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Clinical results of the administration of platelet concentrates in postextraction plots using new protocols for the extraction of platelet-rich plasma

AUTOREFERAT

AT A DISSERTATION ON THE AWARD OF EDUCATIONAL AND SCIENTIFIC DEGREE "DOCTOR"

Scientific specialty "Surgical dentistry"

Scientific supervisor: Prof. ROSEN KOLAROV, MD, PhD

Varna

2023

The dissertation is presented on 180 pages, illustrated with 45 figures, 28 tables and 25 photos. The literature reference includes 264 titles, of which 6 are in Cyrillic and 258 in Latin.

The dissertation paper was discussed and proposed for protection by the Department of Oral Surgery at the Faculty of Medicine of MU-Varna on 29.05.2023. and is directed for public defense before a Scientific Jury in the following composition:

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The public defense of the dissertation will be held on 20.09.2023. from 1.30 p.m.

The materials on protection are available in the library of the Medical University "Prof. Dr. P. Stoyanov" – Varna and in electronic form on the website of MU-Varna

CONTENT

ABBREVIATIONS USED

PRP – platelet-rich plasma

PC – platelet concentrate

OS – oral surgery

SE – surgical extraction

GF – growth factors

WT – wisdom tooth

LTM – lower third molar

DOS – Department of Oral Surgery

OPG – orthopantomogram (X-ray toothshot)

BC - Buffy coat

ECGF - Endothelial growth factor

EGF - Epidermal growth factor

GF - Growth factors

IGF - Insulin like growth factor

IL - Interleukin

L-PRF - Leukocyte- and platelet rich fibrin

PC - Platelet concentrates

PDGF - Platelet-derived growth factor

PPP - Platelet poor plasma

PRF - Platelet rich fibrin

PRP - Platelet rich plasma

RCF - relative centrifugal force

TGF-β-1 - Transforming growth factor beta

INTRODUCTION

The existing problem of tissue healing has plagued surgeons decades ago. Serious obstacles to the normal course of the recovery process remain to this day superponed infection, used foreign bodies in the form of bone or soft tissue grafts, lack of stability in their fixation, e. In the 1950s and 1960s, antibiotics were mainly of the penicillin group, in addition to cephalosporins and lincosamides. Thus, the surgical infection that developed was mainly affected. In the seventies of the twentieth century, the use of various fixing devices – titanium plates and screws, which contribute to increasing the stability of the attachment sites of the allotransplants used, became more and more widespread. In this way, micromovements in the recipient lodge between their own and foreign tissues are reduced, which leads to an increase in cell proliferation and the formation of new blood vessels that have a major role in the normal course of the healing process. In the 1980s, Knighton, Hunt, and Marx defined the leading importance of oxygen, along with macrophage response, in tissue healing. They also conduct research and studies to identify and identify growth factors that support the rehabilitation of damaged tissues. For the first time, in 1990, Knighton introduced into clinical medical practice the use of platelet-isolated growth factors in blood plasma. He, together with collaborators,

proved the contribution of platelet concentrate, called platelet-rich plasma PRP, to stimulate wound healing, mainly bone defects, as well as its anti-inflammatory effect.

Autologous platelet-enriched plasma was first administered in cardiac surgery twenty-five years ago for the purpose of a hemostatic effect. Many directions in general medicine use platelet plasma. In the field of traumatology, it leads to stimulation of cell proliferation and differentiation to restore articular cartilage and connective tissue in arthritis, supports bone healing in defects with the use of auto- or allotransplanant. Plastic and reconstructive surgery uses concentrated platelets to accelerate the healing of soft tissue chronic long-persisting infected or non-wounds. In cosmetic surgery, their use reduces postoperative edema and hematomas, accelerates the healing process after the traditional facelift and blepharoplasty. In neurosurgery, the analgesic effect of platelet plasma is used. For rehydration of the skin and subcutaneous area in the facial area, with anti-aging effect, against hair loss and to reduce cicatrix after excision of skin lesions, it is widely applied in the field of dermatology. Otorhinolaryngology is applied to myringoplasty, tympanoplasty, mastoidoplasty, septo- and rhinoplasty, after adenotone-sylectomies, laryngectomies.

In maxillofacial and cranio-facial surgery, platelet-rich plasma is used in surgical procedures related to reconstructions of the mandible, maxilla, in reconstructions of the middle facial floor, operations on the occasion of congenital slits of the lip, palate and alveolar ridge. In these cases, its placement in the surgical wounds and in the places of bone osteotomies supports the process of bone formation, and shortens the time to reach normal bone maturity.

Since the first years of our century, studies and publications in the foreign press have been documented on the administration of platelet-rich plasma and in the field of oral surgery. Modern dental procedures such as lateral sinus lift, split ridge augmentation, restoration of periodontal pockets, regeneration of post-extraction defects, as well as treatment of severe mucosal losses in the oral ku-hina, increasingly use the actions of PRP. In Bulgaria, presentation of single cases of PRP use began about 10 years ago.

All surgeons are looking for an accelerated and better tissue healing process. The introduction into clinical dental practice of autologous isolated and concentrated from one's own blood platelets, together with the growth factors containing them, under the name platelet-rich plasma (PRP), contributes to stimulating wound healing in the human body and shortens the postoperative period leading to the final anatomical and functional recovery.

There are a number of protocols described in the literature for obtaining platelet-rich plasma. Consensus among the authors exists only with regard to the final concentration

of platelet cells in the resulting clot. While the normal platelet range in the entire blood of healthy individuals ranges from 150,000 to 350,000 platelets/µl, the working definition of platelet concentration is within 1,000,000 platelets/µl of the platelet preparation. In other words, The platelet concentration should reach 3 to 5 times the increase above the baseline.

Marx (2001) proposes a platelet concentration threshold of 1,000,000 platelets/µL as a working definition of PRP, based on scientific evidence regarding the therapeutic effect on bone and soft tissue repair. Rodjeti et al. (2008) studied the relationship between platelet concentration in platelet gel and changes in functional activity of human endothelial cells. The proliferation of endothelial cells, their migration and invasion occurs in a consistent manner. The authors concluded that the level of stimulation for proliferation of endothelial cells has a peak concentration of $1.25x10^6$, and the level for the induction of angiogenesis $1.5x10^6$ platelet/ml. This confirms the fact that platelet concentrates with a value of about 1,000,000 platelets/µL is the working definition of therapeutic enriched blood plasma and rejects criticism of not obtaining the expected optimal PRP results, which may be due to low platelet concentrations.

There are many commercial PRP systems to facilitate the use of platelet-enriched suspensions on an outpatient basis. These systems differ greatly in terms of taking the blood substance, the concentration of platelets depending on the time and method of centrifugation. As a result, suspensions with different levels of platelets and leukocytes are present, which determines the diversity in the concentration of individual growth factors. Differences in platelet and leukocytes concentrations affect the level of growth factors, respectively, have a different clinical effect in medical practice.

The characteristic of platelet enriched plasma yielded by centrifugation technique changes according to the relative centrifugal rotational force, temperature and time. A number of authors believe that the protocol of preparation of two stages (separation and concentration) gives the highest result.

The various protocols have been optimised in terms of a number of variables such as peripheral blood sampling volume, number of centrifugation, rotation time and centrifugal acceleration range. Given the complexity of PRP preparation and the need for quality control for different clinical applications, we believe that standardizing the procedure and reproducing permanent results is crucial.

Despite the differences in protocols, there are several successive stages that consist of

blood collection, initial centrifugation to separate platelets, centrifugation to concentrate platelets and other cellular components, and activation of the resulting sample by adding different types of activators. Amable et al. (2013) studied variations in terms of relative centrifugal force (RCF), temperature, and time to optimize platelet isolation conditions and quantify cytokines and growth factors in PRP before and after platelet activation. All this confronts the clinician with the choice of platelet concentrate obtained by protocol, which will provide the desired healing effect of PRP.

Surgical dentistry is represented by surgical procedures, including both traditional surgical and conventional dental extractions, as well as modern interventions such as the lateral sinus lift, split-ridge cleavage, horizontal and vertical bone augmentation, etc.. They represent a new direction in pre-prosthetic and preimplant surgery, which, together with periodontal surgery, aim to ensure better dental health for patients in shorter terms.

The repair of natural bone and the feeding of bone graft or bone replacement depend on the regeneration of new bone through the mechanisms of cell proliferation and osteoid synthesis (osteogenesis), on the migration of cells into the bone defect (osteoconduction) or on the processes of bone resorption and remodeling (osteoinduction). PRP products, with the high level of growth factors contained in their platelets, promotes and increases the healing of bone wounds. When they are added alone to the surgical wound or together with an autogenic graft, allograft, bone replacement or composite graft, stimulation of bone regeneration is observed.

Platelet concentrates are widely used in surgical interventions, representing the current in the field of oral surgery.

Lateral sinus lift

Sinus lift surgery is a relatively new procedure dating back to the early 1990s, when dental implants were placed in the upper jaw. Its sole purpose is to provide enough bone in the maxilla to support dental implants. The increase should be at least 8 mm, so that if it is possible to obtain an 18 mm vertical size from the ridge of the alveolar ridge to the new elevator level of the maxillofacial cavity.

For this procedure, implantation of autogenous bone is the "gold standard". It allows the formation of trabecular bone with a density of 40% to 60%, depending on various factors – the age of the patient, the presence of systemic concomitant diseases, compromised state of local tissues. Important for the final result in the open sinus lift are also the negative impact of

smoking, taking certain drugs, as well as diseases of the maxillary sinus. The administration of platelet-rich plasma in addition plays an important role in supporting bone regeneration. Another action is to suppress the negative impact of age, compromised general medical health and reduced quality of local tissues in the operated area.

For the needs of sinus lift surgery, about 7 ml of implantation material is needed. This quantity represents a fairly large volume to be obtained by creating a second surgical wound, in most cases from an extraoral donor lodge. Some patients disagree with this surgical procedure, and the techniques of extraoral bone "harvest" are laborious for the surgeon. Therefore, lyophilized and demineralized bone, inorganic bovine bone replacement, hydroxylapatite product and other types of explants and xenotransplants are commonly used in open sinus lift. The density of the trabecular bone obtained in this way is smaller compared to the autogenic graft and varies between 15% and 30%. Adding to this fact the presence of the above aggravating factors – age, concomitant diseases, smoking, decreased immunity, etc., the results in terms of bone regeneration are often unsatisfactory. This requires that when sinus lift procedures use graft other than autogenic, bone regeneration to be stimulated by the application of platelet-rich plasma.

Horizontal and vertical bone augmentation

An increase in the maxillary or mandibular alveolar ridge in order to place dental implants can be realized in horizontal, vertical or only one time in both planes by placing a bone graft. In all cases, however, protection of graft from masticatory forces and/or loading when using temporary dentures is necessary throughout the period of revascularization and cell proliferation.

