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FACULTY OF MEDICINE
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APPLICATION
OF PLATELET-RICH PLASMA
FOR TREATMENT OF PROBLEMATIC SKIN WOUNDS

ABSTRACT OF DISSERTATION

For awarding doctoral educational and scientific degree

Professional field 7.1 Medicine

Higher education field 7. Health and sports,

Doctoral program Orthopedics and Traumatology

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PLEVEN, 2022

The dissertation paper covers 222 pages. It contains 66 tables and 64 pictures and graphs.

The bibliography includes 271 titles, 10 of which are in Cyrillic and 261 in Latin.

The author is an individual doctoral candidate to the Department of Orthopedics and Traumatology, Faculty of Medicine, Medical University - Pleven.

The dissertation paper has been discussed and scheduled for public defense in the extended Department Council of the Department of Orthopedics and Traumatology, Faculty of Medicine, Medical University - Pleven, held on June 15, 2022.

The clinical material related to the dissertation has been collected in the Clinic of Orthopedics and Traumatology of University Multiprofile Hospital for Active Treatment (UMHAT) Kanev in Ruse. The tests have been performed at the Orthopedics and Traumatology Clinic of the Kanev University Hospital in Rousse.

Note: the numbering of the pictures does not correspond to those in the dissertation paper.

The materials for the public defense are available to those who are interested at the Scientific Department with the Faculty of Medicine, Medical University Pleven and on the website of the University - www.mu-pleven.bg

Composition of the Scientific Jury:

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The public defense will take place on Oct 17, 2022, from 1.00 p.m. in Ambroise Pare Hall at TELEC, MU – Pleven.

ABBREVIATIONS USED

CVI	Chronic venous insufficiency
PSW	Problematic skin wounds
ASW	Acute skin wounds
CSW	Chronic skin wounds
PRP	Platelet-Rich Plasma
EG	Experimental group
CG	Control group
BRIF	Blood repositioning, internal fixation
MMP	Matrix metalloproteinases
TAS	Total anatomical score of wound
TWS	Total Wound Score
TSWD	Total Score of Wound Data

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INTRODUCTION

Since ancient times, people have tried to treat skin wounds with various local remedies. Nowadays there are a variety of non-operative (conservative) methods and operative procedures for their treatment. The results of their use are different.

Problematic skin wounds (PSW) do not heal for at least 6 weeks, beginning with the dates of injury, diagnosis, or initiation of initial wound healing. This group also includes those resulting from high-energy injuries, where prominence of bones, tendons or metal have been observed, wounds at patients with concomitant diseases as well and by wounds where reconstructive surgery is not possible. Their treatment is a major therapeutic challenge. Skin ulcers are a clinical problem with a frequency of 0.78%. The annual costs of treating such wounds are enormous. In the UK, £ 40 million have been spent each year, and in the European Union, 2% of the health budget is used to treat unresolved skin injuries. By comparison, the United States spends \$ 1.3 billion on decubitus ulcer treatment. PSW are a serious therapeutic challenge in our country as well. Annually, about 100,000 patients in Bulgaria have this problem.

A number of concomitant diseases can be a prerequisite for receiving such wounds. Chronic venous insufficiency (CVI) is the most common cause of chronic lower limb injuries and it has been thought that it covers 60-80% of cases. There are about 500,000 patients in the United States with such painful and life-threatening wounds. In 2007, the Bulgarian National Society of Angiology and Vascular Surgery conducted a study to determine the frequency of the disease among Bulgarian patients. The authors report that 37% have such a problem at one stage or another - from discomfort to disability and place it in the group of socially significant diseases.

One of the main reasons responsible for the development of chronic wounds, especially in the lower extremities, is the high incidence of diabetes. This disease is a global public health problem and affects approximately 5% of the US population. According to statistics for 2010, more than 6% of Europeans aged 20 to 79 have this disease. In Bulgaria the number of "sweet people" is 6.5%. If this situation remains unchanged, the number of diabetics in Europe is expected to reach 37 million in the next 15-20 years.

Prolonged compression wounds, also known as decubitus ulcers, are localized injuries to the skin and / or underlying tissue that usually occur over bone prominences as a result of pressure or pressure in combination with cutting and / or

friction. Each year, more than 2.5 million people in the United States develop decubitus ulcers. According to a number of studies, there are differences between the countries in Europe and the frequency studied in Italy is 8.3% and 22.9% - in Sweden. No statistical data on the frequency of these wounds in Bulgaria have been found yet.

Chronic and hard-to-heal skin wounds are common and difficult to treat due to the loss of growth factors necessary for the healing process and they are often complicated by superinfection. Conventional therapy includes various types of bandaging, debridement and even skin grafts, but these methods do not allow satisfactory healing because they cannot provide enough growth factors to modulate the process of tissue regeneration. Such patients often have to endure long-term bandaging and subsequent debridement procedures without much success.

In the late 1990s, the term "regenerative medicine" has been introduced. A number of studies related to stem cells, growth factors and the extracellular matrix allow the development of this new therapeutic philosophy, different from the concept of classical tissue engineering, with the aim of restoring fully functioning tissue in the damaged area. This supports and optimizes the process of natural wound healing.

The high concentration of growth factors offers an "ideal environment" for tissue regeneration and this is part of the so-called biological treatment.

The method of application of platelet-rich plasma (PRP) allows modern, biological treatment of PSW. It has not been studied so far in Bulgaria. Therefore, we set ourselves the goal to establish its reliability and significance, to assess its effectiveness and applicability in our country.

I. GOAL AND TASKS

GOAL

The goal of the study is to investigate the reliability and significance of the platelet-rich plasma method, to evaluate its effectiveness and applicability in the treatment of problematic skin wounds.

TASKS

To achieve this goal it is necessary to solve the following tasks:

1. To study, apply and improve the platelet-rich plasma method for the treatment of problematic skin wounds.
2. To systematize the modern indications and contraindications for the application of the method.
3. To evaluate and analyze the results of the application of platelet-rich plasma for in the treatment of acute, difficult to heal and chronic skin wounds.
4. To prove the effectiveness of the improved methodology through statistical analysis of the reported clinical and functional results.
5. To propose a therapeutic algorithm for the treatment of problematic skin wounds by the method of platelet-rich plasma.
6. To create an algorithm for prophylactic application of the platelet-rich plasma method to acute, potentially problematic skin wounds.

II. MATERIAL AND METHODS

2.1. MATERIAL

The study is prospective and was conducted at the Clinic of Orthopedics and Traumatology of University Multiprofile Hospital for Active Treatment (UMHAT) Kanev in Rousse for a period of 90 months: from February 2009 to September 2016. Out of 154 hospitalized patients with problematic skin wounds, 83 patients were examined and treated with the method of platelet-rich plasma, which form the Experimental Group (EG). In the Department of Orthopedics and Traumatology, Surgical Department and Departments of Vascular and Plastic Surgery, 71 patients with similar wounds have been treated by the conventional method (wound debridement and sterile gauze bandage soaked in 0.9% sodium chloride saline) and they form the Control Group (CG).

Main criteria for participation in the present study

The etiology of the skin wounds and concomitant diseases of the hospitalized patients, which represent EG, determine the main criteria for inclusion in the present study. The indications and contraindications for the use of PRP in the PSW are:

INDICATIONS

Problematic skin wounds do not heal for at least 6 weeks from the beginning of the injury, their diagnosis or the start of initial treatment. This group also includes wounds resulting from high-energy trauma, in which bone, tendon or metal prominence has been observed, wounds in patients with concomitant diseases and those in which reconstructive surgery is not possible.

The main criterion for inclusion in the study is the identification of a wound as problematic. We defined the following wounds for problematic:

- Acute, traumatic and postoperative wounds - disorders in the integrity of the skin, and sometimes the underlying tissues, in which the recovery process follows strictly defined successive biological stages. They are particularly difficult to treat when located in the area of bone prominences, underlying tendons, plantar surface of the foot, around joints and skin defects, and can become potentially problematic skin wounds.
- Hard-to-heal skin wounds, traumatic and postoperative wounds - do not heal after the fourth week of their appearance, treated with conventional methods for the respective pathology.

- Chronic, traumatic and postoperative wounds - the recovery process does not go through certain successive stages of tissue regeneration to achieve anatomical and functional integrity within 3 months period.
- Wounds in vascular diseases (venous and arterial insufficiency).
- Wounds in metabolic diseases - diabetes mellitus.
- Decubitus ulcers.
- Potentially problematic skin wounds - caused by trauma or surgery, metabolic and vascular diseases and prolonged pressure (compression). Apart from the etiological factor, their formation is also influenced by the anatomical location, the presence of an infectious agent, the age of the patient and the accompanying diseases.

CONTRAINDICATIONS

- Critical thrombocytopenia;
- Hypofibrinogenemia;
- Platelet dysfunction;
- Hemodynamic instability;
- Sepsis;
- Inflammation at the site of application;
- Acute and chronic infections;
- Chronic liver disease;
- Anticoagulant therapy;
- Neoplasms;
- Non-cooperation or patient refusal.

Main criteria for the assessment of problematic skin wounds (PSW)

Several point scores introduced by Cancela et al. have been used to assess the respective wound. Each of these scores is characterized by an assessment of specific parameters of the wound. All patients included in the study have problematic skin wounds of different ages in each of them and this creates difficulties in their normal daily life and work, accompanied by psychological trauma and pain. A detailed interview with them has been conducted, including clarifying and detailed questions about the therapy applied. Patients who have agreed are included in EG, and those who didn't agree are included in the CG. Each specific wound has been assessed at the beginning of the PRP method

application and then on the 4th, 8th, 12th week and every fourth week until full recovery of wound, or until treatment dropout at week 12 because of bad results.

The distribution of patients by age (Fig. 1) is as follows:

- under 20 years - 2 patients;
- from 20 to 29 years - 4 patients;
- from 30 to 39 years - 5 patients;
- from 40 to 49 years - 9 patients;
- from 50 to 59 years - 18 patients;
- from 60 to 69 years - 24 patients;
- from 70 to 79 years - 17 patients;
- over 80 years - 4 patients.

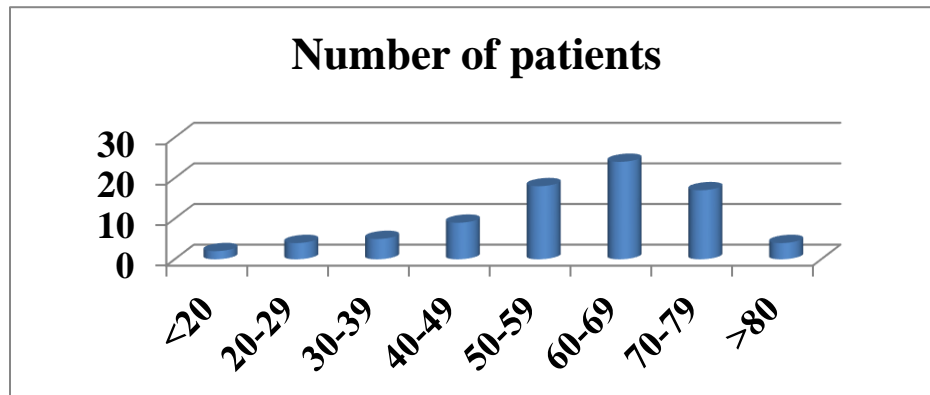


Fig.1 Distribution of patients included in the study according to age

2.2. METHODS

The diagnostic algorithm for patients in the present study includes a detailed medical history, general somatic status and detailed local wound status, and laboratory tests.

2.2.1. Clinical method

For all patients a thorough medical history (anamnesis) and necessary information about the current ailment has been collected. Particular attention is paid to previous diseases and information on hypertension, diabetes, chronic venous insufficiency, arterial insufficiency, conditions leading to difficult or complete immobility with the possibility of developing decubitus ulcers, harmful habits like smoking etc. An in-depth study of somatic status has been conducted. The clinical examination accurately examines the condition of the skin wound (documenting with the help

of photographs, determining its area, depth, location, secretion and microbiological test results). Detailed laboratory tests (full blood count, biochemistry) are performed in order to establish some pathological changes in these indicators. Age and sex have been taken into account.

Based on the literature analysis of existing methods, we have improved an innovative therapeutic algorithm and methodology for the treatment of PSW using the PRP method, which is used in this research and described in the following stages.

Stage 1. Wound treatment

Patient's wound has been treated in hospital conditions (operating room). Swab tests for microbiology is taken initially, and then it is proceeded to wound cleaning (Fig. 2). In the event of acute skin wounds (ASW), devitalized tissues are to be treated and removed. In chronic skin wounds (CSW), there is reduced blood supply and thickening of the area very close to the wound edges and bottom. Adjacent important anatomical structures, which are at risk of damage, are taken into consideration.



Fig.2: PSW with skin necrosis



Fig.3: PSW after surgical debridement

The choice of wound cleaning method is surgical or acute debridement. This means cutting the necrotic tissue right on the border with the healthy tissue, using surgical instruments (scalpel, scissors or sharp curette). This is a selective method because healthy tissue is not to be damaged or - if necessary for prophylactic reasons – it is to be minimally cut up to the limit of bleeding in the same operative time (Fig. 3). In some cases, the wound apparently looks smaller, but in depth there may be extensive undermining of the wound edges with devascularization. This fact should be carefully considered in order to perform adequate debridement to the limit of tissue bleeding. Otherwise, there is a serious risk of wound necrosis followed by infection.

Stage 2. Determining the parameters of the wound

After wound cleaning the exact determination of its area and scope follows. The wound area is to be calculated using sterile self-adhesive foil (Fig. 4). The edges of the wound are outlined with a marker and the area in square millimeters is determined.



