ability of the body to react [15].

Reviewing the results of our research revealed that patients without a positive change in the tissue regeneration process, i.e. with poor results and failure of wound healing with the deterioration of wound parameters are 7.22% (6/83) [7]. Major findings in our research are: tissue regeneration of PSW is not affected by their anatomical depth, whereas anatomical localization and patients' age have a strong impact on the duration of treatment. Infectious agents and concomitant diseases have been shown to have a strong impact by extending the recovery period [7].

There are 4 patients with PSW in our research. The small number of patients does not allow us to draw significant conclusions from our research, but comparative literature analysis proves its effectiveness in patients with CVI. In our series, two patients with PSW and CVI treated with PRP have fully recovered, whereas patients No 2 and No 3, after discontinuation of the PRP treatment, have undergone free skin plasty and enjoyed a positive result.

Patients with diabetes as sole concomitant disease are 21. In 10% of cases with concomitant diseases, diabetes is combined with an additional disease - arterial insufficiency or CVI. No positive outcome is observed in patients with diabetes and arterial insufficiency (3.6% - 1 patient). 50 % of patients with co-occurrence of diabetes with CVI, have been successfully cured (1 patient), 50% have failed to recover (1 patient). Similar results have been observed in patients with CVI as the sole concomitant disease, namely 50% have successfully recovered (1 patient), 50% have failed to recover (1 patient).

The median age of selected patients for the experimental group and the control group has been identical, 58.07 and 58.39, respectively, with a standard deviation of 15.60 and 16.15, again proving that the age-related physiological changes of patients are an important factor for the occurrence of PSW. All 6 patients are over 50 years of age, with a median age (72.7), a fact confirming the conclusion that age is an important factor in the formation and subsequent treatment of these wounds.

PSW in all six patients are located in the lower extremities. The statistical summary of the obtained results allows us to draw the conclusion that the treatment by PRP application of wounds located as specified is actually feasible with duration as follows: Achilles tendon - 12 weeks; shin - 8 to 12 weeks; foot- 12 weeks. This proves that wounds located on shin, foot and Achilles tendon are a significant treatment challenge and lead to a prolonged healing process of problematic skin wounds, due to the anatomical features of these areas [7].

It is observed that the duration of treatment ranges from 16 to 24 weeks for 10.61 % of patients with traumatic etiology. It is observed that the duration of treatment is 16 weeks for 33.33 % of patients with decubitus sores. The summary of statistical data suggests that a greater percentage of patients with decubitus sores have a longer treatment period than patients with traumatic wounds [7].

For our patients, where *S.aureus* and *E.coli* have been isolated we used only PRP to the full recovery of the

PSW. Our data support the above-mentioned conclusions also drawn by other authors. Patients with *Kl. pneumoniae*, *Enterococcus faecalis* and *Ps. aeruginosa*, in addition to PRP, we used the appropriate antibiotics in accordance with the microbiological test [7].

What could be the reason for the poor outcome from PRP use apart from the ones specified above? It is important to differentiate the optimal platelet concentration for their proper functioning when applied to the wound. The effect of the platelet-rich plasma depends on platelet concentration - Jacobson et al. show that the process of angiogenesis starts at concentrations of 1500×10^3 platelets per microliter and proceeds to 3000×10^3 platelets per microliter. Interestingly, inhibition of the vascular formation process is observed at concentrations of 5000×10^3 platelets per microliter, suggesting a paradoxical effect of the increase of platelet count [16]. Our research shows that the use of platelet concentrations about 5 times their normal plasma count, i.e. about 1500000 platelets per microliter will lead to successful results.

The goal of our research is to determine whether the PRP method can be effectively used on problematic skin wounds in which the patients are the comparative base, with wounds in the Control group that have been treated with surgical wound debridement and a soft bandage. The comparison of the other possible alternative methods for the treatment of such wounds would be the target of another scientific research [7].

The PRP is not the panacea of course. The modern treatment of the acute skin wounds and especially contaminated wounds does not include only a wounds debridement, but also other therapeutic methods. We switch to alternative therapies such as different skin flaps, different medications, electrical stimulation, mechanical stimulation-vacuum aspiration, cell-based therapy (epithelial stem cells), gene therapy, or hyperbaric oxygenation, in the event of a poor outcome of PSW treatment with PRP [17, 18, 19].

Based on our own research and literature analysis, we can conclude that the use of activated platelets from PRP for the treatment of PSW is a therapeutic method that greatly supports the tissue-building process. This ensures biological recovery and a normal wound healing process by delivering the necessary growth factors, which play a major role in it.

CONCLUSION

Theoretical and clinical studies prove that the PRP method is a reliable, rational, and modern option for PSW treatment. It should not be seen as the only one and universal. Many of the PRP response mechanisms have not yet been clarified and require further research.

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