After application of platelet-rich plasma in these procedures, graft should be protected in the first 3 postoperative weeks. In cases where platelet concentrate is not used, it is vulnerable 6 weeks after its placement.

Depending on the size and shape that are needed for augmentation, the autogenous bone grafts are mainly used – from the branch of the lower jaw, mentum, calvaria and iliac bone. It is characteristic that they undergo a reduction of their initial volume from 25% to 40% (except for Calvaria – 15% - 25%) in the first 6 months. In order to minimize reduction, it is necessary to immobilize them by titanium screws and the addition of PRP.

Odontectomy of wisdom teeth

Surgical extraction of the third molar teeth is a large volume of surgical interventions in the field of oral surgery. It is often associated with two well-known complications. One is alveolar osteitis, known as dry alveolitis, which occurs between 3% and 25% of cases. The other is the reduction of bone regeneration especially in the area of the distal root surface of the second molar, which leads to the formation of a pathological periodontal pocket. Babbush and Mancuso demonstrated for the first time the remarkable ability of platelet-rich plasma to reduce the development of "dry alveol" and increase bone regeneration in the postextration alveolus of the third of the masticatory teeth molar group.

The use of platelet concentrate in these clinical cases is extremely necessary in women taking the contraceptive pill, smokers, in patients over 30 years of age and those who report the development of frequent pericoronaritis. Patients on steroid therapy or ongoing chemotherapy, patients with prior irradiation in the area of wisdom teeth, diabetics, are also indicated for use of PRP in the postoperative wound.

Treatment of periodontal defects

Periodontal damage is the result of disease processes initiated by subgingival pathogenic microorganisms. Their toxins provoke constant inflammation of the gum tissue around the teeth, which progresses, destroys epithelial and connective tissue attachment and leads to resorption of the underlying bone. Untreated, the inflammation causes advanced bone loss, resulting in loosening of the teeth and their loss.

For the restoration of periodontal defects, it is necessary to fill them with articular autogenous bone or various types of bone substitutes, which will lead to bone regeneration.

The application of platelet-rich plasma as a layer above the surface of the graft placed, causes stimulation of bone repair. In this route of administration, platelet-secreted growth factors come into direct contact with the overlying mucoperiosteal flap and also support the regeneration of gingival tissues.

The problem of bone healing after surgical interventions in the oral cavity, in particular surgical extraction of a lower third molar tooth, requires increased attention on the part of both the patient and the oral surgeon. Here, the addition of PRP, extracted according to certain protocols, would provide prevention of possible inflammation and stimulation of tissue healing.

The literature review shows that platelet-rich plasma can be applied in many areas of general and dental medicine. Autologous platelet concentrate, endo- or exogenously activated, exerts its stimulating effect in the healing processes in the human body; has an impact and improves the repair processes of both soft and hard bone tissues, prevents the appearance of local postoperative complications Along with these data, it becomes clear that there is a lack of standardization in terms of preparation protocols, classifications that are used in practice, as well as the parameters of the obtained enriched plasma products. The choice of a particular product falls to the clinician, depending on the specific needs of the manifested pathology.

The above facts show in some clinical cases the protracted course of the bone and soft tissue healing process. The oral cavity is one of the areas where surgical dental interventions, including post-extraction wounds, especially on the lower third molar teeth, require increased attention in terms of tissue repair. In healthy organisms, osteogenesis and maturation of the cicatrix lasts from 6 months to 1 year after surgery. In compromised patients with concomitant diseases, these processes are protracted and the final recovery occurs much later. This means delayed induction of damaged tissue or organ, which does not meet the modern needs of today's patient. However, the application of platelet concentrate stimulates and accelerates tissue recovery, leads to prevention of possible complications, respectively shortens the time to achieve functional fitness of damaged tissues. This methodology ensures that the necessary quality of life in the dental patient, which is associated with the restoration of the functions of the oral cavity after surgery, is achieved in a timely manner.

Conclusions:

1. The problem of regeneration of the jaw bones and surrounding soft tissues affects much of the diverse pathology subject to oral surgery, requiring surgical intervention. It is directly dependent on the fastest and most complete recovery of patients who have undergone surgical innervation in the oral cavity and their return to their routine daily activities.
2. Every oral and maxillofacial surgeon is expected to be familiar with the process of normal bone repair and different techniques and methods for its improvement, including the algorithm for the application of different types of platelet concentrates.

3. Modern oral surgery is represented by preprotetic, periodontal, preimplant procedures and dental implantology, providing better dental health for shorter periods of time to the patient by applying biological substrates, including platelet concentrates.
4. The application of platelet-rich plasma is a globally accepted modern practice that complements the healing methods used so far in the presence of bone and soft tissue defects, both in general medicine and in oral surgery.
5. Knowledge of the complications of oral healing processes in healthy patients and those with concomitant diseases, their expected frequency and risk factors for their occurrence, helps to minimize them by using PC.
6. The addition of platelet concentrate in bone and soft tissue defects leads to stimulation of tissue regeneration in clinically healthy patients and prevents the development of complications in at-risk patients.
7. The effect of PC is due to the 7 growth factors contained in platelets, which are activated and released during the process of blood clotting. Their concentration in plasma, in comparison with the total blood, is increased by 3 to 5 times.

The literary reference we made provoked us to conduct a scientific study on the most common surgical intervention in the oral cavity, subject to dental surgery and our daily activity - odontectomy of the third lower molar. The healing process is delicate, with a protracted course sometimes with an increased incidence of complications. It can be stimulated by applying TK. In this work we use the established and nationally standardized four new protocols for obtaining PRP products by Ivanova and collaborators (2021), which, after quantitative and qualitative analysis in their scientific development, prove the effectiveness in vivo we are looking for. Applying the methodology with PRP obtained by these new protocols in the post-extraction defects of removed inferior wisdom teeth, we achieved the goal sought – accelerated qualitative restoration of the routine daily rhythm of our patients.

PURPOSE

To present clinical results in postextraction sections after surgical removal of lower third molar teeth, using new, nationally standardized four protocols for extraction of platelet concentrates from whole fresh autologous blood.

TASKS

The following tasks are set to accomplish this objective:

1. To make a summarized analysis of the objective and subjective symptoms associated with the healing process after surgical removal of lower third molar teeth.
2. To analyze the early effects of platelet-rich plasma in postextraction wounds after surgical odontectomy.
3. To evaluate and discuss the influence of platelet concentrate in post-extraction dental defects on bone healing.
4. Based on the 4 new platelet-rich plasma protocols used, a comparison based on postoperative clinical results should be made.
5. To determine if there is a protocol of the new generation with absolute biological significance of growth platelet factors on early and later postoperative clinical symptoms after surgical extraction of third lower molar teeth.
6. Based on the conducted study, an objective assessment of the therapeutic value of the methodology with the application of platelet concentrate in post-extraction dental alveoles should be given.
7. To create a diagnostic and therapeutic algorithm among servicemen in which the use of PC after extraction of lower third molar teeth to ensure stimulation and acceleration of healing of the operative wound and return to the daily workload in a shorter time.

Working hypothesis:

The standardized for our country four new protocols for extraction of autologous platelet concentrates, with the quantitative fluctuations of the blood components in them, have different degrees of impact on the healing process in the post-extraction regions after odontoectomy of lower third molars. This brings sufficient clarity to the clinician about the need to apply PRP and what method to obtain it, according to the clinical results sought.

MATERIALS AND METHODS

Materials

To achieve the goal - to present clinical results in post-extraction areas after surgical removal of lower third molar teeth, when using platelet concentrates obtained by new protocols by extraction from whole fresh autologous blood, blood from healthy donors is used – patients requiring surgical extraction of lower third molar teeth – people with their informed consent and knowledge. The scientific study is with the permission of the Commission Research Ethics at MU-Varna (125/26.01.2023).

The study is combined. Retrospectively, it covers 1278 patients who have passed through the hospital of the Department of Oral Surgery at the Military Medical Academy - Sofia. Sofia for the period: January 2020 – March 2023, on which surgical procedures in the oral cavity were performed.

From the conducted retrospection and analysis of the "History of the disease" of the patient, it was found that the largest share of the operated patients in our ward falls on the surgically removed lower third molar teeth. The remaining surgical activity is distributed between the extraction of upper third molar teeth, cystectomies, removal by bone trepanation of other teeth and tooth roots, surgical treatment of periodontal diseases, as well as operations on orthodontic, prosthetic and preimplantation indications.

Prospectively, the present study involved a total of 150 patients with extracted inferior wisdom teeth for the period October 2022. – March 2023, as a result of various causes – follicular or periodontal tooth cyst, keratocyst, exacerbated periapical process or complicated caries.

All patients participating in the study are servicemen from different units in the system of the Ministry of Defense, treated in the inpatient of our Department of Oral Surgery.

The participants are divided into five groups of 30 people. In the first group, PC is not used, while in the other four groups an allocation is made regarding the protocol of extraction of PC, which is applied in post-extraction bone defects obtained by four different new methods.

Methods

To develop the topic and fulfill the goal and tasks we set ourselves, we used the following methods.

1. Diagnostic methods

The methods of general medicine are used, applying specific to the field. The aim of the clinical examination is to make an accurate diagnosis, to select the best surgical method for the patient and to develop a strategy for subsequent monitoring.

1.1. History

The anamnesis aims to provide a collection of information obtained during the questioning of the patient and to clarify not only the problems related to his specific complaints (anamnesis morbi), but also to identify possible concomitant diseases that may have a negative impact on surgery, medication, past surgical interventions, allergic episodes.

1.2. Physical examination

1.2.1. Status present tense generalis

1.2.2. Status present tense specificans

a) Extraoral examination – attention is paid to bumps on the neck and face; the presence or absence of asymmetry is detected; Palpation is performed in order to establish soreness, dimensions, boundaries, surface, consistency, condition of the surrounding tissues, temperature, fluctuation.

b) Intraoral examination – the volume of opening and closing of the mouth is determined first. It normally opens from 4 to 6 cm. Restrictions in opening are indicated by three-smus, I degree (opens 3 cm), trismus II degree (ovarya up to 2 cm) and trismus III degree (opens less than 1 cm). They are associated with trauma or inflammation of the teeth and/or jaws. The measurement is carried out at the cutting edges between the central incisors of the jaws. The following is a view of the dentition and occluso—articulation ratios. The dentition is examined for the type of bite (pricus) and the condition of the individual teeth (number, color, position in the tooth arch, the presence of tartar, caries). We distinguish physiological (orthognatic) and

pathological (deep, open, tubular, progeny, prognostic) pricus. With pathological access, there are disorders in the functions of chewing and speech, as well as changes in appearance. The study continues with a comprehensive view and palpation of the mucous membrane of the vestibulum, gingiva, oral floor, cheeks, palate, tongue. Then percussion – horizontal or vertical of individual teeth. The pressure test with two instruments (dental probe and mirror) is applied to establish dental mobility. It is defined in three degrees: I degree – mobility in mediodistic or vestibulooral directions less than 1 mm; II degree – mobility in mediodistic or vestibulooral directions greater than 1 mm; III degree – mobility in horizontal and vertical direction. In the diagnosis of dental diseases, probing with a dental probe is also applied, looking for a defect in the hard tooth tissues or establishing the magnitude of the periodontal pockets.