Fig.4 PSW with foil and outlined wound



Fig.5 PSW with measuring ruler edges

Another way to determine the area of the wound is with a measuring centimeter ruler (Fig. 5). During performing wound debridement, an attempt is made, if possible, to bring the shape of the wound closer to the shape of known geometric shapes, to make it easier to calculate the area, for example a shape of rectangle, and then calculate its surface. When the wound is like ellipse, its area is calculated by the following formula:

$$\text{Area (sq.mm)} = \text{length (mm)} \times \text{width (mm)} \times 0.25 \times \pi,$$

When it looks like a circle:

$$\text{Area (sq.mm)} = \pi \times r^2.$$

The depth of the wound could be measured by the reached anatomical structures in depth – subcutaneous tissue, fascia, tendon, muscle, bone. Another way to measure the depth of the wound is with a centimeter ruler in mm. The three-dimensional shape (volume) of the wound could be measured using a certain amount of saline injected under the self-adhesive film (foil) and read in cubic milliliters. After determining these parameters, the wound is covered with a sterile gauze bandage soaked in physiological serum.

Stage 3. Preparation of PRP

Step 3.1. Collection of autologous venous blood from the patient

On the second postoperative day, the first application of activated PRP is to be administered. It begins with the collection of autologous venous blood from the patient (Fig. 6). Cubital vein is usually used, but in some cases (obesity, concomitant diseases, deteriorating skin and vascular system), all possible venous sources are used. The skin is cleansed with a swab soaked in 70 per cent alcohol.



Fig.6: Collecting venous blood in vacuette



Fig.7: Vacuettes with sodium citrate

Step 3.2. Preparation of vacuettes for centrifugation

Standard laboratory vacuettes with 3.2% sodium citrate with a volume of 3 ml are used (Fig.7). The amount of venous blood collected is determined by the area and depth of the wound. After filling the vacuette with blood, it is carefully inverted several times to obtain a homogeneous mixture of venous blood and sodium citrate. They are then placed on a stand until the required amount of vacuettes is obtained. The required amount of venous blood for the respective wound is determined as follows: per 1 cubic cm of wound - 1 vacuette (about 3 ml) blood. After processing the blood, the amount of plasma is about 1.5 ml.

Step 3.3. Centrifugation

The next step in the treatment of venous blood is centrifugation of the vacuettes. A centrifuge with time and speed sensors is used (Fig. 8).

The correct, symmetrical arrangement of the vacuettes is very important for the normal course of the process. Otherwise, strong vibrations occur, which can

negatively affect the location and distribution of the layers in the vacuette and can lead to hemolysis and unfit contents. Centrifuge speed at 1800 rpm for 8 minutes. Selection of these technical characteristics is in accordance with the recommended technology of Endoret® (prgf®) and the used medical equipment. After completion of the procedure, several layers are observed in the vacuettes (Fig.9).



Fig.8: Centrifuge

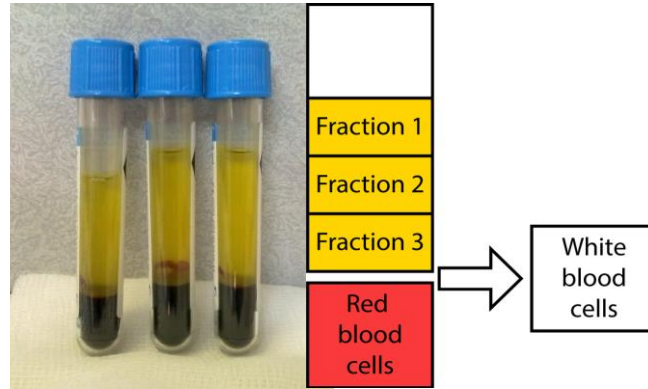


Fig.9: Vacuettes after centrifugation

The bottom is the level of erythrocytes. This is followed by a layer of white blood cells, about 1 mm thick. At the top is the plasma layer. Plasma layer is divided into three levels: bottom-up - rich in platelets (fraction 3), medium, with a normal number of platelets (fraction 2), as in normal venous blood and superficial, poor in platelets, but rich in fibrinogen (fraction 1).

Step 3.4. Separation of PRP

The centrifuged vacuettes are placed on the holder stand again. Their caps are opened and with the help of laboratory micropipettes (Fig. 10) plasma is separated (take all three layers) from that with red blood cells. We use all three levels because top-level fibrinogen is converted by plasma thrombin to fibrin, which acts similarly to the tissue glue (enriched with activated platelets) and fibrin scaffolding, which is needed to fill a wound defect. It is very important in our method to leave the layer of white blood cells in vacuettes, because they are not needed. This is because activated platelets secrete antimicrobial peptides and anti-inflammatory mediators. The presence of leukocytes leads to a number of side effects - neutrophils can damage muscle, connective and bone tissue because they release degrading MMPs-3, 8, 9 and 13 and free radicals.



Fig.10: Lab micropipettes



Fig.11: Activator ampoules of 10 pct calcium dichloride



Fig.12: Activator ampoules 10% 10ml calcium gluconate

Step 3.5. Activating PRP

The amount of obtained plasma is read in milliliters. It can be placed in a syringe or other container, as required for the specific wound. The finished plasma is then activated. For activators are used:

- ampoules of 10% calcium dichloride, the ratio is 1 milliliter of plasma per 50 micrograms of 10% calcium dichloride (Fig. 11).
- ampoules of 10% 10 ml of calcium gluconate, the ratio is 0.1 milliliters of calcium gluconate per 1 milliliter of plasma (Fig. 12).

In both modes of activation, an equivalent activated PRP is obtained.

After mixing the plasma with the activator, the reconstituted mixture is allowed to stand for 5 minutes required for platelet activation. During this time, platelets change their shape, starting contraction and releasing growth factors, antimicrobial peptides, and other substances. The activated platelet-rich plasma is then ready for infiltration and instillation into the wound edges and bed (Fig. 13).



Fig.13. Syringes with activated PRP



Fig.14. Fibrin clot

If it is necessary to obtain an activated platelet-rich fibrin clot, we wait for about 40 minutes to obtain this 3D gel shape (Fig. 14). It is distinctive, that fibrin clot takes the form of the vessel in which the activated plasma is placed.

Step 4. Application of activated platelet-rich plasma in the wound bed

Step 4.1 Application in the wound

Depending on the anatomical location of the wound, patient should take such a position that the wound is in a horizontal position in order to prevent spillage of activated PRP.

In wounds with complicated bottoms, with many pockets and branches, it is preferable to use platelet-rich plasma in the liquid phase to completely cover and fill the wound surface. We have to wait about 40 minutes according our experience, and during this time, patient does not change their position until the formation of fibrin clots (Fig. 15). In this way, it sticks to the wound surface (due to the available fibrin) and it is not possible for the plasma to leave the treated wound. With the activated plasma, the wound edges are always infiltrated with a 26 G needle throughout the circumference (Fig. 16).



Fig.15. Fibrin clot in the wound



Fig.16. Infiltration of the wound edges

Step 4.2. Wound bandaging

A sterile gauze bandage moistened with 0.9% sodium chloride is applied. The procedure is repeated every week because the activated platelets are secreting and releasing growth factors every 7 days. After removing the bandage, the surrounding skin is cleaned with Iodopovidone and the wound with saline. The activated, platelet-rich plasma is applied again and then covered with a sterile gauze bandage soaked in 0.9% sodium chloride. According to our observations, in wounds with abundant secretion, this process of preparation of the plasma and its

application has to be repeated every three days. After the initial treatment of the wound and the use of activated plasma in a hospital conditions, subsequent manipulations may be performed in outpatient practice.

2.2.2. Methods for assessing the specific parameters of the wound

Several point scores introduced by Cancela et al are used to assess the relevant wound. Each of these scores is characterized by an assessment of specific wound parameters.

The score with general wound parameters -Total Wound Score (TWS) is used to assess the following wound parameters: edema and erythema around the wound, purulent secretion, fibrin, granulation, wound bottom edema, ochre edema. Each of these parameters has a certain number of points, depending on the degree of manifestation. At the end, the total number of points for each specific wound in each patient has been counted.

Similar is the following scale for assessing the total anatomical score (TAS) of wound. Indicators related to the presence of open bone (tibia), open tendons, pulsations of the a.dorsalis pedis, pulsations of a.tibialis posterior has been taken into consideration here. Each of these parameters has a certain number of points, which are placed depending on the degree of wound damage. At the end, the total number of points for each specific wound in each patient has been counted.

The Total Score of Wound data (TSWD) is used to estimate the dimension (area) of the wound in sq.mm, the depth of the wound in mm, the wound undermining in mm, and the period of non-healing of the wound in weeks, months and years. Again, each of these parameters has a certain number of points, which are set depending on the degree of wound damage. At the end, the total number of points for each specific wound in each patient has been reported.

Each wound was evaluated at the beginning of treatment with platelet-rich plasma and then on the 4th, 8th, 12th week and so on every four weeks until complete wound healing or wound treatment refusal in the 12th week at bad result.

2.2.3. Statistical methods

The statistical analyzes were performed and researched through the software product SPSS (Statistical Package for the Social Sciences). In terms of its structure and organization, SPSS is a product that processes and analyzes information from social, medical and marketing research. In SPSS, an array of information has been structured so as to make the most of the built-in technologies for data processing

and statistical analysis. The main features of the product that make it suitable for processing this type of data are:

- The ability to relatively easily obtain aggregate distributions of the values of a certain variable, and together with them are derived most of the statistical indicators characterizing these distributions;
- The possibility to modify and restructure the data set;
- The possibility to summarize the data set in section by each of the variables in it;
- The ability to relatively easily apply complex methods of statistical analysis of relationships and dependencies (correlation, regression, factor, etc. analysis).

For all dissertation related tests, $p < 0.05$ values were predicted to be statistically significant.

III. OWN RESULTS

3.1. Study of treatment results by a PRP method using statistical analysis

The subject of the study is successful treatment of patients by the method of PRP. It consists of 154 patients treated at UMHAT Kanev Rousse, which defines it as a random sample study.

Our study is the first prospective study in Bulgaria for the treatment of patients with problematic skin wounds using the PRP method.

The elements of research are divided into two homogeneous groups - control and experimental. CG includes patients treated by the conventional method (debridement and soft, sterile, gauze bandage soaked in saline), and EG - by the PRP method. The random sampling meets all the requirements for sampling in medical research for statistical processing.

For the purposes of the study, information for the status of the observed characteristics of the studied cases has been collected. A variable has been defined for each feature and their values and meanings corresponding to the features have been imported.

Through in-depth analysis of the data obtained in the process of healing the PSW (problematic skin wounds), for CG and EG, all tasks were solved according to the applied statistical method of research. As a result, the effectiveness of the experimental method of PRP in the treatment of PSW has been proven.

Our data show that the initial two groups - EG, in which patients were treated by PRP method, and CG, in which patients were treated by conventional methods, have significant differences at the beginning and end of the experiment related to the treatment results of problematic skin wounds in relation to the initial condition of these wounds.

It has been statistically proven that the selected groups of patients have an "equal start", i.e. those included in the experiment have the same initial condition of PSW. The conducted statistical and medical research shows that both in EG and in CG achieved real improvements in the process of healing the wounds of patients by applying the methods used. Follow-up patients had a significant difference in the final state of the PKR treatment. In both groups, EG and CG, significant changes in wound condition were observed at the end of treatment.

Statistical significance in the results was established until complete recovery of the wound at the end of the experiment at the therapy of patients treated by the PRP

method and the conventional method for both groups, which proved the higher effectiveness of the experimental methodology compared to a conventional therapy.

3.2. Variation analysis

In order to achieve the goal and solve the tasks of the research, a variation analysis of the variables containing information about the initial and final data of the experiment was performed. The purpose of the variation analysis is to determine the main numerical characteristics of the variables and through comparative analysis to check the trend of their change in EG and CG for each of the groups of the experiment.

The coefficients of variation at the beginning of the experiment have been calculated. This is necessary to comply with the requirement that the experimental and control groups to have approximately equal opportunities at the beginning of the experiment. At values of the coefficient of variation up to 10%, the random sampling is homogeneous, from 10 to 40% - approximately homogeneous, and over 40% - inhomogeneous.

3.2.1. Variation analysis based on "Age groups"

For the patients from the experimental and control groups, a comparative analysis by age groups has been made, in order to determine the distribution of patients.

The obtained in Fig.17 and Fig.18 distributions show that the studies cover the entire age group of patients in both EG and CG who could develop problematic skin wounds.

The percentage distribution of patients in EG from Fig.19 shows that 20.48% of patients are in the age group 70-79 years, 28.92% are in the age group 60-69 years, 21.69% are in the age group 50-59 years. The largest share of patients with PSW fall into age groups over 50 years, which is associated with changes in the biological condition of patients due to physiological changes. As the patient's age increases, the number of concomitant diseases that create conditions for the occurrence of PSW (problematic skin wounds) increases as well.