Making a precise clinical diagnosis, which is inherently preliminary helps, to determine by the further healing algorithm. In our case, the determination of local status is associated with the establishment of the etiological factor that led to surgery on the third lower molar tooth.

1.3. Paraclinical tests

1.3.1. Laboratory blood parameters

In diseases of the teeth, peridental tissues and jaw bones, changes in hematological indicators may occur. For the needs of our study, they need to be within the limits of their reference values. These are the amount of formed elements of the blood – platelets (150 – 450 x 10^{9} / l), erythrocytes (4.2 – 6.2 x 1012 / l), leukocytes (3.5 – 10.5 x 109 / l) with differential counting, ESR (11-20 mm/h), hemoglobin (120-160 g/l), bleeding time (2-5 min) and clotting (5–10 min), fibrinogen (2-4 g/l), INR (International Normal Ratio – 0.9 – 1.2), blood sugar (2.75 – 5.55 mmol/l).

Of utmost importance are the normal values of platelets studied in fresh whole blood, the effect of which in terms of acceleration of healing processes is the basis of our clinical trials. Another important indicator is the value of fibrinogen, the increase of which can be a sign of peripheral blood vessel disease and an increased risk of venous thrombosis, which is unfavorable for our research. Of particular importance to us as oral surgeons performing the study is also the value of the prothrombin index (INR). Its norm is the assessment of the external coagulation pathway, especially with treatment with indirect anticoagulants. It is necessary that it is within its reference range, which is a sign of a normal course of blood

clotting processes in an organism. It is the initial, uncomplicated and immediate hemostasis that is a factor for the manifestation of the tissue regeneration effect of the platelet concentrate inserted in the surgical wounds sought in this dissertation.

1.3.2. X-ray methods

In everyday practice, for the diagnosis of diseases of the teeth and peridental tissues, segmental target radiography and panoramic radiography are most often applied. Less often the radiography in a bite is used.

In the diagnosis of diseases of the jaw bones, a review radiography is used – full face and profile and computed tomography (CT) without contrast amplification, which gives a three-dimensional real image in millimeters of the spread of the pathological process in the three planes.

The patients in our study were given pre- and postoperative orthopantomograms, at the 3rd and 6th postoperative months. Their aim is to establish the clinical pathology, the presence of inferior wisdom requiring surgical extraction – the subject of this work, as well as to analyze the postoperative results related to bone recovery in post-extraction wounds in the observed five study groups according to the application of PC.

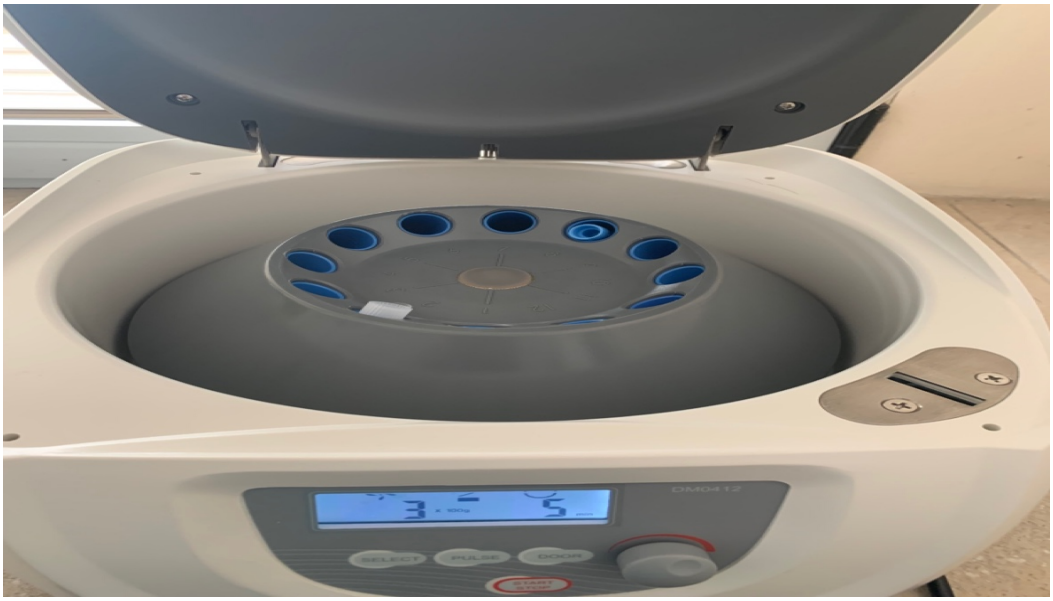
2. Methods of obtaining platelet-rich plasma

For the practical implementation of the tasks set in the dissertation, the following laboratory consumables are used: the first – vacutainer 8 ml with separating biocompatible inert gel (cycloaliphatic polymer gel), composed of a mixture of polymers for plasma separation (eliminating red blood cells), the second type – monovete 8 ml (S-Monovette, Sarstedt) without the presence of separating gel. Each vacutainer and monovete contain an anticoagulant 3.2% trisodium citrate. Symmetrical location of Test tubes and weight calibration are a mandatory element before centrifugation.

Venous blood is drawn from each patient in a comfortable position, subject to sterility conditions and good laboratory practice. Venipuncture is performed with a 22G sized needle to avoid premature platelet activation. The amount of blood withdrawn by each participant is about 10ml. It is distributed in vacutainers and monovettes with anticoagulant trisodium citrate for each patient in the four experimental groups and subjected to treatment of four experimental Method. Each blood unit was processed within up to 2 hours of its withdrawal. SCILOGEX small bench laboratory centrifuge WT 0412 was used for the purposes of scientific

investigation, the rotation angle of the samples in the rotor being 32° (Picture 1.a,b). It has a socket for 12 ordinary or vacuum tubes (7/15 ml), angle rotor, pulse centrifugation function, speed control and centrifuge rotation force (RCF), timer in minutes, protective shutdown in case of disturbed balance, start function – melt mode and protective anti-corrosive coating against chemicals and mechanical damage. Symmetrical arrangement of the test tubes and calibration of the weight are a mandatory element before centrifugation.

Effective separation and the required high concentration of vital bioactive platelets are the product of gravitational centrifugal forces for a certain time, usually measured in minutes, when the centrifuge operates with the whole blood-filled tubes placed in it. Different methods are used regarding the number of times of blood centrifugation, as well as different times in minutes and different spin speeds. We, based on the development of Ivanova et al., used and introduced in our clinical practice the following new protocols for obtaining platelet-rich plasma.



Picture 1a,b. Laboratory working centrifuge

The first protocol of blood treatment involves a single centrifugation of monovetes without gel, at room temperature with a centrifuge force of 1500 ×g for 10 minutes. The second method is under the same conditions of single centrifugation with a centrifuge force of 1500 ×g for 10 min, but using a vacutainer with a separating gel. The third protocol involves a process of double centrifugation of monovetes without gel. The first rotation (separative) has a centrifugal force of 1150 ×g for 10 minutes, the second – concentration rotation with centrifuge force 350xg for 5 min. The fourth method treats 8 ml of blood in a vacutainer with separating gel under conditions as in method three – first rotation with centrifugal force 1150xg for 10 minutes and second concentration rotation with centrifugal force 350xg for 5 min (Table 1).

Table 1. Protocols for obtaining PRP by four different methods (Ivanova et al.)

Methods		**Separating rotation**		**Concentrating rotation**	
		Centrifuge force (g)	Time (min)	Centrifuge force (g)	Time (min)
1	without gel	1500	10		

2	with gel	1500	10		
3	without gel	1150	10	350	5
4	with gel	1150	10	350	5

In all four methods, the blood is centrifuged at room temperature 20-22°C. The working steps for the extraction of platelet-enriched plasma are assigned to the so-called. "buffy coat" technique, namely the first rotation has high values of RCF (Relative centrifugal force) – relative centrifugal force. After the first separating rotation, in all four methods, three visible layers are formed: the lowerest, rich in red blood cells; medium, rich in platelets and white blood cells – the so-called. "buffy coat"; platelet poor plasma (PPP) (Fig. 1).

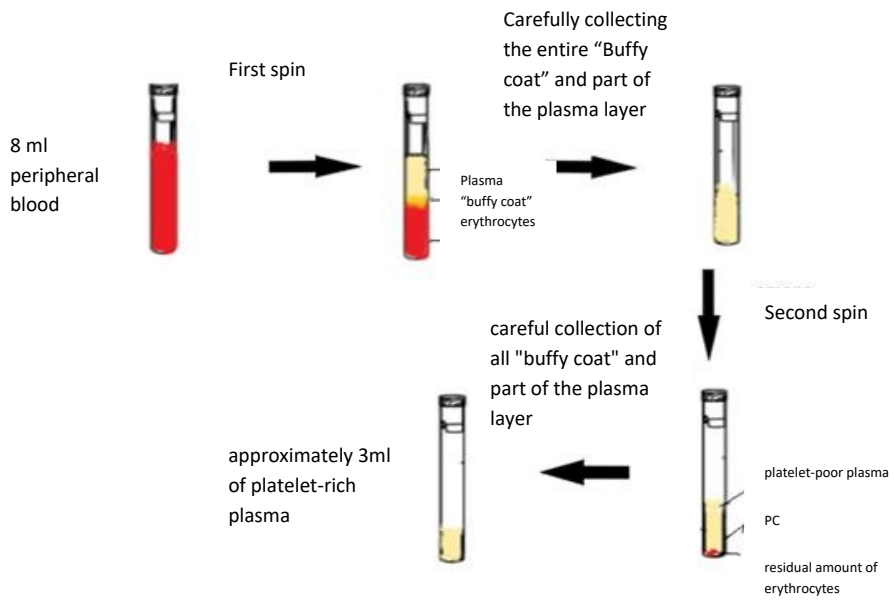


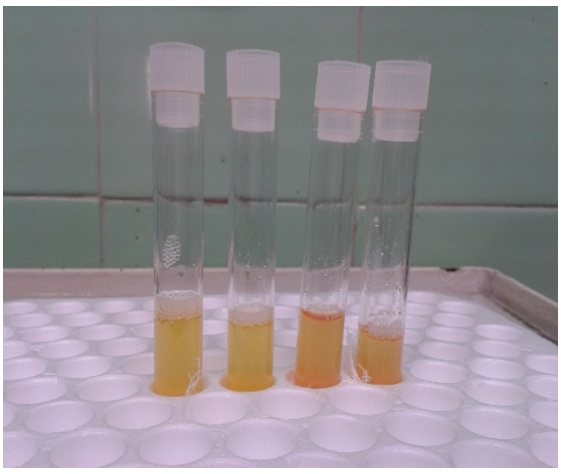
Fig. 1. Schematic representation of the stages in PRP production using the "Buffy coat" technique (Ivanova et al.)

In method 1 and 2 – single centrifugation, removal of part of the surface platelet poor layer (PPP). At Protocol 1, we carefully pipette 3 ml of the remaining above erythrocytes product for subsequent activation and administration to experimental group 1.

In Method 2, some of the remaining plasma (PPP) in the tube is aspirated and returned to "peel off" the adhering layer of cells on the separating gel. After the "washing" of the adherent cells from the gel, we aspirate the product (also with a volume of 3 ml) for its application to patients of experimental group 2, after prior exogenous activation.

In Protocol 3 and 4, the middle layer and most of the platelet-poor plasma (PPP) were carefully pipetted and transferred to a new tube already without the presence of an anticoagulant for subsequent second concentration centrifugation. After the second centrifugation, a small number of erythrocytes precipitated in the lower part of the tube, platelet concentrate above it and again a platelet-poor plasma layer at the top is detected. We carefully aspirate part of the surface layer – platelet-poor plasma, which is eliminated. The remaining amount of plasma (3 ml volume) is evacuated by pipette in order to capture the platelet concentrate as much as possible, the resulting product being administered, post-extractively, to patients in experimental group 3 and 4.

As a final product in each vacutainer and monovette, after each of the four protocols there is a 3 ml PRP volume that is transferred for subsequent exogenous activation (Picture 2).

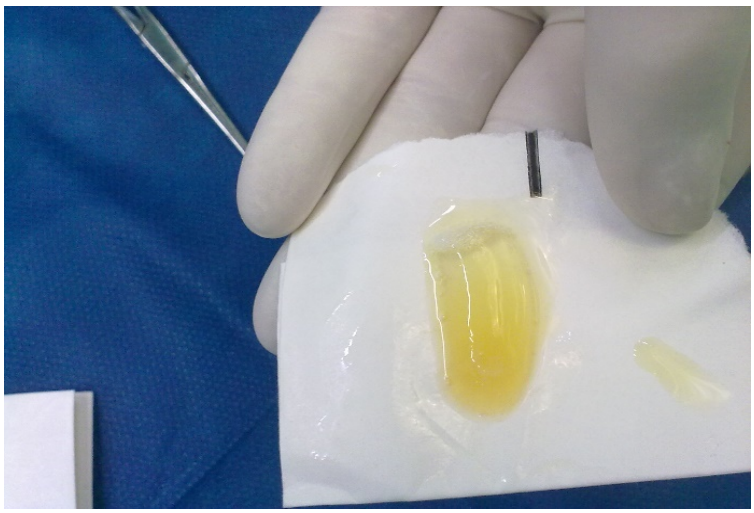


Picture 2. Uncoagulated PRP volume for subsequent exogenous activation

The platelet-rich plasma thus extracted is in an uncoagulated state and remains in such a state until the process of blood clotting is initiated, in which platelets secrete their granules with growth factors and thus stimulate healing processes in the operative field. Bioactivity and viability of platelet concentrate were maintained for 8 h at room temperature. This makes it possible to use it in clinical cases requiring prolonged surgical interventions or those in which the surgical procedure must be postponed by several hours. It is recommended that the PRP remain anticoagulated until it is placed in the surgical wound. It is not practiced to put it in the refrigerator or freeze it, because this causes the destruction of the platelet membrane and compromises the action of growth factors.

For an exogenous activator we choose calcium gluconate (Calcium gluconicum amp. 10%). In a test tube without anticoagulant we put the extracted plasma, which we mix with 1ml of calcium gluconate, that is, in a ratio of 3:1.

Homogenize the mixture and leave at rest at room temperature for 20 minutes. When applying the activated platelet concentrate after the extraction of one tooth, we found that we need about 4 milliliters of quantity, which we extract through the above protocols of 8 ml autologous venous whole blood. In the course of our study, we found that the activated biological product thus obtained has an extremely suitable gel-like consistency for application in the post-extraction wounds of patients (photo3, 4).



Picture3. Final biological product – PC, post-extraction dental alveoli has been applied

Cn.4. Intraoperative application of PC after extraction of lower third molar tooth

3. Surgical methods

Extraction of impacted or semi-impacted lower third molars

In the present study, we applied conventional surgical extraction of lower third molar teeth, one of the main surgical methods in the field of oral surgery.

This surgical procedure, regardless of the etiological factors that led to its need, is usually associated with a longer and more aggravated healing process, as well as various complications (broken jaw, inflammatory, etc..) than the extraction of other teeth.

The impacted or semi-impacted lower wisdom tooth is covered with a different amount of bone. He, depending on the position of its long axis relative to a second lower molar, is medial, distal, bucal, horizontal, lingual, vertical or reversed (Winter,G.B.,1926; Archer, W.H., 1975; Kruger, E., 1984). Depending on the depth of retention and the distance between the second molar and the jaw branch, there are different classifications – Pell – Gregory, Archer – Kruger. In all these cases, surgical extraction is necessary, under local or general anesthesia. It begins with the removal of a triangular or envelope-shaped muco-periosteal flap, with a dispatorium opening the crown and removing the bone above and / or around it. This is achieved with a consuming bone milling machine or with a round bur the size of the bone window is marked,

with a fissure bur the perforation holes are joined and the crown bone is removed. Once it is released, proceed to the extraction of the tooth.

When the lower wisdom tooth is located deep vertically, medio-angular, medio-distal or horizontal, extraction is performed by flying and removing the cranial and vestibular bone located around the tooth. In case of medio-angular, disto-angular or horizontal location of the lower wisdom tooth and dense contact of the chewing surface of its crown with the roots and neck of the second molar, in order not to remove unnecessarily strong bone, cut and separate the crown from the roots, removing them separately. Next is the treatment of the bone wound – curettage, smoothing of uneven bone edges, adaptation and suturing of the muco-periosteal flap. In the vast majority of clinical cases, wisdom teeth are associated with a follicular or periodontal cyst that is extirpated before suturing the wound.

Despite the improvement of surgical technique over time, the introduction of cutting-edge ultrasound and other new generations of bone drills, despite the use of systemic antibiotics administered even intraalvellar, surgery of the third lower molar is associated with two main well-known postoperative complications. The first is alveolar osteitis, often known as dry alveolitis, whose incidence rate reaches 18%. In second place is the reduced bone regeneration in the area of the lower wisdom tooth, especially in close proximity to the second lower molar, which leads to food retention and the formation of a pathological periodontal pocket. Numerous studies (Babbush, Mancuso et all) over the past decade have demonstrated the significant influence of PRP placed intra-alveolar, which reduces the incidence of dry alveolitis and stimulates bone recovery. Thus, the main complications of surgery of the third lower molar are reduced, with the prevention of pathological conditions associated with the distal surface of the second molar.

Alveolar osteoitis is a primary contamination of the blood coagulum from which bacteria pass through and colonize the bone walls of the alveolus. Such is the mechanism of provoking inflammation, leading to reduced bone regeneration in the alveolus of the lower wisdom tooth. Inflammation explains postoperative pain, and blood clot breakdown and bacterial colonization explains the bad breath and taste associated with the condition. What distinguishes alveolar osteitis from true osteomyelitis is the fact that bacteria do not invade the bone marrow spaces of the lower jaw. In rare cases, especially in immunocompromised patients or adult patients with a number of systemic concomitant diseases, alveolar osteitis progresses into various states of osteomyelitis.

When alveolar osteositis develops and persists, the focus of the patient and the surgeon is dealing with the pain symptom and preventing the development of osteomyelitis. Local lavages of the post-extraction alveolus, application of various anti-inflammatory and antibacterial drugs in it, and sometimes the appointment of systemic antibiotics, leads to the elimination of complications within a week. The wound is filled with granulation tissue, the epithelium from the gingiva adjacent migrates along the surface and closes the post-extraction defect. Bone is slowly regenerated by migration through granulation tissue (osteoconduction) of osteocytes from the bone walls of the alveolus. However, in the extraction wounds complicated and developed osteitis, a reduction of bone height and the absence of bone on the back surface of the distal root of the second molar occurs. The consequences are caries of the root and intractable periodontitis. The reduced amount of bone in the sage alveolus is a direct result of the loss of the blood clot and growth factors secreted by platelets in it, as well as the loss of fibrin-fibronectin-vitronectin adhesion molecules that normally stimulate osteoconduction through osteoprogenitor cells from the bone walls.

There is a number of scientifically documented evidence of a significant reduction in clinical symptoms in dry alveol, when treating the alveola with platelet-rich plasma up to four times. Our working hypothesis aims to confirm these data and to prevent patients requiring surgery of the third lower molar from developing alveolar osteostheitis and damage to the second lower molar.

The mechanism by which PRP protects against alveolar osteitis and leads to an increase in bone regeneration is discussed at this time. It is borne in mind that the ratio of formed elements in the ordinary blood coagulum and platelet clot differ significantly. The PRP-clot is represented by 94% platelets, 5% red blood cells (erythrocytes) and 1% white blood cells (leukocytes), while the normal blood clot contains 94% erythrocytes, 1% leukocytes and only 6% platelets.

On the other hand, the PRP clot has pH values of 6.5-6.7 against the pH 7.4 of the normal clot, the effect of which is inhibition of bacterial growth. The lower pH values in PRP result from the anticoagulant in vacutainers (sodium citrate). In addition, the content of white blood cells having an anti-inflammatory effect, especially depending on the PC yield protocol, is also greater in the PRP-clot. Administration of PRP intraalveolar also leads to faster development of granulation tissue with early formation of blood capillaries inhibiting bacterial growth. The explanation is the induction of macrophages and neutrophils, which are in increased quantity

in the PRP-clot, creating a richer oxygen environment in the alveola, inhibiting the development of anerobic microorganisms. It is believed that the main fact that explains the healing effect of PRP is the increased concentration of platelets, the growth factors released by them and the cellular adhesion molecules.

In summary, the oral surgeon should refine and approach individually in each clinical case requiring surgery of the third lower molar regarding the administration of platelet concentrate intraalveolar. It reduces the risk of developing alveolar osteitis in smokers, women on hormone contraception, patients over 30 years of age with a history of frequent peri- and paracoronitis (soft tissue inflammation with difficulty in the breakthrough of inferior wisdom tooth), patients on corticosteroid therapy or having undergone chemotherapy and/or radiotherapy in the oral cavity, patients with type I and type II diabetes, as well as patients with impacted lower third molar teeth, in whom greater traumatism than usual is assumed. We include another group with testimony for the application of PC and these are servicemen whose positions require a slight postoperative period, without complications, for a timely return to the work process.