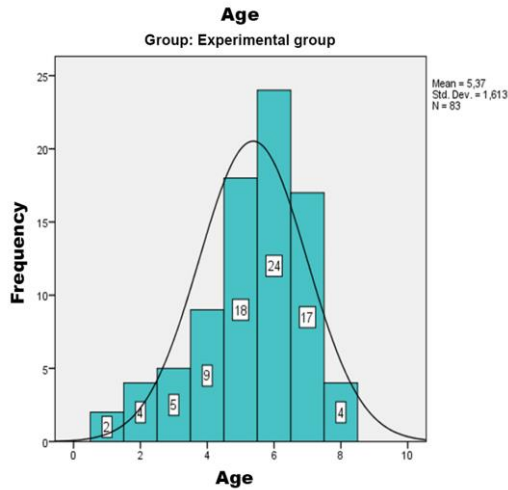


Fig.17. Variation analysis on basis Age groups in EG

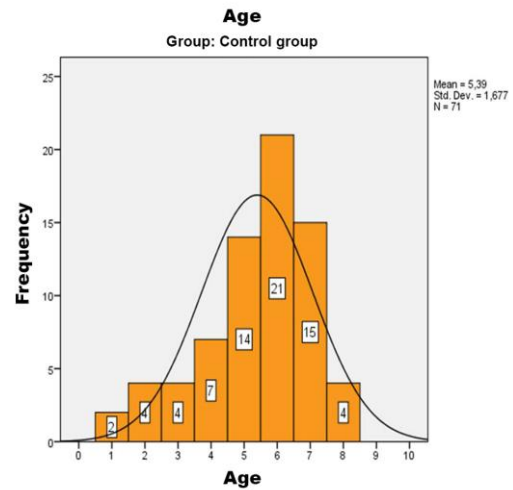


Fig.18. Variation analysis age groups in CG

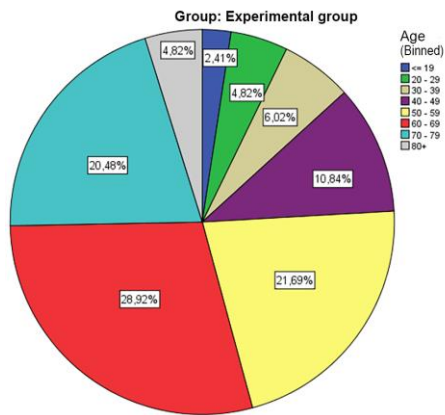


Fig.19. Variation analysis on basis age groups in EG in %

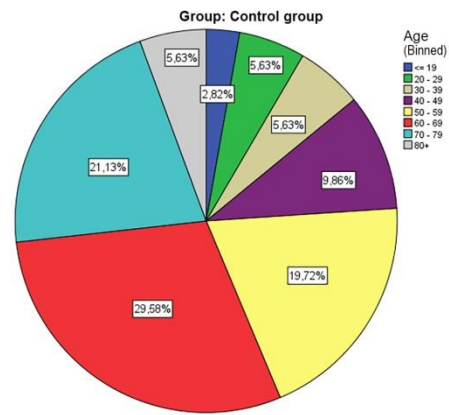


Fig. 20. Variation analysis for age groups in CG in %

The percentage distribution of patients in the control group Fig.20 similarly shows that 21.13% of patients are in the age group 70-79 years, 29.58% in the age group 60-69 years, 19.72% in the age group 50-59 years, which proves the equal start of the studied groups in terms of age distribution.

3.2.2. Variation analysis based on the "Age" feature of the patients

The obtained in Fig. 21 and Fig. 22 data show that the average age of the patients in both the experimental and control groups were 58.07% and 58.39%, respectively, which proves again that the change in the biological status of patients due to age physiological changes is an important factor in the appearance of problematic skin wounds.

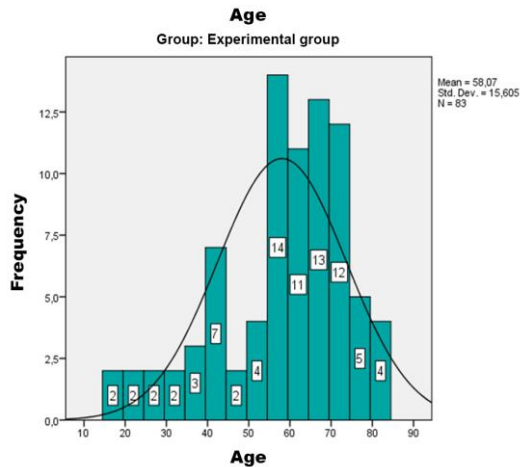


Fig.21.Variation analysis based on age groups in EG

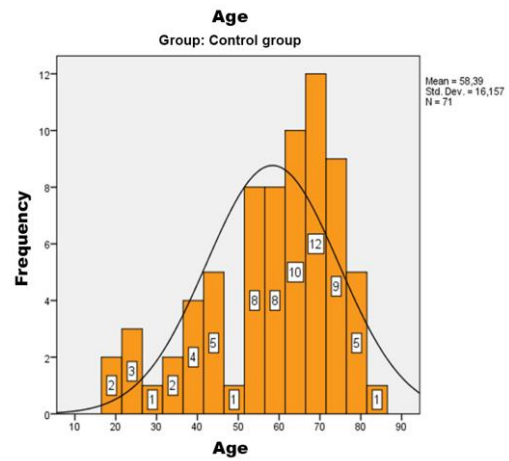


Fig.22.Variation analysis based on age groups in CG

3.2.3. Variation analysis based on "Type of wound"

For the patients with EG and CG a comparative analysis of the type of wounds has been made in order to show their breakdown by duration of existence of the wound before the proposed experimental treatment by PRP method according to current dissertation has been performed.

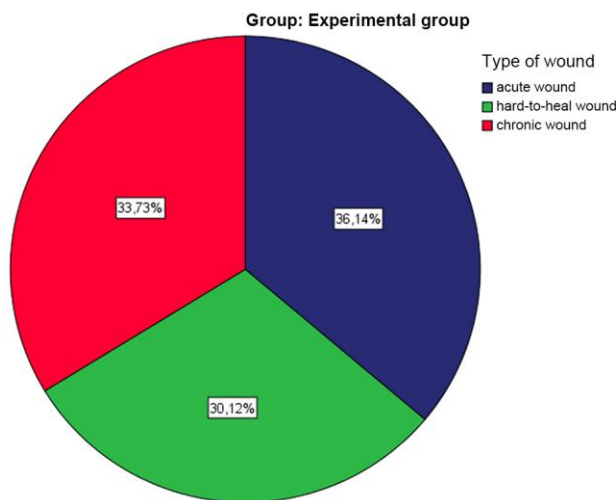


Fig.23 Variation analysis on basis Type of wound for EG in percentage

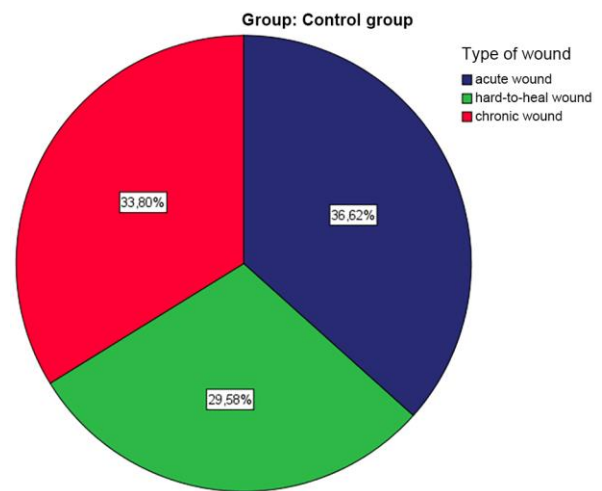


Fig.24 Variation analysis on basis Type of wound for CG in percentage

The obtained in Fig. 22 and Fig. 23 percent distributions by wound type show that the patients studied were evenly distributed in the three categories of groups, respectively:

- The experimental group: with acute wounds - 30 patients (36.1%); with hard-to-heal wounds - 25 patients (30.1%) and with chronic wounds - 28 patients (33.7%);
- Control group: with acute wounds - 26 patients (36.6%); with hard-to-heal wounds - 21 patients (29.6%) and with chronic wounds - 24 patients (33.8%).

3.2.4. Variation analysis based on "Sex"

The patients from EG and CG are classified based on their Sex

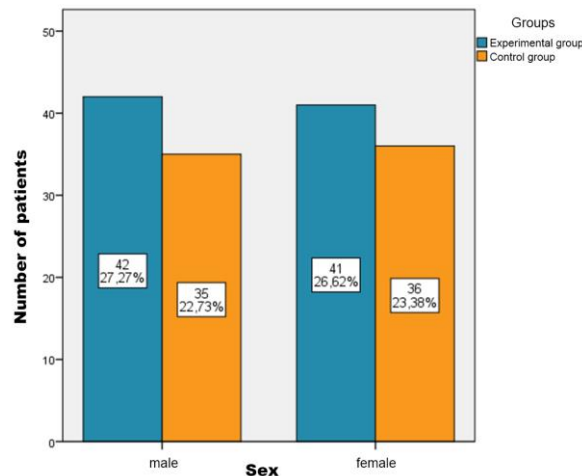


Fig.25 Variation analysis based on Sex for EG and CG compared to the total number of patients

The analysis of the results from Fig.25 shows that women and men with problematic skin wounds (PSW) are distributed in equal proportions in the two groups, respectively:

- The experimental group (EG): men 50.6% and women 49.4%;
- Control group (CG): men 49.3% and women 50.7%.

3.2.5. Variation analysis based on "Etiology"

The analysis of the results from Fig.26 shows that the most common etiology for patients in EG is:

- Decubitus cutis defectus cutis - 13 patients;

- PS Wound cutis, defectus cutis - 5 patients;
- Fractura malleolus lateral extr clavi defectus cutis - 9 patients;
- Fractura cruris, ORIF (open reposition internal fixation), defectus cutis – 6 patients;
- Fracture fracture malleolus lateral, ORIF(open reposition internal fixation), defectus cutis – 5 patients;

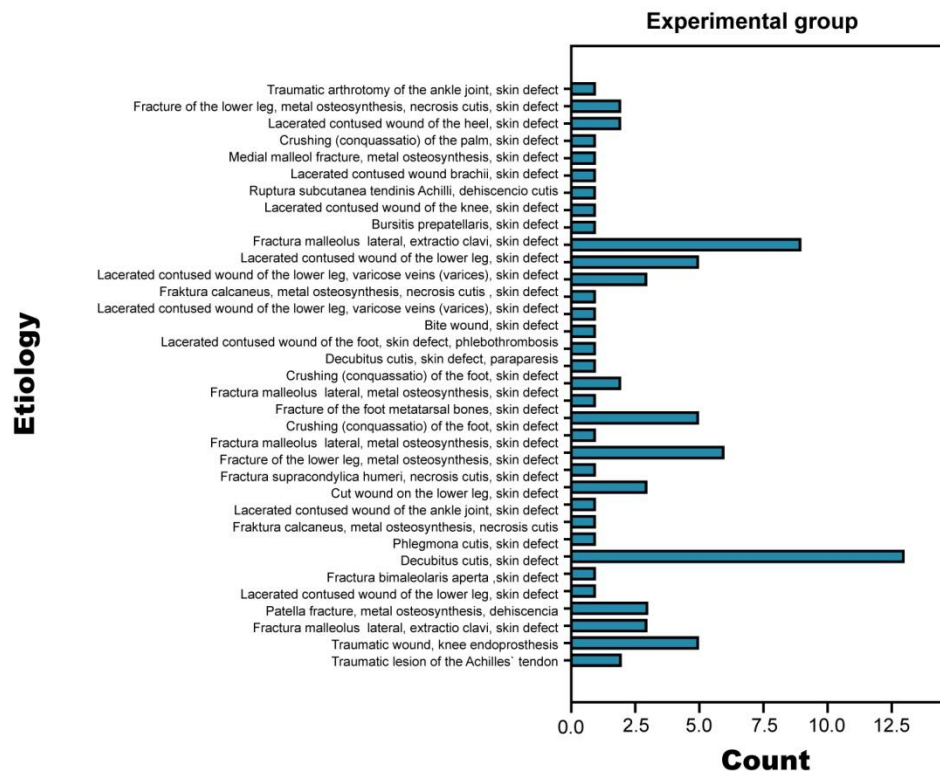


Fig.26 Variation analysis based on Etiology for EG

The analysis of the results from Fig. 27 shows that the most common etiologies for patients in CG are:

- Traumatic wound, knee prosthesis – 5 patients;
- Decubitus cutis defectus cutis - 11 patients;
- Fractura cruris, ORIF (open reposition internal fixation), defectus cutis – 7 patients
- PSW cutis, defectus cutis – 4 patients

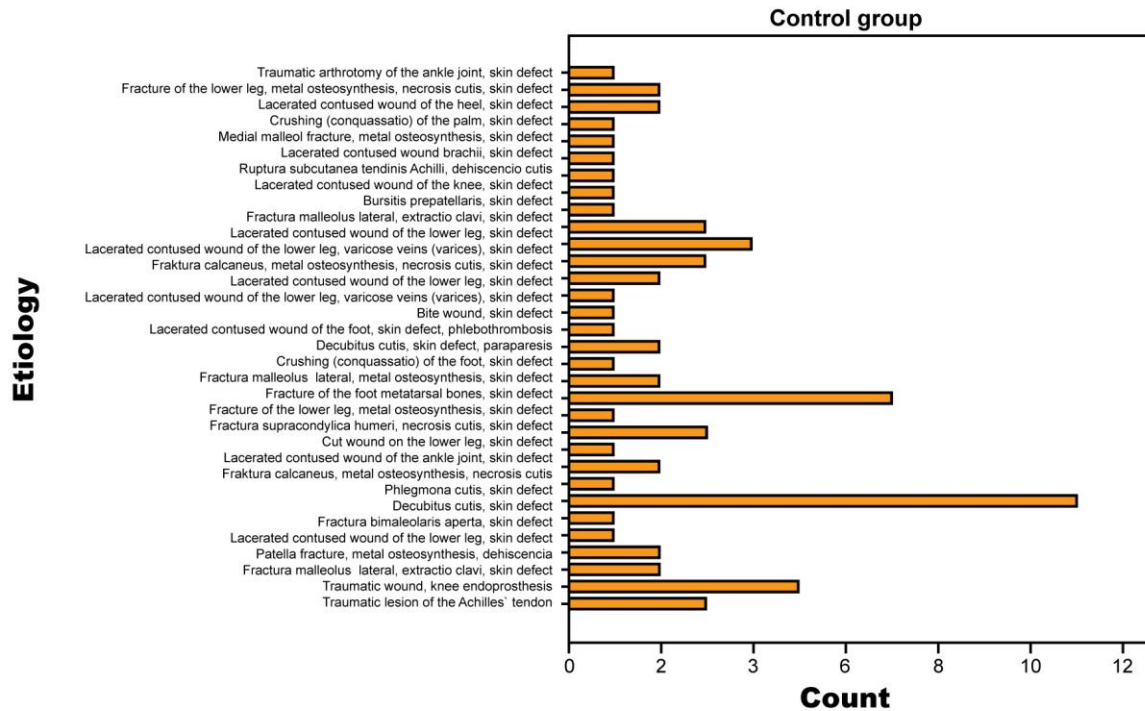


Fig.27 Variation analysis on basis Etiology for CG

3.2.6. Variation analysis based on "Etiological groups"

Examined patients from EG and CG based on "Etiological groups" are presented in Fig. 28.

Analysis of its results shows that traumatic wounds, decubitus wounds and wounds of inflammatory origin are distributed relatively equally in the two studied patient groups. The distribution by groups is as follows:

- EG: traumatic wounds 79.5%; decubitus ulcers 18.1% and wounds of inflammatory origin 2.4%;
- KG: traumatic wounds 80.3%; decubitus wounds 16.9% and wounds of inflammatory origin 2.8%.

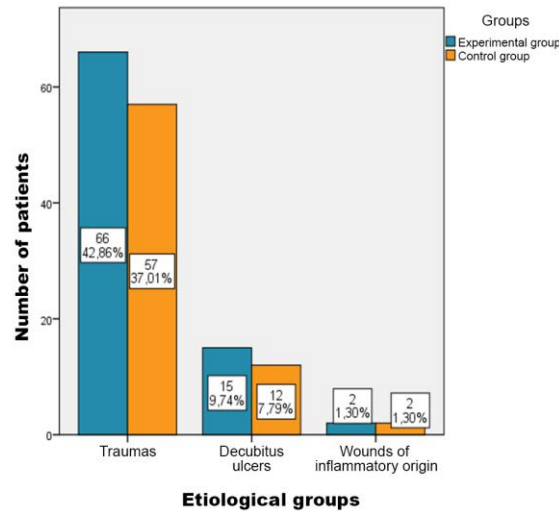


Fig.28 Variation analysis based on Etiological groups compared to the total number of patients

3.2.7. Variation analysis based on "Treatment weeks"

The examined patients from EG and CG based on "Weeks of treatment" are systematized and presented in Fig.29 and Fig.30. The Variation analysis of Pic. 29 based on "Treatment Results" versus "Weeks of Treatment" for EG showed that most patients were completely cured in 8 and 12 weeks, respectively 34.94% and 28.91%, which represents over 63.85% successfully recovered by all patients in EG. Hence, the applied PRP (platelet-rich plasma) method gives the best results for a treatment in the period of 8 to 12 weeks. Only 7.22% of the patients treated by a.m. method have a poor result due to concomitant diseases at an advanced stage.

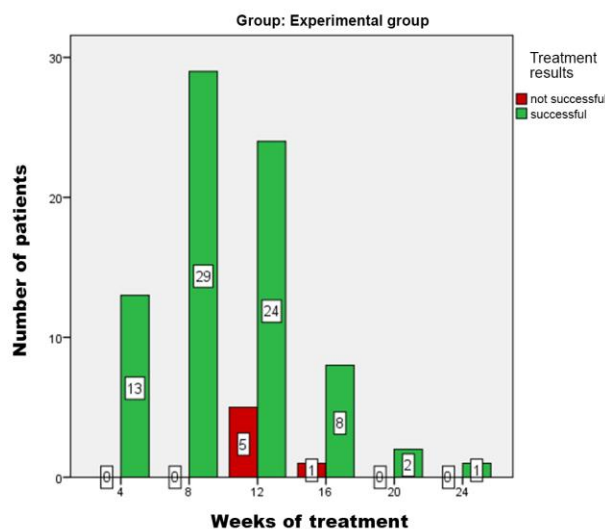


Fig.29 Variation analysis based on Treatment results versus Treatment weeks for EG

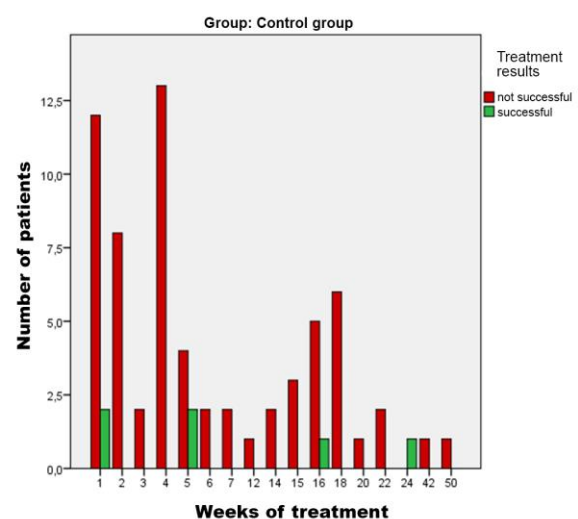


Fig.30 Variation analysis based on Treatment results versus Treatment weeks for CG

The Variation analysis of Pic.30 on the basis of "Treatment Results" versus "Treatment Weeks" for CG showed that only 8.45% of patients were completely cured by conventional treatment, and the remaining 91.55% did not achieve a positive result. Hence, the conventional method does not give good results in the treatment of PSW.

3.2.8. Variation analysis based on "Treatment results"

The examined patients with EG and CG based on "Result of treatment" are presented in Fig.31. The analysis confirms that 92.78% of EG patients have been successfully cured by the PRP method and have achieved a positive result until complete wound recovery. While the conventional method of treatment of PSW, the successful result is only 8.45%.

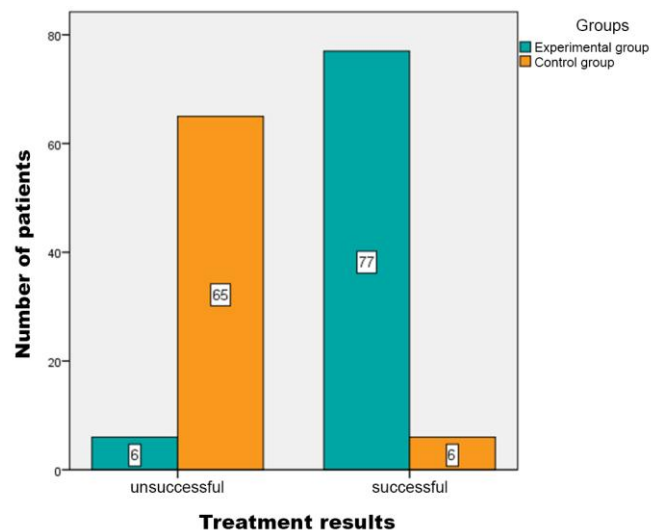


Fig.31 Variation analysis based on Treatment results for EG and CG

3.3. Study of correlation between qualitative characteristics

In order to achieve the goal and solve the tasks of research of the present dissertation, an analysis of the variables containing information about the patients with the initial and final data from the PRP treatment method was performed to prove possible relationship between the different features. The correlation dependencies have been examined only for EG in order to find connections between various features.

3.3.1. Study of correlation between features "Result of treatment" and "Concomitant diseases"

Patients in EG have been examined in order to establish correlation between the characteristics "Result of treatment" and "Concomitant diseases". The results are presented in Fig. 32.

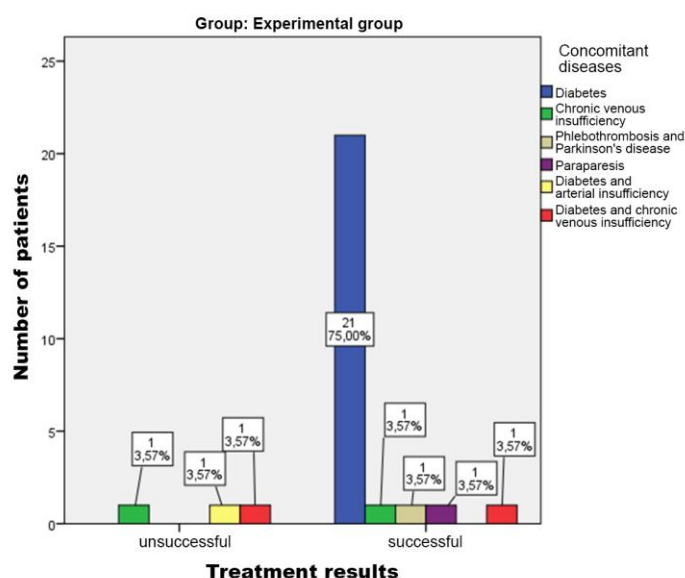


Fig.32 Study of correlation between features Treatment result and Concomitant diseases

The study of the correlation dependence between "Treatment result" and "Concomitant diseases" features shows that the most common concomitant disease is diabetes (as the only concomitant disease) - a total of 75% of all patients examined. The used PRP method gives 100% healing to patients with the only concomitant diabetes. Upon single concomitant diseases such as phlebothrombosis of the lower leg and Parkinson's disease, as well as upon paraparesis of the lower extremities, successful treatment was established using the method of PRP.

3.3.2. Study of correlation between characteristics "Treatment results" and "Sex"

Patients in the experimental group were examined to determine the relationship between features "Result of treatment" and "Sex". The results are presented in Fig. 33.

The study of the correlation between features "Result of treatment" and "Sex" shows an even distribution of patients, respectively, treated men are 50.6% and treated women - 49.4%. The total number of patients cured by the PRP method is

92.77%, of which the cured men are 92.85% and the cured women - 95.12%. The used method gives equally good results in the treatment of both sexes, which means that the method is not influenced by sex.

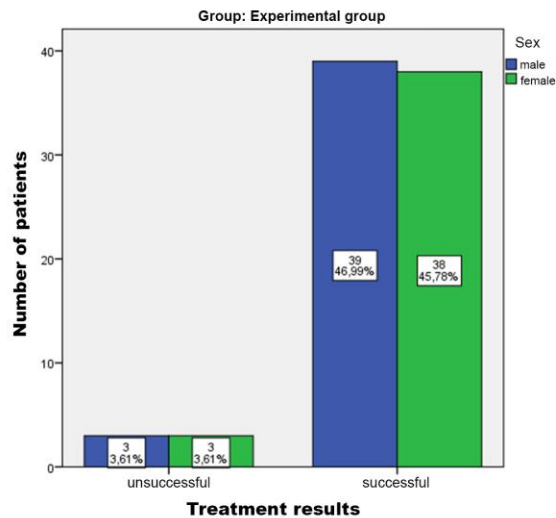


Fig.33 Study of correlation between features Treatment result and Sex

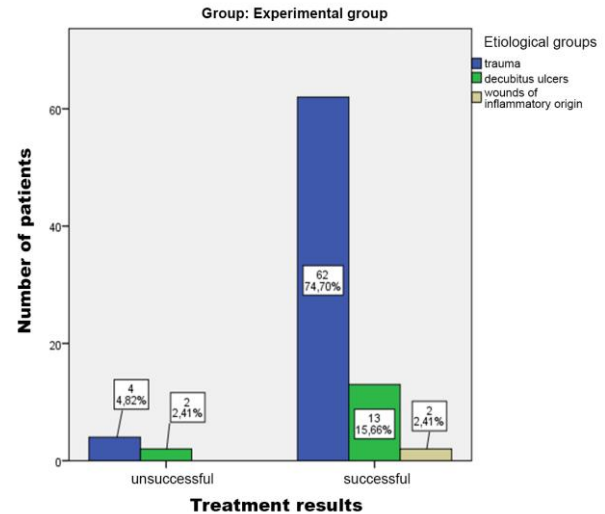


Fig.34 Study of correlation between features Treatment result and Etiological groups

3.3.3. Study of correlation between "Treatment result" and "Etiological groups" features

Patients in EG were examined in order to establish relationship between features "Treatment result" and "Etiological groups". The results are presented in Fig. 34.

For the experimental group of patients with PSW treated by the PRP method, positive results with fully healed wounds were respectively: 74.70% with traumatic wounds of all 79.5% treated with such wounds; 15.66% with decubitus ulcers of all 18.1% treated with such wounds; and 2.41% with wounds of inflammatory origin of all 2.41% treated with such wounds.

The result for all patients with PSW treated by the PRP method was successful for 93.9% of patients with traumatic wounds, 88.8% with decubitus wounds and 100% for wounds of inflammatory origin.

3.3.4. Study of correlation between features "Causative agent" and "Treatment result"

Patients in the experimental group have been examined in order to establish the relationship between features "Causative agent" and "Treatment result". The results are presented in Fig. 35. Antibiotic treatment has not been used with patients where

S. aureus and *E. coli* had been detected by microbiological test (antimicrobial culture). On the contrary with those patients where the causative agents *Kl.pneumoniae*, *P.aeruginosa*, *E.faecalis* have been found, antibiotic intravenous treatment according to the obtained microbiological result has been applied simultaneously with the application of the PRP (platelet-rich plasma) method.