4. Methods for assessing the therapeutic ouPCome

4.1 Radiological assessment

Imaging methods – mainly orthopantomogram (panoramic dental picture of both jaws) taken on the 3rd and 6th postoperative months were used to assess the therapeutic outcome of the application of autologous platelet-rich plasma in bone wounds. With the help of panoramic radiographs from Digital Transtomograph ProMax – Planmeca (64,0kV 7,0mA 15,8s 79,1mGy/cm^2) were objectified and compared the clinical results obtained after the application of platelet concentrates as an adjunct to the standard surgical treatment obtained according to four standardized new protocols for the extraction of PRP in order to stimulate healing processes in the oral cavity, prevent the discussed complications and optimal bone recovery. We used a subjective visual assessment concerning the regenerated bone structure by species – dense homogeneous structure, heterogeneous structure, bone with few trabeculae. In a small number of patients, we used the data from the CT test – on the density of the restored bone postoperatively, after the application of PRP, compared to the cases in which such was not used. The reason for only a few CT scans made with a Siemens Somatom III generation device is the high financial value of the study, which not all patients included in this experiment could afford, and it is not reimbursed by the health insurance. The use mainly of X-ray diagnostics postoperatively in front of CT - the results for establishing the effect of PRP action is also due to the following facts. When the bone matures, when it has recovered quantitatively after

surgery and only the mineralization processes continue, the increase in its mineral composition is associated with the accumulation of adipose tissue between the bone slats. It is less dense than the other soft tissues and in the Hounsfield scale is placed below the 0th occupying negative values. The spongious bone of CT is estimated at about 130 Hounsfield units (HU), the compact at over 250 HU, and the adipose tissue at about 65 HU. Thus, due to the above, the density of mature bone can be visualized to a lesser extent compared to a fresh surgical bone wound filled with young osteoid tissue. Therefore, the postoperative assessment of the bone regeneration stimulating effect of platelet concentrate by the methods of CT – the study is not accepted with one hundred percent confidence.

4.2. Questionnaire method

We also analyzed the results with the help of questionnaires that patients filled in a week and 3 months after the surgical procedures, giving them detailed explanations and explanations regarding the answers to the questions in them.

4.3. Objective assessment by an oral surgeon of Status lokalis intraoralis postoperativa

The degree of restoration of soft tissues around the teeth and jaws in the operative field and their objective condition was also assessed by the Landry Healing Index, as well as the presence of post-extraction complications according to the Cheung criteria. This is realized during the control examination by oral surgeons one week after the surgical intervention and added as a method for determining the therapeutic effect of the presented biological substrate (PRP) in this dissertation.

4.4 Instrumental studies

With the help of a periodontal tooth probe, we made instrumental measurement at the 3rd and 6th postoperative months in all study groups, the analyzed criteria being sensitivity on the distal root surface of the second lower molar, the presence of a periodontal pocket at the distal root of the 7th tooth, as well as food retention due to the possible pathological condition of the tissues in the area of the removed inferior wisdom tooth.

5. Statistical methods

The data were entered and processed with the SPSS 16.0.1 statistical package. When performing the statistical analyses for a critical level of significance, 0.05 was adopted.

Methods used:

One-dimensional and two-dimensional distributions with calculation of number and relative share for category variables, and for quantitative - determination of arithmetic, median, fashion, standard deviation and 95% confidence interval.

Hi square analysis.

Fischer's exact-test to assess the relationship between category variables.

t-test to determine whether there are significant differences in two mean of two independent samples.

Kolmogorov-Smirnov test to determine whether the distributions of quantitative variables differ from the normal distribution.

For the comparison between the results, Wilcoxon tests (in three categories of the compared variable) and McNemar (in two categories) were applied.

The graphical images of the studied variables are presented in the form of histograms and sector charts (bar chart, pie chart).

STUDY DESIGN

The current study is a randomized, controlled and reported trial with parallel groups of patients. From the patients who have passed through our ward for the period October. 2022 – March 2023 we selected 150 servicemen indicated for surgery on the third lower molar, regardless of the etiological factor. They were divided into five groups of 30 patients – one control and four experimental. Patients in each of the individual four experimental groups were administered PRP, in the postextraction defect obtained by four different protocols for the extraction of platelet concentrate (Ivanova et al.): experimental group undergoing surgical extraction of a third lower molar and administered PC obtained by method 1; experimental group with surgical extraction of third lower molar and administered PC obtained by method 2; experimental group with surgical extraction of a third lower molar and administered PC obtained by method 3; experimental group with surgical extraction of a third lower molar and administered PC obtained by method 4. To a total of 120 patients, we applied autologous, platelet-rich plasma in addition to their surgical treatment in order to fulfill our dissertation goal.

In the selection of patients, we formed criteria and conditions for inclusion and exclusion from the study that operated patients had to meet. These are the requirements for participation in the study:

- Healthy men and women aged 18 years and over
- Patients with demonstrable need for surgical removal of inferior wisdom teeth who will undergo local or general anesthesia

Servicemen who were willing to cooperate with us and met the requirements related to the study protocol were provided with the appropriate informed consent in writing, after a detailed explanation by our medical team.

Patients meeting one or more of the following criteria were not admitted to the study:

- Concomitant disease – platelet dysfunction syndrome or critical thrombocytopenia
- Concomitant treatment with nonsteroidal anti-inflammatory drugs, including aspirin, antibiotics, systemic corticosteroids, anticoagulants or immunosuppressive agents
- Diabetes mellitus
- Cardiovascular disease, including a history of rheumatic fever or other conditions requiring antibiotic prophylaxis
- Neoplasia or malignant haematological diseases
- Kidney, liver or endocrine diseases
- Metabolic bone disease such as osteomalacia, hypocalcaemia or hypercalcaemia
- Pregnancy and breast-feeding
- Alcohol intake over 50 ml / day; smoking – over 10 cigarettes, daily
- Participation in another survey

The participants in our 5 target groups of patients were initially examined in the diagnostic and consultative office of the Department of Oral Surgery at the Military Medical Academy,

Sofia. They were given information regarding the purpose of the survey and given sufficient time to assess their willingness to participate. They were then adopted planned in the DOS.

Preoperatively, radiographic inspection is made, expressed in the need for available panoramic or targeted, segmental radiographs. They determined the cause of the applied surgical method, for example: complicated caries, localized or generalized periodontitis, peri- and paracoronaritis, orthodontic cause, endodontic insufficiency, fractured tooth or tooth root, tooth in malposition, teeth implanted in the jaws, teeth with periapical pathology.

Data were also collected for each patient, including gender, age, smoking (non-smoker, moderate smoker – up to 10 cigarettes per day, heavy smoker – more than 10 cigarettes per day), alcohol consumption in units, assuming for one unit the equivalent of 10 ml of alcohol (sober, moderate drinking up to 5 units of alcohol and alcoholic with an intake of more than 5 units). Regarding cigarettes and alcohol, we selected patients falling into the first two groups.

Immediately before the operative intervention, the autologous platelet-rich plasma was prepared by our team for the patients in the four experimental groups. It was applied to bone wounds in the final stage of operations, before suturing the muco-periosteal flaps.

The extraction of PRP is obtained by separation and concentration of platelets from autologous whole fresh blood. This process starts with aseptic and minimally traumatic phlebotomy technique usually on the antecubital vein, from where about 10 ml of blood is drawn. The amount of blood taken is determined depending on the expected estimated volume of the bone wound that will occur during surgery. It is important to note that the process of taking blood and obtaining platelet concentrate precedes surgery. It is performed immediately before the patient is inserted into the operating room or after placing it on the operating table, before the start of the preparation of the surgical field associated with facial skin treatment and disinfection of the oral cavity.

In the majority of our surgical procedures for the purpose of this study, we used local infiltration conduction or terminal anesthesia. In some of the clinical cases, patients underwent general endotracheal anesthesia. In order to avoid the entry into the general circulating blood of local and/or general anesthetics that would affect the desired action of platelets, as well as in order not to activate the platelet function and coagulation system by the operation itself, the withdrawal of all the venous blood precedes all this.

As mentioned, various protocols exist for the extraction of PCs. For the purpose of this work, to study the clinical effect of PRP on the healing process after SE of LTM, we used PC obtained by four new PRP extraction protocols, presented by Ivanova et al. The first protocol of blood treatment involves a single centrifugation of monovetes without gel, at room temperature with a centrifuge force of 1500 ×g for 10 minutes. The second method is under the same conditions of single centrifugation with a centrifuge force of 1500xg for 10 min, but using a separating gel vacutainer. The third protocol involves a process of double centrifugation of monovetes without gel. The first rotation (separator) has a centrifugal force of 1150 x for 10 minutes, the second – concentration rotation with a centrifuge force of 350 ×g for 5 minutes. The fourth method treats 8 ml of blood in a vacutainer with a separating gel under conditions as in Method Three – first rotation with centrifugal force 1150 ×g for 10 minutes and second concentration rotation with centrifugal force 350 ×g for 5 minutes. The blood containers we centrifuged in a small bench laboratory centrifuge WT 0412 of SCILOGEX (detailed in chapter Methods for the preparation of PRP). Finally, we obtained an exogenously coagulated blood substrate with a gel-like consistency and pale yellow color, which we very easily applied in the bone wounds of the patients of the four experimental groups, after removal of the lower wisdom teeth. In the control group of patients we did not add PRP and the operations ended with curettage, bone hemostasis with revision and soft tissue suture of the wounds. All patients were prescribed analgesic and anti-inflammatory therapy per os - for 3 - 5 days.

A week after the operation, we conducted the first check-up. After a detailed explanation on our part, all patients in the control and experimental groups were provided with an anonymous questionnaire, which they filled out. For the purposes of this study, the questions in it were related to health, assessed by the perception of recovery of the patient after the surgical procedure, with or without platelet plasma, in four main areas: pain, oral function, general activity and other symptoms. The severity of pain is associated with the consumption of analgesics. Oral function is concerned with the ability to eat food, with swallowing, opening the mouth and speech speech. A change in overall activity was sought in terms of the ability to perform daily routines – work, sleeping, self-care. Other symptoms include the presence of edema, bruising, bleeding, bad taste or halitosis.

The soft tissue condition in the operative field was also assessed using the treatment index of Landry et al., showing any deviations from the normal course of the early healing process (Table 2). It includes the degree of color of the gingiva, epithelialization of the corolla edges, bleeding on palpation, granulation and suppuration.

Tab. 2. Criteria for a soft tissue healing process

Treatment index	Criteria
Very bad 1	Gingiva color: more than 50% red Palpation response: bleeding Granulation tissue: there are Surgical wound edges: non-epithelialized, with loss of epithelium Suppuration: there
Bad 2	Gingiva color: more than 50% red Palpation response: bleeding Granulation tissue: there are Edges of the surgical wound: non-epithelialized, with open connective tissue
Good 3	Gingiva color: less than 50% red Palpation response: no bleeding Granulation tissue: none Edges of the surgical wound: no open tissue
Very good 4	Gingiva color: less than 25% red Palpation response: no bleeding Granulation tissue: none Edges of the surgical wound: no open tissue
Excellent 5	Gingiva color: pink Palpation response: no bleeding Granulation tissue: none Edges of the surgical wound: no open tissue

On the 7th postoperative day, post-extraction complications were also assessed in the study groups, where available. Persistent postoperative bleeding and trismus (restriction in mouth opening) after 48 hours were reflected; difficulty eating, speech and, as a consequence, disturbances in the quality of the regular daily work process.

A normal healing process is one in which, in the secondary healing of the alveoli, they are filled with granulation tissue, in the presence or absence of pain. Criteria for complications, according to Cheung et al are:

- Alveolar osteitis/localized osteomyelitis – diagnosed by the presence of persistent pulsating postoperative pain in and around the extraction wound that is not affected by prescribed analgesics, including increased body temperature requiring systemic antibiotics
- Dry alveolitis – acute inflammation of extraction wounds, painful but without exudate or fever

At the 3rd postoperative month, a second clinical examination of the operated patients (all study groups) of our team was conducted. They had to present an up-to-date orthopantomogram on which we, by objective visual evaluation, classified the regeneration of the bone structure in the area of the removed LTM as follows: dense homogeneous, heterogeneous and bone with little trabeculae. During the objective examination, we focused on the local status in the area of the already missing LTM, and by probing, we established whether there is food retention and the presence of a periodontal pocket >/< of 5mm, distal in the second lower molar, as well as whether or not there is sensitivity on the back surface of its distal root, preceding the development of a carious defect.

On the 6th postoperative month, we again collected data to analyze the methodology we are considering. Similarly, as during the visit of the 3rd postoperative month, the servicemen from the five studied groups appeared with a new OPG and during the objective examination, our interest was focused on the local status associated with possible complications from the distalia root of the lower second molar.

RESULTS OF OWN SURVEY

150 servicemen who passed through the DOS at the Military Medical Academy in Sofia for the period October 2022 were tracked. – March 2023, undergoing surgical extraction of the third lower molar tooth and divided into 5 groups of 30 patients. One group we define it as a control group – in these patients we do not use platelet concentrate. The remaining 4 - are as follows: group with extracted lower wisdom tooth and applied PC obtained by method 1; group with extracted lower wisdom tooth and applied PC obtained by method 2 group with extracted lower wisdom tooth and applied PC obtained by method 3; group with extracted lower wisdom tooth and applied PC obtained by method 4.

Just over half of the patients (56.7%) were men and the remaining 43.3% were women, Fig. 2.

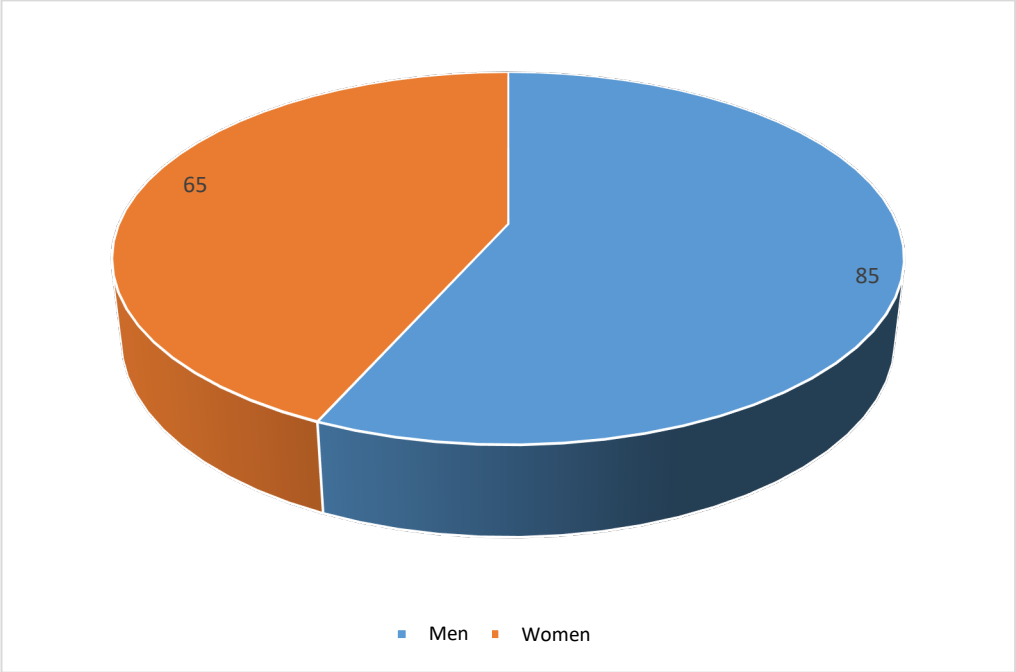


Fig. 2 Distribution of patients by sex (relative share, n=150)

The average age of the patients is 28.8 years, the median is 27 years, the youngest patient is 19 years old and the oldest – 40 years. The standard deviation is 6.74 years, and the 95% confidence interval varies from 27.06 to 30.54 years. Every fourth patient (28.3%) is in the age group between 20 and 25 years, as many persons (25%) are aged 36-40 years, and

23.3% are between 26 and 30 years old. The representatives of the group 31-35 years are 15%, and those under 20 years – only 8.3%, Fig. 3.

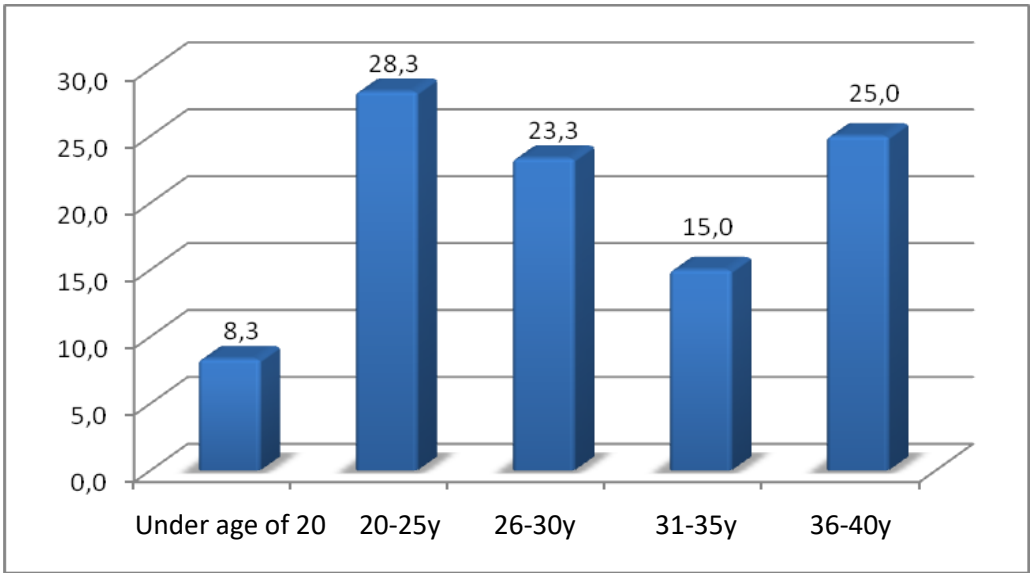


Fig. 3. Distribution of patients by age (relative share, n=150)

A T-test for independent samples found that there was no statistically significant difference for the arithmetic mean of the ages of men and women: t=-1.311, p.0.05.

A check for the normal distribution of the persons examined by age was carried out by the Kolmogorov-Smirnov tests, D=0.154. p>0.05 and Shapiro-Wilk, D=0.927, p>0.05, with the results indicating that the two tests were not statistically significant, i.e. the age distribution of the persons examined was normal.

The statistical analysis of the data presented below was made on the basis of the questionnaires filled in by all study patients with the help of members of our team, together with a captured objective status, 7 days after the removal of the wisdom teeth.

In 4/5 of all studied patients was administered in the post-extraction areas PC received by 4 different protocols, while in the remaining 1/5 – post-extraction wounds were sewn with the presence of a normal blood coagulum in them, Fig. 4.

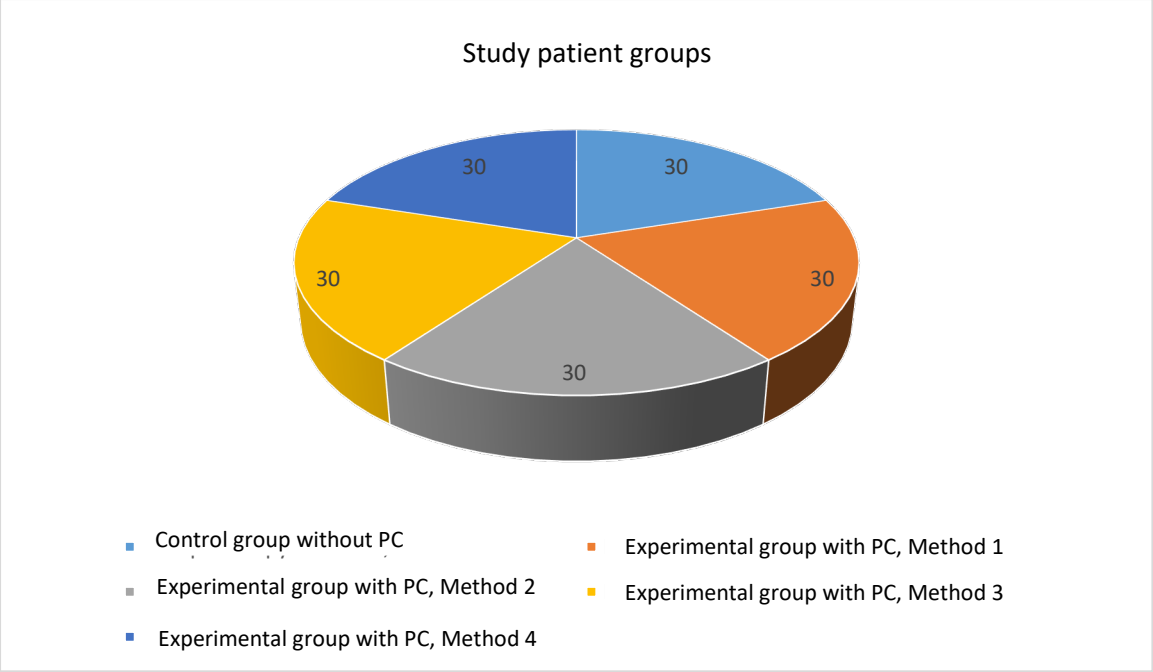


Fig. 4. Distribution of patients according to the application of PC (relative share, n=30)

1.1. Comparative analysis of the results in the study groups of patients – first follow-up examination, 7th postoperative day

Compare each group with the others

Statistical methods: results were presented as absolute frequencies (n) and relative rates (%) of patients in individual groups. The comparison between groups was made with chi square analysis (exacter test of Fischer when applicable).