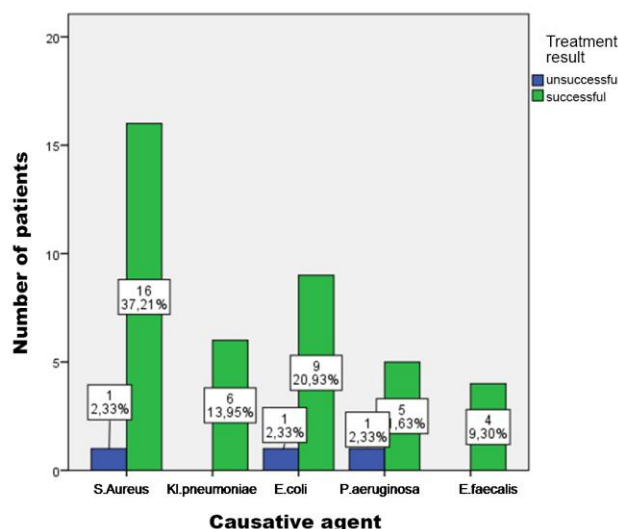


Fig.35 Study of correlation between features Causative agent and Treatment result

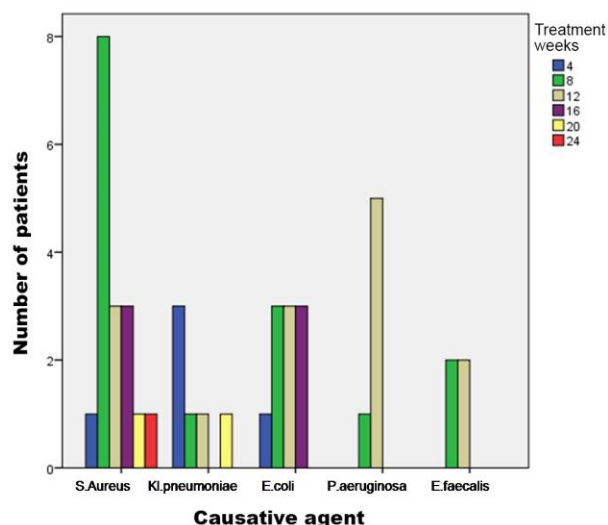


Fig.36 Study of correlation between features Causative agent and Treatment weeks

Therefore, there is no strong dependence between the two features "Causative agent" and "Result of treatment" and it cannot be argued that the causative agent has a significant impact on the treatment result.

Despite the presence of the examined infectious agents, the PRP method gives significant results in the treatment of PSW.

3.3.5. Study of correlation between “Causative agent” and “Treatment weeks”

Patients in EG have been examined in order to establish the relationship between features "Causative agent" and "Weeks of treatment". The results are presented in Pic. 36, there is a moderate dependence between the two symptoms and it can be argued that the causative agent has a moderate effect on treatment duration. The causative agent *S. aureus*, identified by microbiological test, is observed in all patients with this agent, which prolongs the duration of treatment to 24 weeks due to its difficult response to medication. Successfully cured patients with *S. aureus* are 37.21% distributed in all 4-week ranges. The other common problematic skin

wounds causative agent is *E. coli*, which extends the therapeutic period to 16 weeks. A similar effect has been observed in patients with the causative agent *Kl. pneumoniae*, where the therapeutic period prolongs up to 20 weeks.

3.3.6. Study of correlation between features "Causative agent" and "Concomitant diseases"

Patients with EG have been examined in order to establish the correlation between the "Causative agent" and "Concomitant diseases". The results are presented in Fig. 37, there is a strong dependence between the two features and it can be argued that the causative agent has a strong influence on the treatment of concomitant diseases. The study of the correlation between features "Causative agent" and "Concomitant diseases" shows that 51.8% of all studied patients have causative agents, and 37.20% of them have concomitant diseases.

The most common concomitant disease is diabetes mellitus (as the only concomitant disease), which combines with all causative agents and extends the duration of treatment. 43.75% of patients with diabetes fall into a group of *S. aureus* and *E. faecalis*.

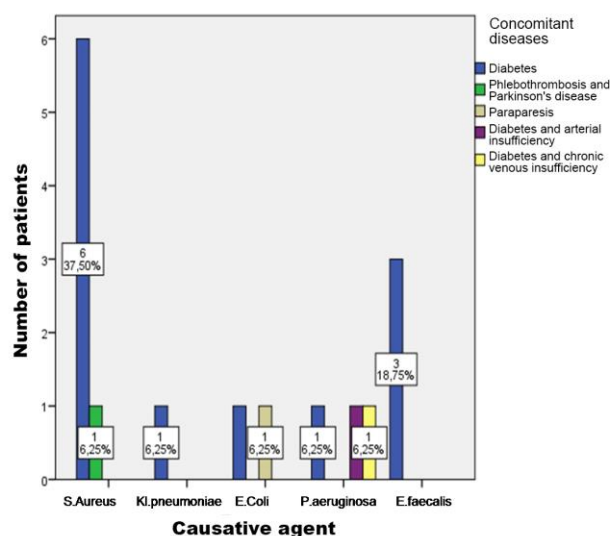


Fig.37 Study of correlation between features Causative agent and Concomitant diseases

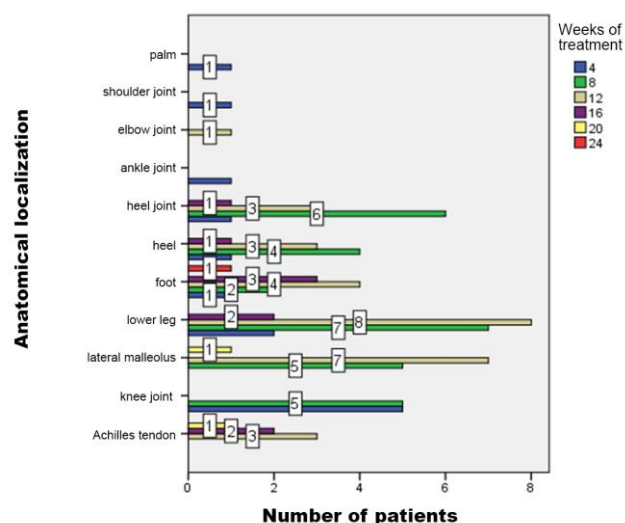


Fig.38 Study of correlation relationship between features Anatomical localization and Treatment weeks

3.3.7. Study of correlations between characteristics "Anatomical localization" and "Weeks of treatment"

Patients in EG have been examined in order to establish correlation between features "Anatomical localization" and "Weeks of treatment". The results are

presented in Fig. 38. There is a strong relationship between the two features "Anatomical localization" and "Treatment weeks" and it can be argued that anatomical localization has a strong influence on the duration of the treatment.

The resulting correlation between Anatomical Localization and Weeks of Treatment shows that patients with PSW in the foot area, who represent 13.25% of EG, cover five of the six 4-week treatment ranges and are the most - long-term treated patient (24 weeks) with a positive result until complete healing of the wound in this group. The anatomical localization Achilles tendon includes 7.23% of EG patients who are successfully cured patients by the PRP method, and covers 12 to 20 weeks of the 4-week treatment ranges. The anatomical localization *Lower leg* includes 22.89% of EG patients who successfully cured patients by the PRP method, and covers 8 to 16 weeks of the 4-week treatment ranges. The localizations in lower extremities require a longer period of treatment than those in the upper extremities. The statistical summary of the obtained results allows us to make a conclusion that the treatment of the following localizations by the applied method of PRP is really achievable in the following number of weeks: for Achilles tendon - 12 weeks; for lower leg - 8 to 12 weeks; for the foot - 12 weeks. This shows that the anatomical localization ***Lower leg, foot and Achilles tendon*** is particularly difficult to treat with extended period of healing of PSW, associated with the anatomical features of these areas. These localizations are among the wounds that most often fall into the category

Incurable by conventional method.

The achieved results are prerequisite for the statement that the application of the PRP method in the treatment of PSW leads to complete recovery of the treated wound, as well as proves the statement that the anatomical localization affects the duration of treatment.

3.3.8 Study of correlations between features Anatomical depth and Treatment weeks

Patients in EG group have been examined in order to establish the correlation between features "Anatomical depth" and "Weeks of treatment". The results are presented in Fig. 39, there is a moderate relationship between the two features "Anatomical Depth" and "Treatment weeks" and it can be argued that anatomical depth has a moderate effect on the duration of the treatment.

The statistical summary of the obtained results allows us to conclude that the treatment of the following anatomical depths by the applied method is really achievable in the following number of weeks: for fascia - from 8 to 12 weeks;

subcutaneous tissue - from 8 to 12 weeks; bone - from 8 to 12 weeks; tendon - from 12 to 16 weeks.

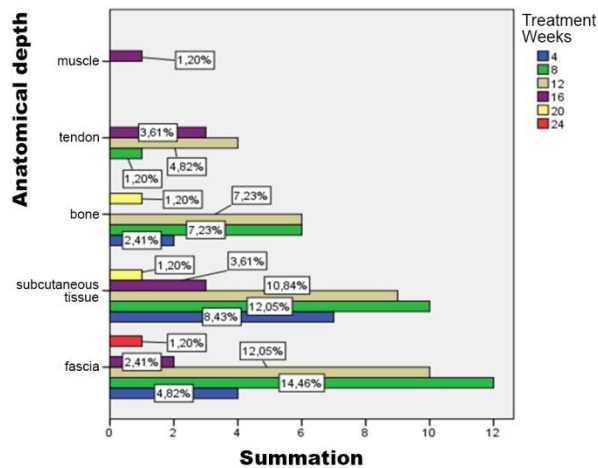


Fig.39 Study of correlation between features Anatomical depth and Treatment weeks

The relationship between anatomical depth and treatment weeks indicates that wound depth does not determine the time period for skin ulcers healing. At each of the examined anatomical depths, patients with extended wound healing period have been observed.

3.3.9. Study of correlations between features "Etiological groups" and "Treatment weeks"

Patients in EG have been examined in order to establish correlation between features "Etiological groups" and "Weeks of treatment". The results are presented in Fig. 40, there is a moderate relationship between the two characteristics "Etiological groups" and "Weeks of treatment" and it can be argued that the etiological group has a moderate effect on the duration of treatment. In patients with traumatic etiology, it was observed that for 10.61% of patients in the "Trauma" etiological group duration of treatment is in the range from 16 to 24 weeks.

In patients with decubitus ulcers, it was observed that for 33.33% of patients in the "decubitus ulcers" etiological group duration of treatment was 16 weeks. The summary of statistical information allows us to conclude that a higher percentage of patients with decubitus ulcers have longer treatment periods than patients with traumatic wounds.

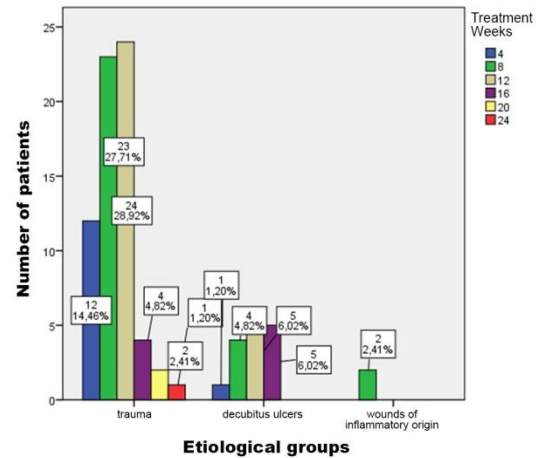


Fig.40 Study of correlation between features Etiological groups and Treatment weeks

IV. DISCUSSION

The goal of our study is to investigate the reliability and significance of the PRP method, and to evaluate its effectiveness and applicability in the treatment of PSW among Bulgarian patients. The subject of the study is the successful treatment of patients by the PRP method. The total number of patients treated at UMHAT Kanev Ruse is 154, which defines the study as a random sampling study.

The term PSW is not widely used in the scientific literature. Only two articles were found in the literature. In our study we analyze acute, hard-to-heal and chronic skin wounds based on their etiology, physiology, pathophysiology and treatment method and we unite them in the so-called "Problematic skin wounds" (PSW), which is a novelty for Bulgaria.

Hard-to-heal and chronic skin wounds are problematic in themselves. Acute skin wounds (ASW) heal primarily in the normal way of recovering of a skin wound, but when they are accompanied by intrinsic and extrinsic factors (age, concomitant diseases, taking various medications, the presence of a pathogenic infectious agent, wound localization, etc.) they can become chronic, which has been published by Lindholm et al. They prove that about 15% of acute skin wounds (ASW) are becoming chronic skin wounds and fall into the group of PSW (problematic skin wounds). The main reason for this difficult and delayed healing is the lack of growth factors in the wound bed under the influence of various causes and this negatively affects the normal tissue regeneration. Activated platelets in PRP deliver the necessary growth factors in the wound bed and peripheral wound edges, stimulate the normal wound process and achieve wound healing. **So far in Bulgaria there is no data on the treatment of problematic skin wounds by the use of PRP method**, which prompted us to research and examine the PRP method in this study. The results of our research show that 92.78% of patients achieve complete healing and excellent results, while 7.22% of patients have no positive change in wound healing (unhealed wound).

While developing this thesis, we created a methodology for the use of PRP method for prevention of acute, potentially problematic skin wounds, because we have proven that in patients over 50 years of age, with concomitant diseases, infection agents and anatomical localization on the lower limb, treated by the method of PRP, a positive effect and complete recovery until the complete wound healing is achieved.

For the first time in Bulgaria the current paper, containing our research and analysis **examines in detail the possibility of digitizing the criteria for assessing the condition and status of PSW**, both in the beginning at first contact with the

wound and upon weekly follow-up until the end of tissue regeneration, **and the possibility to give an estimated value forecasting wound recovery as well.**

In order to achieve the goal and solve the tasks of the study, we performed a Variation analysis of the variables containing information about the initial and final data of the experiment, where we determined the main numerical characteristics of the variables and checked the trend of their change for each group in this experiment by using comparative analysis.

We made a comparative analysis of the examined patients based on Age groups in order to determine their distribution. The obtained results show that the studies cover the entire age range of patients where the problematic skin wounds could occur. Their distribution in % reveals that 20.48% of patients are in the age group 70-79 years; 28.92% in the age group 60-69 years; 21.69% in the age group 50-59 years. **The largest share of patients, 71.09% of the total number the are in age group over 50 years, which is associated with changes in their biological condition due to physiological changes.** This is also proven by Nunan et al, Mustoe et al, Martin et al in the researched literature sources. One of the factors that can create appropriate conditions for occurring such wounds is the advanced age of the patient. Their skin is easily damaged. Also a lack of adequate response to stress in terms of gene regulation of stress-related proteins has been observed. The possibility of various concomitant diseases (diabetes mellitus, chronic venous insufficiency, various diseases that lead to impaired mobility and long lasting compression and risk of decubitus ulcers, systemic diseases) has been also reported. Various medications (non-steroidal anti-inflammatory drugs or steroids) can have a significant influence. Numerous changes have been observed in the cellular and molecular characteristics of adult skin - delayed cell proliferation, changes in production, composition of the extracellular matrix and the response to the action of the growth factors and all this worsens the wound healing process.

We performed a comparative analysis of the examined patients based on Type of wounds, in order to present their distribution by duration of the wound existence before the proposed experimental treatment by the method of PRP. The obtained distributions in % based on wound type show that the **treated patients are evenly distributed in the three categories of groups**, respectively for EG - acute wounds - 36.1%; hard-to-heal wounds - 30.1% and with chronic wounds - 33.7%. In patients in CG, the values are similar - acute wounds - 36.6%; hard-to-heal wounds - 29.6% and with chronic wounds - 33.8%. The statistical analysis regarding the type of wound proved that the examined patients were evenly distributed in the three categories of groups, which gives grounds for reliability and

for summarizing the results. The treated patients according to the proposed method are distributed in equal proportions, respectively men 50.6% and women 49.4%.

Our analysis of the results obtained based on etiology proves that the most common etiological reasons are of traumatic nature and long lasting compression (pressure) and **traumatic wounds, decubitus ulcers and wounds of inflammatory origin are relatively evenly distributed** in the two examined patient groups. For EG - traumatic wounds are 79.5%; decubitus ulcers are 18.1% and wounds of inflammatory origin are 2.4%. The ratio for CG is similar - traumatic wounds 80.3%; decubitus wounds 16.9% and wounds of inflammatory origin 2.8%. The data allowed to compare and evaluate the results in the three etiological groups.

A thorough analysis of the treatment **results of problematic skin wounds compared to the treatment weeks** showed that the optimal period for treatment to complete wound healing is 8 and 12 weeks, respectively 34.94% and 28.91%, which gives over 63.85 % of the 77 patients successfully cured and treated by the PRP method. Only **7.22% of patients treated with the method have poor results due to concomitant diseases in the advanced stage**. A review of the literature sources reveals publications corresponding to the optimal period for treatment of PSW (problematic skin wounds) with PRP in our study conducted for the first time in Bulgaria. Tran et al. reviews the result of wound healing in patients with diabetic foot after the use of activated platelet-rich plasma. The obtained results showed that 100% of these wounds heal completely within 7 weeks. The authors conclude that this is an effective method of treating non-healing foot wounds. These results are similar to the results of our study where most patients are completely cured in 8 and 12 weeks.

Sakata et al. evaluate the results of standard care compared to the use of local treatment with PRP in the form of a gel in various complicated wounds. The result of wound healing in 39 patients with 40 chronic, non-healing wounds of the lower limb shows a healing period of 20 weeks in 83% of cases. Our data show a healing period of 20 weeks in only two patients, which is 1.66% of all cases, which highlights the better results of treatment with our advanced method of PRP.

Frykberg et al. use PRP in the form of a gel obtained at physiological platelet concentrations and found that the product can be used in complex chronic skin wounds, even those not amenable to other treatment and in elderly patients, deterioration values of laboratory tests and the presence of concomitant diseases. The quoted results have significant clinical consequences and the authors propose this treatment method for such wounds, which supports the data from our study, because they consider the wound reaction by reducing its parameters. **The results**

of our study show the complete healing of wounds and prove the applicability of the treatment method.

Dellinger et al. report results in the use of autologous platelet gel for the treatment of diabetic skin wounds. They do not report complications, report a wound healing period of 5 to 8 weeks, regardless of the size of the skin wound being treated, report a reduced risk of subsequent amputation, and observe an improvement in patients' quality of life. **Our study shows similar results.** Successfully cured patients by the method of platelet-rich plasma with concomitant diabetes mellitus are 75% (21/28) of all, which gives reason to conclude that the method used leads to complete healing, regardless of aggravated status.

Carter et al. analyze and prove that PRP therapy is the preferred method for complete recovery of chronic skin wounds. Upon acute skin wounds the chance of developing infection is reduced and complete or partial healing with improvement in local status compared to the control group is reported. Based on research from the last 10 years, they revealed that this method can lead to the healing of chronic and acute skin wounds and eliminate pain and infection. **Our study proves results to complete wound recovery**, both in the treatment of ASW and in the application of the PRP method in difficult-to-heal and chronic skin wounds.

The results of our examined patients from EG and CG on the basis of "Result of treatment" confirm that 92.78% of patients in the experimental group were successfully cured by the method of PRP and have achieved a positive result until complete wound healing. With the conventional method for the treatment of PSW, the successful outcome is only 8.45%. This fact is confirmed in the studies of a number of authors. Martinez-Zapata et al. perform a systematic review of randomized controlled clinical trials in adult patients to assess the safety and efficacy of the autologous PRP method. They conclude that the rate of complete healing in treated by PRP wounds is increasing compared to control wounds, but more new studies are needed to confirm these results. Villela et al. perform a systematic review and the results of the meta-analysis show that PRP is favorable for the healing process. In conclusion, the authors note that there is scientific evidence on the favorable results in using the method of treatment of diabetic wounds and is a good choice for chronic skin wounds treatment.

The comparative analysis of the achieved results by leading scientists and our study confirms the role of PRP and in particular of the growth factors in the healing process of PSW. From the review of the literature sources and on the basis of our study of tissue regeneration it can be concluded that the application of PRP method allows optimal treatment of wounds, leading to their complete healing due to biological effects of the process.

To achieve the goal and solve the tasks of the study, we analyzed the variables containing information about patients with initial and final data from the treatment with PRP, to prove the possible relationship between the individual features. The summary of our data from the conducted research on the interaction between the various indicators shows the following:

When reviewing the *interaction between treatment results and comorbidities*, the most common comorbidity is diabetes mellitus (as the only comorbidity) and the used PRP method achieved a 100% positive result until complete wound healing. This method is also successful in those treated with single comorbidities. The following analysis from the literature studies gives similar data, but in most of the results lower rates of successful treatment until complete wound healing have been achieved. Tran et al. investigated the effect of wound healing in patients with diabetic foot with non-healing wounds in which they use activated PRP. The results show that 100% of these wounds heal completely in a period of 7 weeks. The authors conclude that it is effective in treating non-healing foot wounds. Driver et al. report a study on the use of autologous PRP in the treatment of diabetic foot wounds. The effectiveness of plasma in the form of a gel is compared with that of a gel with normal saline for 12 weeks. The authors found that 68.4% (13/19) of the patients in the group with PRP method and 42.9% (9/21) in the control group have completely healed wounds. The wounds in the first group healed after an average of 42.9 days against 47.4 days in the control group. This study is limited to some extent because it excludes wounds in patients with mild to moderate vascular disease of the limbs, with exposed tendons or prominent bone, in patients with hyperglycemia and / or poor nutritional status. Picard et al. perform a literature review in order to summarize the knowledge related to the use of PRP in the treatment of chronic skin wounds in patients with diabetes. In 87.5% of controlled studies, a significant benefit of PRP was found in the treatment of such wounds. It is recommended to use PRP for diabetic wounds that have not healed after standard treatments. Margolis et al. evaluate the effectiveness of PRP used to treat diabetic lower extremity neuropathic ulcer. They concluded that this treatment was more likely to be used in more severe wounds and was more effective in these wounds than in standard wound care. A great advantage of autologous PRP in clinical practice is the lack of side effects.

In the review of the interaction between treatment outcome and etiological groups in our study, it has been proved that the outcome of therapeutic behavior for all patients treated by PRP method was successful for 93.9% of them with traumatic wounds, 88.8% with decubitus ulcers and 100% for wounds of inflammatory origin. The obtained extremely high percentage of successful treatment of decubitus wounds directs the attention for literature comparison of this type of

wounds in order to prove the effectiveness of the method in them. Ramos-Torrecillas et al. observe a clinical case of a female patient with a decubitus heel wound and describe their experience with platelet-rich plasma. Complete healing of the wound was reported over a period of 54 days. The authors conclude that this is an easily applicable and cost-effective method, but more studies are needed to evaluate the effectiveness of this treatment. Sell et al. present the result of the use of PRP in the treatment of grade 4 decubitus wounds in three patients with spinal cord injury. They concluded that plasma demonstrated a stimulating effect on chronic long-term compression wounds in patients with lower paraparesis with spinal cord injury. Frykberg et al. investigated the use of PRP in the form of a gel obtained by physiological platelet concentrations and found that the product can be used for the treatment of complex decubitus CSW, even those not amenable to another treatment, as well as in elderly patients with deteriorating values from laboratory tests and in the presence of concomitant diseases. Singh & et al. conducted a study of a series of cases in patients with decubitus ulcers. Their aim is to assess the local application of PRP to these wounds in one group compared to a control group with such saline-treated wounds, and each patient has both wounds. Improvement was reported in 96% of these patients and only 1 patient had wound deterioration. The authors conclude that this is a promising alternative to a standard saline technique. The technique is easy to perform, with good bio-tolerance, low cost and significant clinical efficacy, but extensive studies are needed to confirm the positive effect of this therapy. Our results are close to those of Singh et al. as **we observed complete wound healing in 88.8% of our patients with decubitus ulcers**, and not "improvement in 96%", as noted above. This comparison proves the positivity of the method used and the effectiveness of treatment and provides a reason for recommendation in therapy and prevention.

There are 4 *patients with problematic skin woPSW* and chronic venous insufficiency in our study. The small number of patients does not allow strict confirmation of our studies, but comparative literature analysis proves its effectiveness also for chronic venous insufficiency. Sarvajnamurthy et al. have studied the efficacy of platelet-rich autologous plasma in the treatment of chronic venous wounds. Twelve patients with 17 wounds were treated with PRP and the result was measured by the percentage of improvement in the area and volume of the wound. The average percentage of improvement in the field and volume of the wound was 94.7% and 95.6%, respectively. The authors conclude that this is a safe, simple and effective treatment procedure. O'Connell et al. present the results of a study involving the treatment of CSW in patients with chronic venous insufficiency in lower extremities with an autologous, platelet-rich and platelet-rich fibrin membrane. Although this study is limited, the authors made a

conclusion that the use of the product has potential in patients who have failed to recover by the conventional method.

It is especially important for the normal healing of a wound without complications, that the wound does not contain a pathogenic infection agent, which requires prophylaxis of wounds, both after receiving them and in postoperative wounds, especially in elderly patients with comorbidities. The microbial causative agent must be controlled according to the results of microbiological culture. Based on analysis of literature sources it was found that platelet-rich plasma inhibits the growth of *S. aureus* and *E. coli*, while in *Kl. pneumoniae*, *E. faecalis* and *Ps. aeruginosa* no similar effect has been observed. This is confirmed by a paper by Cieslik-Bielecka et al. They reported that PRP in gel form inhibited the growth of *S. aureus* and *E. coli*, but no similar effect was observed in wounds with *Kl. pneumoniae*, *E. faecalis* and *Ps. Aeruginosa*. No correlation between leukocytes and platelet concentration and antimicrobial activity has been observed. The activity of antimicrobial peptides against bacteria is more pronounced compared to their activity against fungi. Plasma in gel form induces in vitro growth of *Ps. aeruginosa* thus deteriorating the wound infection. Authors believe that the combination of the inductive and antimicrobial properties of PRP in gel form improves the treatment of infected delayed adhesion or non-adhesion. Anitua et al. defend the idea of obtaining PRP, which is free of leukocytes due to the proinflammatory effects of proteases and acidic hydrolases. Authors emphasize that the term Platelet Rich Growth Factors (PRGF) is based on the principle that any platelet concentrate after activation will release growth factors that are major agents of wound healing. They evaluated the effect of Platelet Rich Growth Factors (PRGF) on CSW in 14 patients and reported good wound response to the wounds in 80% of cases after 8 weeks of administration, compared to 20% in the control group. No leukocytes have been detected in the analyzed products.

Their explanation for the good results is the high concentration of growth factors. A similar result of PRP influence on the development of *S. aureus* and *E. coli* in wounds reported Singh et al. in their paper. The authors report a study aiming at determining the source of microbial colonization of prolonged compression wounds and the antimicrobial properties of autologous PRP in such wounds. The most common microorganisms isolated in them were *S. aureus* and *E. coli*. The researchers found a significant relationship between the colonization of wounds from prolonged compression and bacteria found in urine cultures and feces. The local application of autologous PRP changes their "biological environment" through its antimicrobial properties, which leads to a reduction in bacterial colonization. Moojen et al. use platelet-leukocyte gel. They investigated the hypothesis if platelet-leukocyte gel has antimicrobial properties and studied its

activity against *S. aureus* and the involvement of myeloperoxidase by leukocytes in this process. The gel shows strong antimicrobial capacity, but the role of myeloperoxidase in this capacity is questionable and it could be used strategically against postoperative infections. According to them, further research is needed to determine the exact antimicrobial activity.