150 patients were followed, divided into 5 groups, as follows: control group-30 patients with extraction of inferior wisdom teeth without the application of platelet concentrate; a group of 30 patients with extracted inferior wisdom teeth and platelet concentrate administered by method 1; group with extracted wisdom teeth and platelet concentrate applied obtained by method 2; group with extracted lower wisdom teeth and platelet concentrate applied obtained by method 3; group with extracted inferior wisdom teeth and platelet concentrate applied obtained by method 4.

Determination of the extent of the healing process by the index of Landry et al (7th postoperative day)

Tab. 3. Distribution of patients according to the degree of the healing process through the index of Landry et al, by groups

		control group without platelet concentrate		platelet concentrate group by method 1		platelet concentrate group obtained by method 2		platelet concentrate obtained by method 3		group with platelet concentrate applied obtained by method 4	
		n	%	n	%	n	%	n	%	n	%
Determination of the extent of the healing process through the index of Landry et all (7th postoperative day)	very bad	2	6,7%	1	3,3%	0	0,0%	0	0,0%	0	0,0%
	Bad	3	10,0%	1	3,3%	4	13,3%	0	0,0%	0	0,0%
	Good	17	56,7%	15	50,0%	10	33,3%	2	6,7%	2	6,7%
	very good	4	13,3%	8	26,7%	10	33,3%	14	46,7%	10	33,3%
	It's cool	4	13,3%	5	16,7%	6	20,0%	14	46,7%	18	60,0%

Considered for the whole sample, a link between the results the Landry et all index and the group ($p<0.001$) was demonstrated. In Fig. 5. it can be seen how with increasing the number of the group increases and the share of patients rated as excellent and very good, and decreases badly and very badly (the last group is missing in methods 3 and 4).

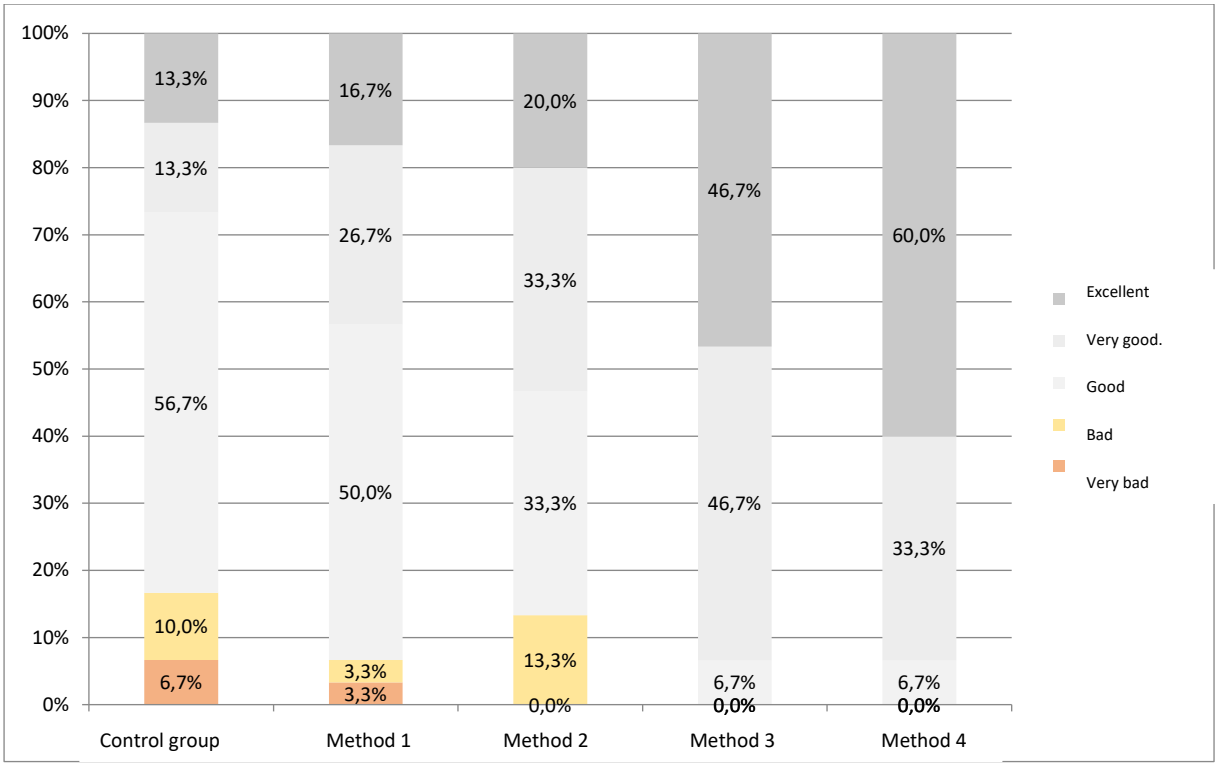


Fig. 5. Distribution of patients according to the degree of the healing process through the index of Landry et al, by groups

Statistical data from our clinical results confirm the opinion on the contribution of PC obtained under the 4 protocols. PRP in the first two experimental groups had little effect on prolonged bleeding, pain and difficulty opening the mouth after 48 hours. The results of the control group almost coincided with that of the PC group by method 1. The data regarding the index of Landry et al in patients with PC from group 3 and 4 are identical – we have over 60% "excellent" soft tissue condition in the operative field within the first postoperative week.

The relationship between results and groups in pairs was investigated.

Tab. 4. Study of the relationship between the degree of the healing process through the index of Landry et all and the groups in pairs

Pairs of groups		p
control group	Method 1	0,547
control group	Method 2	0,140

control group	Method 3	<0,001
control group	Method 4	<0,001
Method 1	Method 2	0,391
Method 1	Method 3	0,001
Method 1	Method 4	0,001
Method 2	Method 3	0,004
Method 2	Method 4	0,002
Method 3	Method 4	0,558

Evaluation of ostextural complications according to the criteria of Cheung et al (7th postoperative day)

Tab. 5. Distribution of patients according to ostextural complications according to the criteria of Cheung et al, by groups

		control group without platelet concentrate		platelet concentrate group by method 1		platelet concentrate group obtained by method 2		platelet concentrate obtained by method 3		group with platelet concentrate applied obtained by method 4	
		n	%	n	%	n	%	n	%	n	%
alveolar osteoitis - throbbing pain, swelling, fever	not	25	83,3%	28	93,3%	30	100,0%	30	100,0%	30	100,0%
	yes I do	5	16,7%	2	6,7%	0	0,0%	0	0,0%	0	0,0%
	not	13	43,3%	15	50,0%	26	86,7%	28	93,3%	28	93,3%

alveolitis - pain, without swelling and exudation, without fever	yes I do	17	56,7%	15	50,0%	4	13,3%	2	6,7%	2	6,7%
postoperative bleeding	not	16	53,3%	26	86,7%	28	93,3%	30	100,0%	29	96,7%
	yes I do	14	46,7%	4	13,3%	2	6,7%	0	0,0%	1	3,3%
persistent trismus after 48 hours	not	9	30,0%	11	36,7%	21	70,0%	28	93,3%	28	93,3%
	yes I do	21	70,0%	19	63,3%	9	30,0%	2	6,7%	2	6,7%
difficulty eating, speaking, working process after 48 hours	not	4	13,3%	10	33,3%	20	66,7%	26	86,7%	26	86,7%
	yes I do	26	86,7%	20	66,7%	10	33,3%	4	13,3%	4	13,3%

Each of the four indicators studied is related to the group variable (p=0.006 for alveolar osteitis and <0.001 for the other indicators). The direction of the connection is evident in Fig. 6-10, with increasing group number decreases the share of patients with the presence of the estimated parameters.

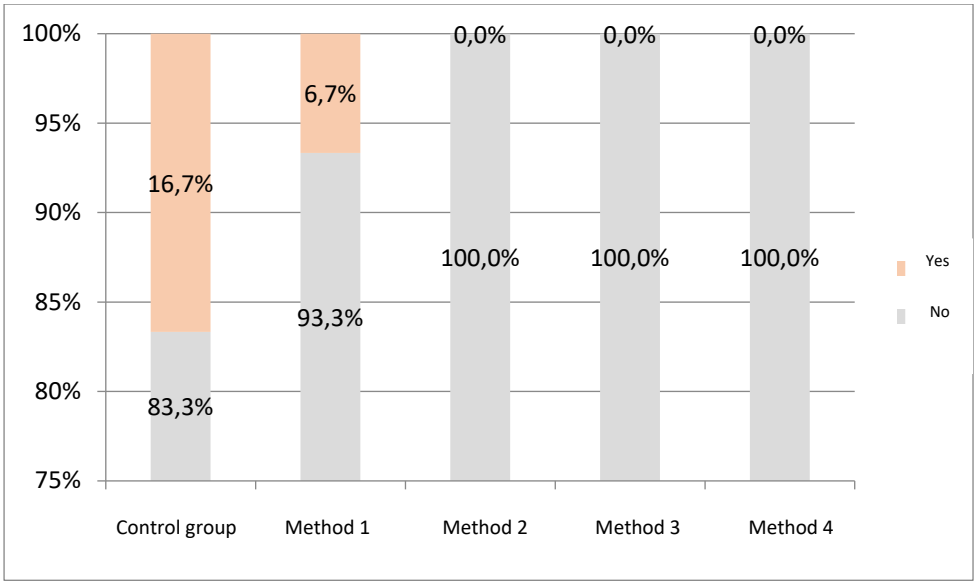


Fig. 6. Distribution of patients according to the presence of alveolar osteitis, by groups

The most severe postoperative complication in SE of LTM is the development of alveolar osteitis, which could pass into localized osteomyelitis of the mandibular bone. Symptomatically, the inflammatory process is characterized by a swelling, throbbing pain, difficult to respond to analgesics, exudation from the wound and even increased body temperature. The data of Fig. 6 showed that <1/5 of the patients in the control group developed alveolar osteitis, only 2 of the experimental group with PC by method 1 had such a complication in the other groups, there was no prevalence,