According to literature, it is questionable whether leukocytes are necessary in PRP or only antimicrobial peptides contained in platelets are sufficient to inhibit wound infection. The comparative analysis of studies performed with and without leukocytes, determined our choice in the study and treatment of patients by the method of PRP. Tang et al. evaluate the potential antimicrobial properties of platelet proteins. The platelets have structural and functional similarities to granulocytes, which are involved in human antimicrobial protection. It has been proved that the peptides are more powerful against bacteria than against fungi; their activity was higher at acidic pH, and their antimicrobial properties were dependent on the dose. These results show the direct antimicrobial role of activated platelets in response to trauma or mediators of inflammation with the release of antimicrobial peptides. Drago et al. have studied the antimicrobial effect of pure PRP against oral microorganisms in the oral cavity *E. faecalis*, *Candida albicans*, *Str. agalactiae*, *p. oralis* and *Ps. aeruginosa*. The pure PRP does not inhibit the growth of *Ps. aeruginosa* strains. According to the authors, plasma is potentially useful in the fight against postoperative infections. This could be a valuable extra quality in addition to improving tissue regeneration. Anitua et al. report the antimicrobial potential of growth factor-rich plasma when used against both methicillin-sensitive and methicillin-resistant *S. aureus* and *S. epidermidis*. Effect of incorporated leukocytes from the patient in growth factor-rich plasma has also been reported. The researchers found that all types had a strong bacteriostatic effect, especially in the first 4 hours after application. All types had an antibacterial effect at the 4th hour for three of the four strains, with the exception of methicillin-sensitive *S. epidermidis*. The researchers concluded that growth factor -rich plasma could be used to fight and prevent postoperative wound infections. A similar study was reported by El-Sharkawy et al. The presence of leukocytes in PRP would lead to additional tissue damage by leukocyte proteases and make wound tissue recovery more difficult. For this reason, we join the authors using leukocyte-free plasma.

Taking into account the results of the *interaction between treatment result and weeks of treatment against the causative agent*, we found that in patients with causative agent *S.aureus*, the duration of treatment is extended to 24 weeks due to its difficult response to medication. The other causative agent present, *E. coli*, prolongs the duration of treatment to 16 weeks and, accordingly, to *Kl.pneumoniae*

is reached up to 20 weeks. In patients with isolated *S. aureus* and *E. coli*, we used only PRP until complete recovery of PSW. Our data support the results of the above mentioned authors. In patients with *Kl. pneumoniae*, *E. faecalis* and *Ps. aeruginosa*, in addition to PRP, we used appropriate antibiotic treatment in accordance with the microbiological test. 37.21% of our patients with *S. aureus* distributed in all 4-week ranges were successfully cured. According to the results obtained, the type of causative agent affects the duration of treatment. **Our study showed that the PRP method gives significant results in the treatment of problematic skin wounds, despite the presence of the studied infectious agents.** In addition, the review of the interaction between the *causative agent and concomitant diseases* revealed a strong dependence and we can say that the causative agent has a strong impact at presence of concomitant diseases on the treatment of PSW by the PRP method. The most common concomitant disease is diabetes mellitus (as the only concomitant disease), which combines with all causative agents and extends the duration of treatment of PSW.

An examination of the interaction between *anatomical localization and treatment weeks* proved that the anatomical localization of wounds had a strong effect on the duration of treatment. In our study, it was found that a huge percentage of wounds are located on the lower limb, as confirmed by Callam et al. and VanDenKerkhof et al., who believe that the prevailing CSW localization is lower limb and presumed involvement of active leg in patients in Europe is 0.1 - 0.3%. **The anatomical localization in lower leg, foot and Achilles tendon make a therapeutic response particularly difficult, with prolonged healing of problematic skin wounds associated with the anatomical features of these areas.** These localizations are among the ulcers that usually fall into the category of incurable by conventional methods. Based on obtained results, we can conclude that the application of the PRP method leads to complete recovery of the treated wound.

When reviewing the interaction between anatomical depth and treatment weeks, we reported that the anatomical depth has a moderate effect on the duration of treatment. The speed of healing of skin ulcers is moderately affected by the depth of the wound. At each of the studied anatomical depths, single patients with prolonged healing time period has been observed. These results differ from the opinion of Nurden et al. and Anitua et al. that wounds affecting deeper structures often require more time for recovery. **Some of the patients in our study with deeper wounds had shorter period** of complete healing than superficial wounds.

Examination of the interaction between etiological groups and wound depth in mm revealed that patients with traumatic wounds had wound depth of 5 mm and

represent 33.33%, and those with depth of 10 mm represent 21.21%. There are 13 different depths in this group, which is probably related to the diversity of injuries. In patients with decubitus ulcers, 46.67% of those treated are with depth of 5 mm and the rest have been distributed in 6 different depths.

When *examining the interaction between etiological groups and area* (sq.mm) it was found that in traumatic wounds 15.15% are patients with a wound area of 1600 sq.mm. There are 22 different wounds in size and area, which is determined by the specific etiology. In the group with decubitus ulcers, patients were divided into 10 different areas of the wound, and 20% of those treated patients had PSW with an area of 1200 sq. mm. There are no data in the literature sources for such a detailed statistical study of etiology, groups, area and depth, which determines the lack of respective comparative analysis.

Examination of the interaction between etiological groups and weeks of treatment revealed that the etiological group had a moderate effect on the duration of PSW treatment. This allows us to conclude that a higher percentage of patients with decubitus ulcers have a longer treatment period than patients with traumatic wounds.

The conducted statistical research proves that the PRP method gives significant results in the treatment of PSW regardless of aggravating factors such as causative agent, concomitant diseases and localization, and for 92.77% of patients in EG complete wound healing has been achieved.

There is a copious amount of contemporary scientific evidence on the positive role of PRP in tissue regeneration and wound healing, as well as few studies using a variety of platelet-rich products that show no significant improvement in the recovery of patients with chronic skin wounds. Margolis et al. assess risk factors in patients with diabetic neuropathic lower extremity ulcers. They report that patients with PRP adjuvant therapy or skin graft have wounds that are less likely to heal and there is no change in the assessment of each risk factor by more than 10 percent, suggesting that the methods had small effect on study results. Authors suggest that this effect is due to prejudiced selection of inappropriate patients and that not all patients have received a complete therapy of 20 weeks. In order to support the method, they quote a previous study by Margolis et al., which has showed that PRP method has been successful in treating even the most severe wounds of diabetic polyneuropathy of lower extremities. Martinez-Zapata et al. report the benefits of PRP in the treating CSW (chronic skin wounds). Their report includes randomized controlled studies comparing PRP with placebo or alternative treatment in adult patients. They conclude that there are currently no convincing data on the benefits of PRP. New, better-designed studies are needed to

demonstrate the benefits of this therapeutic application. Krupski et al. study the effect of using an autologous platelet-derived wound healing formula (PDWHF). Although random sampling is limited, researchers suggest that results of treating CSW with an autologous platelet formula is not better than in patients treated with traditional therapy.

A review of results of our study found that patients without a positive change in the process of tissue regeneration, i.e. with poor results and non-healing of the wound with deterioration of its parameters are 7.22% (6/83). These patients have a number of additional factors that complicate the wound healing process. These are advanced age, concomitant diseases, wound localization and etiological causes. **Their percentage is extremely low and this allows the PRP method for treating PSW to be considered as successful.**

According to Lindholm et al. about 15% of ASW treated by standard methods are transforming to CSW, which leads to an extension of the tissue recovery time, with all the adverse mental, emotional, social and economic consequences associated with the treatment of a CSW.

Because of our research, examination and summarization of its results, we present prophylactic and therapeutic algorithms in order to support the work of physicians in both out-of-hospital and hospital care. The algorithm for prophylactic application of PRP in potentially problematic skin wounds, Fig. 41, provides an opportunity to reduce the risk of transforming ASW to CSW. The proposed algorithm should be part of the work of each practicing physician in order to avoid complicating patient's condition due to an inappropriate treatment. The algorithm for therapeutic application of PRP in PSW, Fig. 42, allows a correct and accurate treatment of patients with different PSW in order to improve the quality of treatment and achieve complete recovery.

The contribution of our work to the researched area could be discussed based on the conclusions derived from the literature review and from the analysis of our results. We use PRP prepared by single centrifugation of the patient's blood and the separated plasma is leukocyte-free. We support authors who use leukocyte-free plasma. We confirm the published data that PRP inhibits the development of some infectious agents without the need for antibiotic treatment and a complete wound healing by the used method can be achieved.

The discussion leads to the conclusion that a number of still unresolved issues are on the agenda, which require further research for clarifying and precisizing. Improving the level of scientific evidence requires new research proving the real benefits of this truly promising therapeutic method. It is important to clarify the

optimal concentration of platelets for their proper functioning when applied to the wound. Depending on their concentration in PRP, the effect of their action is different – Jacobson et al. that the process of angiogenesis starts at concentrations of 1500×10^3 platelets per microliter and goes up to 3000×10^3 platelets per microliter. Interestingly, inhibition of the process of vascular formation is observed at concentrations of 5000×10^3 platelets per microliter, which suggests a paradoxical effect of increasing platelet count. A consensus is needed on the necessity for activating platelets and the way to do it - with physical, chemical or biological agents. The question of the form of plasma application is still unresolved. The liquid form of PRP has been recommended for infiltration of wound edges, while the gel form is used to fill wound defects. It is necessary to review the issue related to the specific application of the activated mixture, as well as the depth of infiltration in the wound edges and bottom.

The presence of leukocytes in PRP is also debatable, knowing their side effects and this allows the use of a certain cell line. Another important unresolved issue is the frequency of the applications in the wound. It is known that the life of activated platelets, during which they secrete growth factors, is 7 days. This supports the thesis of weekly administration. Further precise clarification of clinical indications and contraindications is needed. It is also an important question how and when to conduct rehabilitation and physiotherapy during treatment and after resolving the wound problem, as well as to determine the need for the use of auxiliaries. Last but not least is the assessment of the primary role of nutrition, the patient's history and psychological factors. It is important to specify the type of bandage that should be used after the manipulation – should it be moisturizing or not, impregnated with additional medication or not, how often to change and determine the need for additional immobilization of the damaged anatomical area. It is advisable to confirm the long-term safety of this therapeutic method and to consider the possible side effects of PRP application.

Analyzing the literature sources and taking into account the results of our study, we can conclude that the use of platelet-rich plasma for the treatment of problematic skin wounds is a secure, effective, efficient, safe and optimal therapeutic method. It should not be considered as a unique and universal for the complete recovery of any wound. Unlike other therapeutic methods, it follows the principles of biological treatment (delivers an increased amount of growth factors in the damaged area and accelerates the recovery process) and with precise indications allows a high percentage of complete tissue regeneration of problematic skin wounds. We join the supporters of the application of platelet-rich plasma in the treatment of problematic skin wounds.

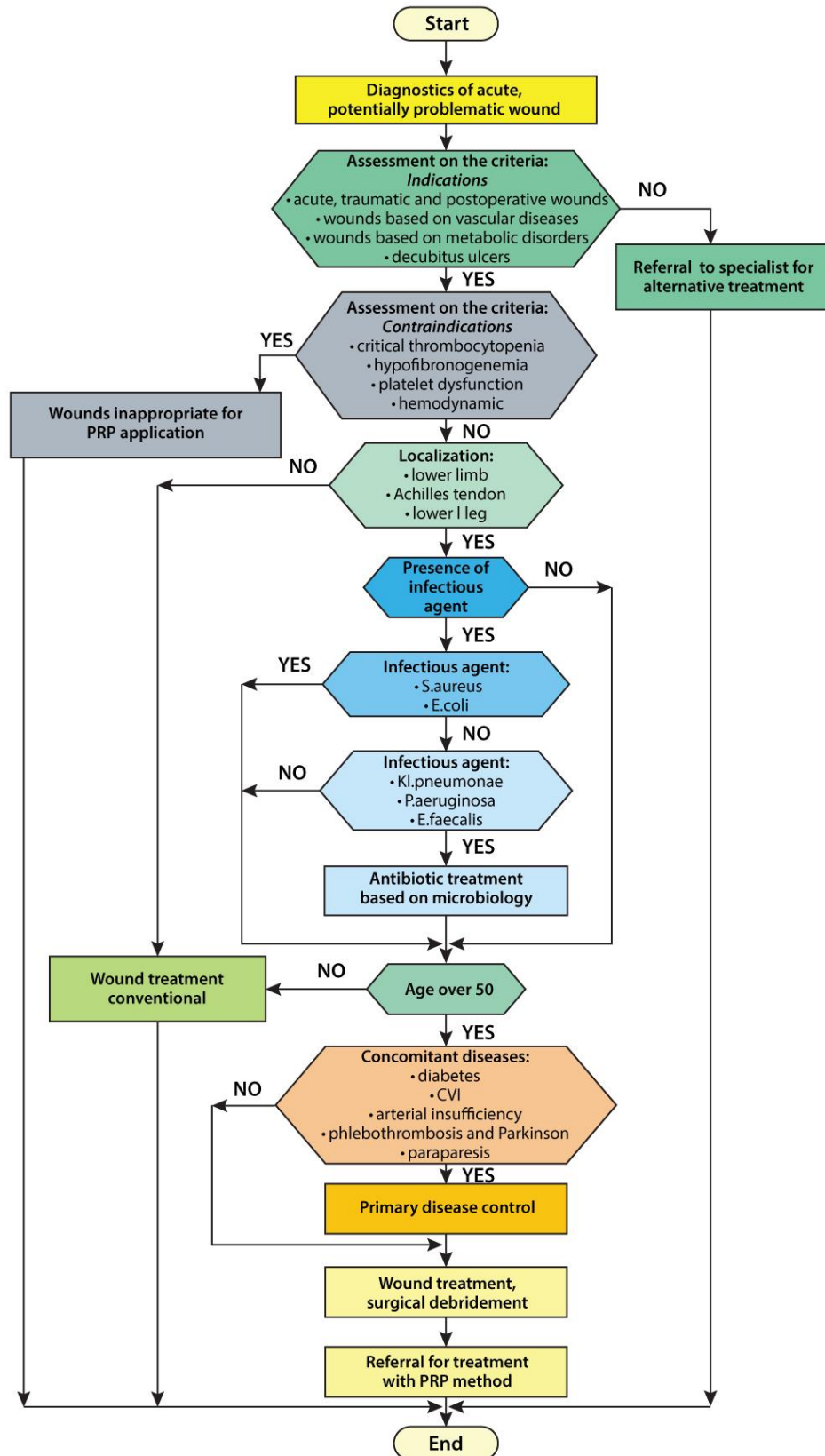


Fig.41: Own algorithm for prophylactic application of PRP to acute, potentially problematic skin wounds

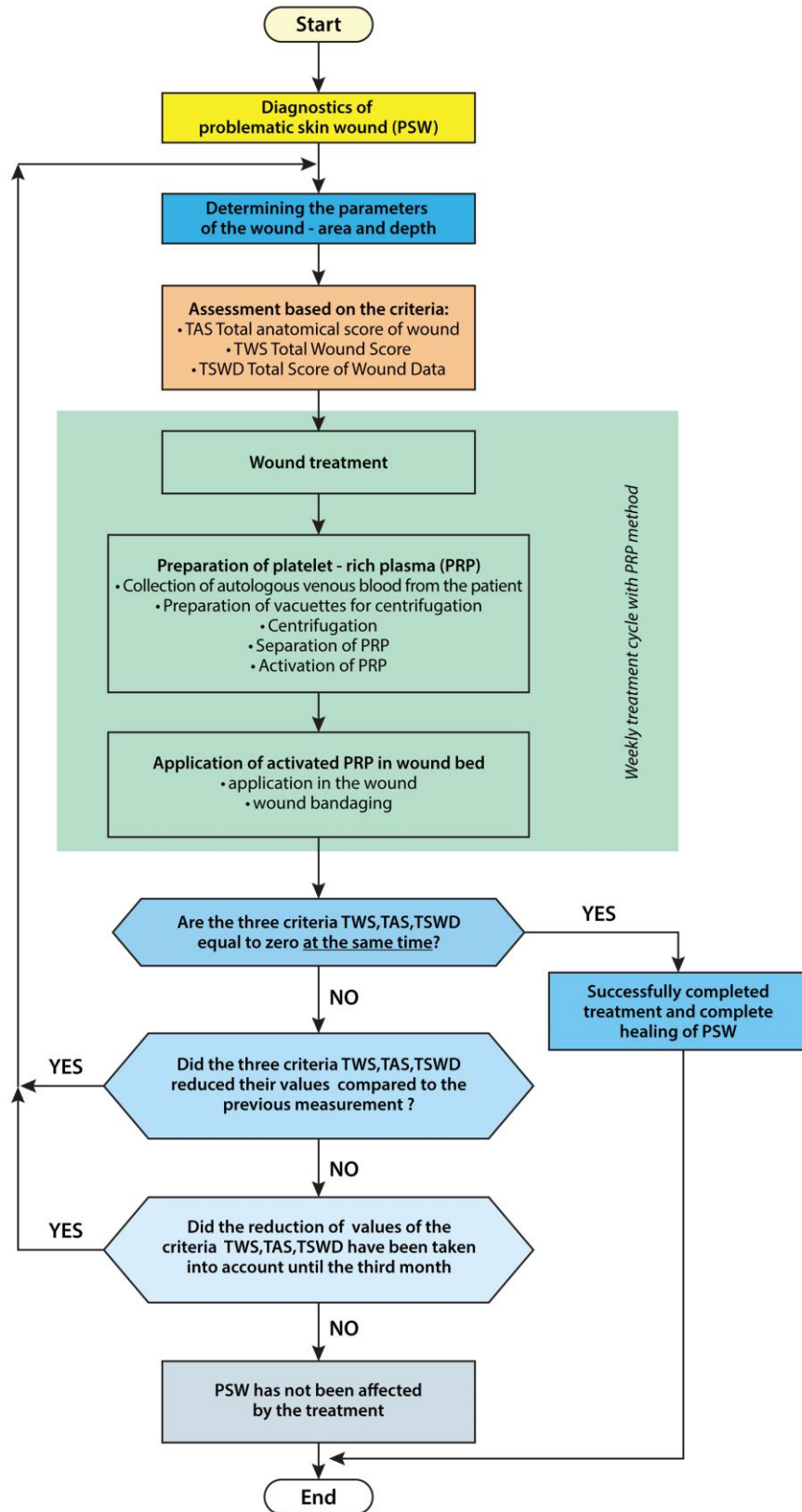


Fig.42: Own algorithm for therapeutic application of PRP to problematic skin wounds

V. CONCLUSION

In the current prospective study, we followed-up 83 Bulgarian patients with problematic skin wounds treated by the method of platelet-rich plasma for a period of 7 years (2009-2016). This is the largest followed-up series of patients in a clinical center among those published in the available literature. The 92.78% of our excellent results (complete wound healing) correspond to and are fully comparable to those achieved in large multicenter studies or in centers with similar experience to ours.

Problematic skin wounds are a major therapeutic challenge with growing importance to global public health. It is no coincidence that the term "silent epidemic" is used to refer to this problem. It has been proven that 15% of acute wounds are untreatable by the conventional method and transform to difficult-to-heal and chronic wounds. There are about 100,000 patients with such unhealed skin wounds in Bulgaria. The application of our improved method of platelet-rich plasma together with the developed own algorithms for prophylactic application of platelet-rich plasma in acute, potentially problematic skin wounds that can turn into problematic skin wounds, and therapeutic application of platelet-rich plasma in problematic skin wound would reduce this number and achieve a wholesome way of life for patients. In addition, the platelet-rich plasma method is cost-effective, easy to implement and usable in outpatient practice, making this procedure possible for any physician practice.

By analyzing our results, we can conclude that the use of platelet-rich plasma for the treatment of problematic skin wounds is a secure, effective, optimal, safe and modern therapeutic method. Our study showed the importance of the method for the treatment of problematic skin wounds.

However, it should be noted that new research is needed to improve the level of scientific evidence, revealing the real benefits of this truly promising therapeutic method. It has its place for the treatment of problematic skin wounds following precise indications.

VI. INFERENCES

1. The analysis of the biological and clinical characteristics of the problematic skin wounds shows the obvious advantages of platelet-rich plasma.
2. Defining a wound as problematic allows systematization of modern indications and contraindications.
3. The statistical analysis of the results proves the reliability, therapeutic significance and effectiveness of the methodology we have improved.
4. Digitization of the current condition of the wounds on the established scores optimizes the successful monitoring and evaluation of regeneration.
5. The results show that 92.78% of patients were successfully cured by the studied method, while the percentage with the conventional method is 8.45%.
6. Tissue regeneration of problematic skin wounds is not affected by their anatomical depth, while the anatomical localization, age of patients, infectious agent and concomitant diseases have a strong influence on the duration of treatment.
7. Own algorithms for prophylactic and therapeutic application of the method in acute, potentially problematic and problematic skin wounds optimize the quality of treatment.
8. The theoretical and clinical studies and the achieved results prove that the platelet-rich plasma method is a reliable, rational, effective and modern choice in the treatment of problematic skin wounds.

Based on the inferences drawn, we consider that the goals of the dissertation have been achieved.

VII. CONTRIBUTIONS

The main contributions of this paper can be formulated as:

1. For the first time in Bulgaria the platelet-rich plasma method for treatment of problematic skin wounds has been studied, applied and improved.
2. For the first time in our country we defined which skin wounds are problematic and indicated for treatment by the modified technique.
3. An innovative method for wound assessment has been proposed, which allows changes in clinical parameters to be digitized and formed in tabular groups in order to make prognosis for the end result of treatment.
4. The specific indicators of the problematic skin wounds during the whole period of tissue regeneration have been studied and evaluated by the established point scales and the prognostic time for treatment has been systematized.
5. A detailed statistical analysis of the obtained clinical results was performed, proving the effectiveness of the improved methodology.
6. The achieved results by the application of platelet-rich plasma are thoroughly, systematically and critically evaluated.
7. Modern, own prophylactic and therapeutic algorithms for the treatment of problematic skin wounds have been proposed.
8. This prospective study for followed-up patients with problematic skin wounds is the largest in the world, according to the available literature sources.

VIII. SCIENTIFIC SOURCES RELATED TO THE DISSERTATION PAPER

PUBLICATIONS

1. Sokolov Ts., Valentinov B., Andonov J., Angelov S., Kosev P. Platelet-rich plasma (PRP) and its application in the treatment of chronic and hard-to-heal skin wounds. A review. J of IMAB 2015 Oct-Dec; 21 (4): 982-986.
2. Sokolov Ts., A. Manukova, V. Kovachev, M. Kovachev. Treatment of Problematic Skin Wounds Based on the Platelet-Rich Plasma Method. Our own Algorithms for Application. Journal of IMAB.2020; 26 (4): 3436-3442.
3. Sokolov Ts., A. Manukova. Research of Medical Factors and Reasons for Healing Failure of Problematic Skin Wounds Treated with Platelet – Rich Plasma. Journal of IMAB.2020 Oct-Dec; 26 (4), 3463-3468.

PARTICIPATIONS IN SCIENTIFIC FORA

1. Sokolov Ts., Kosev P., Valentinov B., Andonov J. Application of PRP (platelet rich plasma) in hard-to-heal wounds. XX anniversary days of BOTA, Tryavna September 25-27, 2014
2. Sokolov T. Application of platelet-rich plasma (PRP) in hard-to-heal and chronic skin wounds 'treatment. 17th ESSKA Congress, Barcelona, 4-7 May 2016
3. Sokolov Ts., Valentinov B., Kosev P., Filipov V. Results of platelet-rich plasma application on problematic skin wounds. XIII National Congress of BOTA. Sofia, September 29 – October 2, 2016
4. Sokolov Ts., Andonov J., Boyan Valentinov B., Kosev P., Manukova A.
Statistical dependencies of the results from problematic skin wounds treatment with activated platelet-rich plasma. XIV National Congress of BOTA. Varna, October 3 - October 6, 2019
5. Sokolov Tsv., A. Manukova. Application of the method of platelet-rich plasma in an innovative software system for the treatment of problematic skin wounds. In: XXIV BOTA Conference, October 2-4, 2020, Plovdiv, 2020

Scientific-research projects related to the dissertation

Participation in the Scientific Research Fund and development of algorithms for prevention and treatment of problematic skin wounds under project 2020-FOZZG-02 "Development of algorithms for diagnosis and treatment of problematic skin wounds by the platelet-rich plasma method" led by Prof. Dr. Ivanichka Serbezova and Assoc. Prof. Dr. Anelia Manukova.

IX. ANNEXES

Clinical cases

Several clinical cases from the followed -up EG(experimental group) patients with problematic skin wounds (PSW) have been presented, including examples from the three groups – acute skin wounds (ASW), hard-to-heal and chronic skin wounds (CSW), treated by the PRP method. All patients showed in the picture material have complete wound healing.

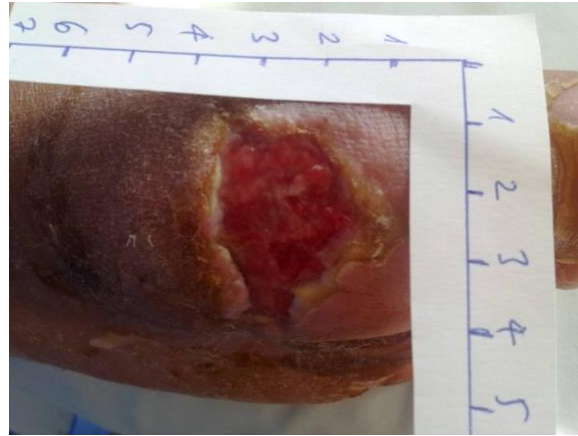
Male, 42 years old Crushing of the lower limb, fracture malleolus medial, repositio sanguinea osteosynthesis metallica, acute skin wound	
	
At the beginning	At the beginning
	
First week	4 th week

Male, 45 years old

Foreign object in the wound, phlegmon, hard-to-heal skin wound



At the beginning



Second week



4th week



8th week

Female, 17 years old

Hemangioma r.ahili, dehiscentio vulneris, acute skin wound



At the beginning



4th week



8th week



12th week

Male, 40 years old

Traumatic arthrotomy of the ankle, acute skin wound



At the beginning



4th week



12th week



16th week

Male, 67 years old

Traumatic lesion of Achilles tendon, dehiscentio vulneris, hard-to heal wound



At the beginning



4th week



8th week



20th week

Female, 72 years old

Traumatic wound, phlebothrombosis of the lower leg, chronic skin wound



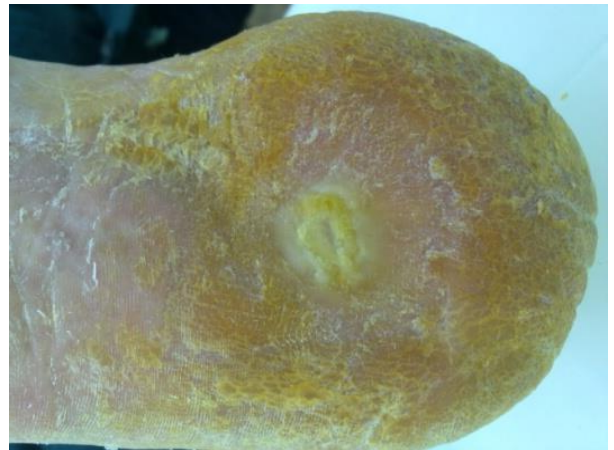
At the beginning



4th week



16th week



24th week

DECLARATION OF ORIGINALITY

I declare that in my work on my dissertation paper, I have respected all ethical and legal frameworks and all studies and developments used in the dissertation have been conducted in accordance with the principles set out in the Declaration of Helsinki.

I declare that I have no conflict of interest related to my dissertation paper.

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