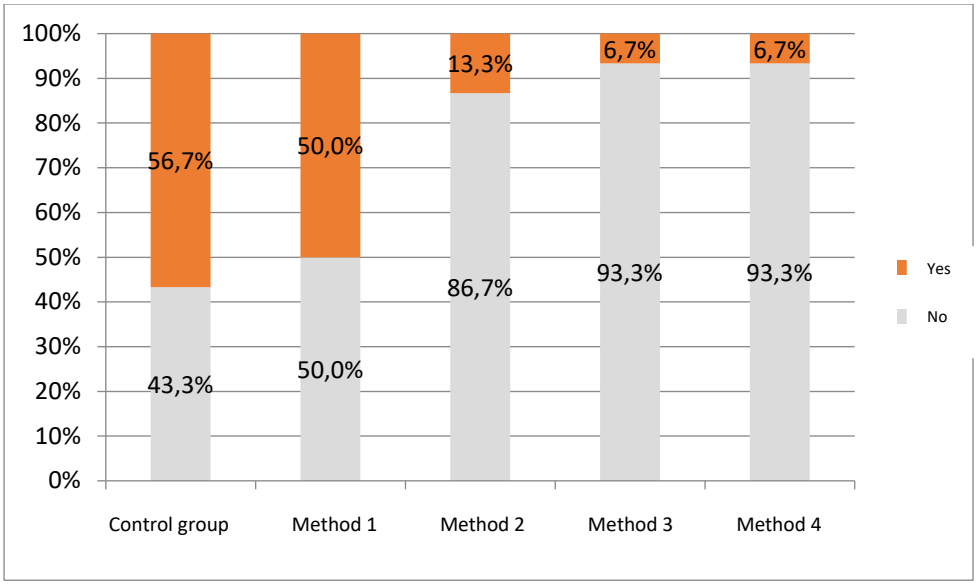


Fig. 7. Distribution of patients according to the presence of alveolitis, by groups

The data are identical by groups and according to the presence of dry alveolitis (Fig.7-10) postextraction complication with the following symptoms: chronic pain of low intensity, no swelling and exudation in the operative field; without increased overall temperature. The results show the definite healing and prophylactic effect of PC obtained by method 3 and 4.

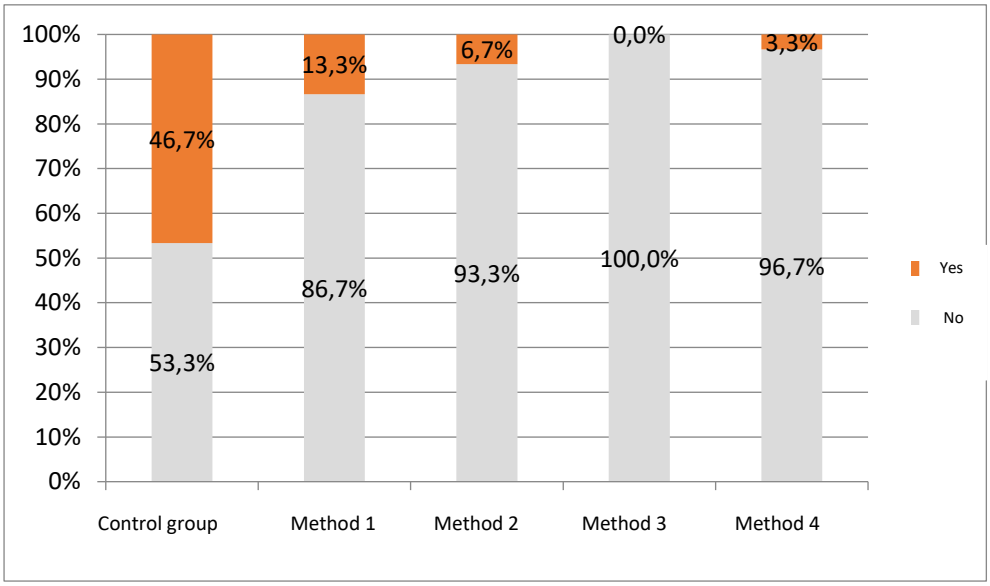


Fig. 8. Distribution of patients according to the presence of postoperative bleeding, by groups

Fig. 8 shows the hemostatic effect of PC in patients of all experimental groups and almost 50% of cases in the control group (without TK) with persistent, after 48 hours, bleeding.

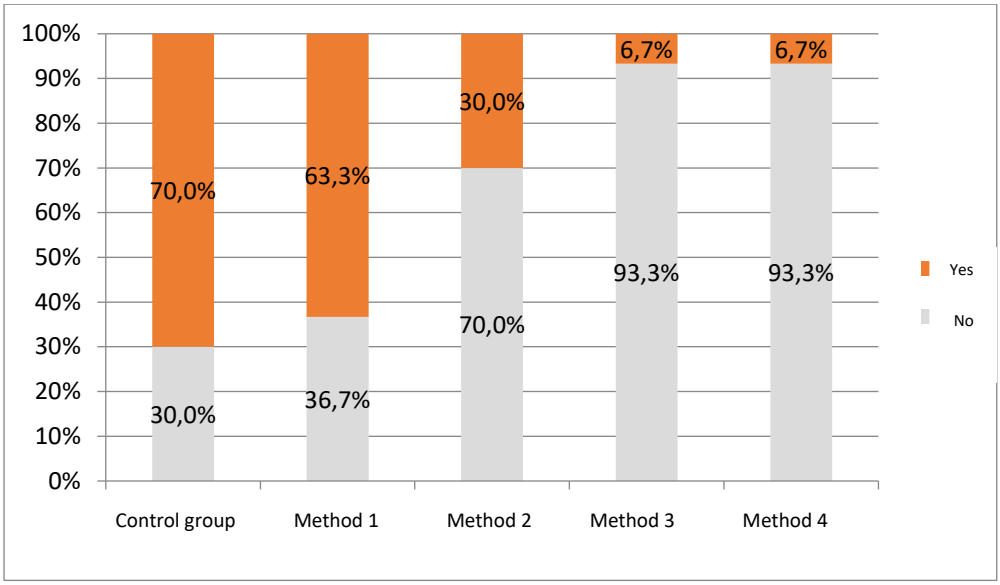


Fig. 9. Distribution of patients according to the presence of persistent trismus after 48 hours, by groups

The more frequent occurrence of trismus among patients is due to the usual traumatism that accompanies SE to LTM. However from Fig. 9 was seen to be minimally prevalent in the TL groups by method 3 and 4, whereas in method 1 and the control group, there was almost no difference in results.

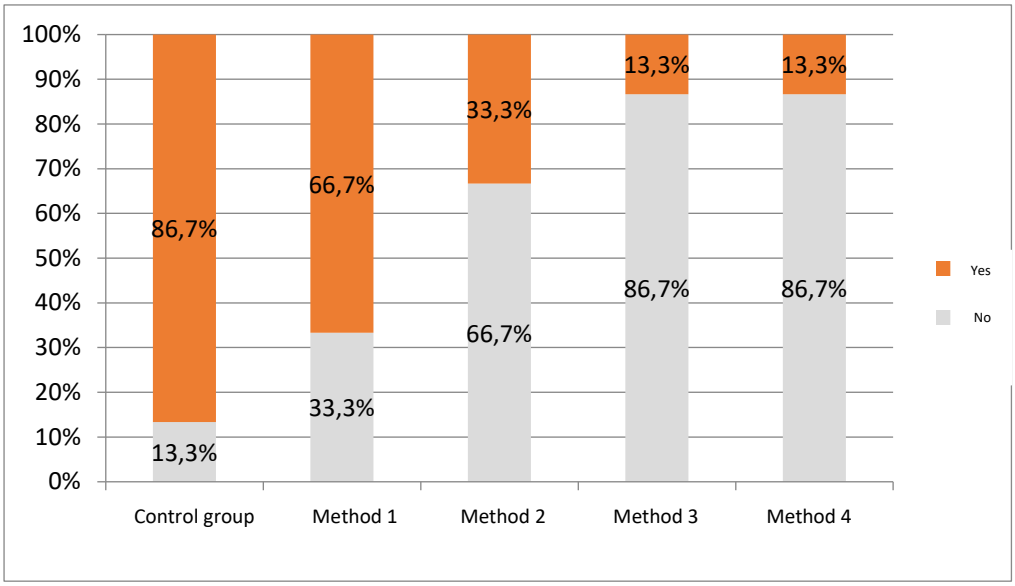


Fig. 10. Distribution of patients according to the presence of difficulty eating, speech, work process after 48 hours, by groups

The analysis of the prevalence of impaired functions in the oral cavity and the delay in restoring the normal daily activities of patients (Fig. 10) is identical to that of the previous criterion studied.

The relationship between results and groups in pairs was investigated.

Tab. 6. Study of the relationship between the presence of alveolar osteitis and pairwise groups

Pairs of groups		p
control group	Method 1	0,248
control group	Method 2	0,020
Control Group	Method 3	0,020
Control Group	Method 4	0,020
Method 1	Method 2	0,150
Method 1	Method 3	0,150
Method 1	Method 4	0,150
Method 2	Method 3	
Method 2	Method 4	
Method 3	Method 4	

Tab. 7. Investigation of the relationship between the presence of alveolitis and paired groups

Deuces of the group		p
Control Group	Method 1	0,605
Control Group	Method 2	<0,001
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,002
Method 1	Method 3	<0,001
Method 1	Method 4	<0,001

Method 2	Method 3	0,389
Method 2	Method 4	0,389
Method 3	Method 4	0,999

Tab. 8. Investigation of the relationship between the presence of postoperative bleeding and paired groups

Deuces of the group		p
Control Group	Method 1	0,005
Control Group	Method 2	<0,001
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,389
Method 1	Method 3	0,038
Method 1	Method 4	0,161
Method 2	Method 3	0,150
Method 2	Method 4	0,554
Method 3	Method 4	0,313

Tab. 9. Study of the relationship between the presence of persistent trismus after 48 hours and the groups in pairs

Deuces of the group		p
Control Group	Method 1	0,584
Control Group	Method 2	0,002

Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,010
Method 1	Method 3	<0,001
Method 1	Method 4	<0,001
Method 2	Method 3	0,020
Method 2	Method 4	0,020
Method 3	Method 4	0,999

Tab. 10. Study of the relationship between the presence of difficulty eating, speaking, working process after 48 hours and groups in pairs

Deuces of the group		p
Control Group	Method 1	0,067
Control Group	Method 2	<0,001
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,010
Method 1	Method 3	<0,001
Method 1	Method 4	<0,001
Method 2	Method 3	0,067
Method 2	Method 4	0,067
Method 3	Method 4	0,999

1.2. Comparative analysis of patients from the study groups – second follow-up examination, 3rd postoperative month

Clinical analysis of postoperative orthopantomograms by subjective visual assessment by oral surgeon (3 postoperative month)

Tab. 11. Distribution of patients according to the clinical analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon (3rd postoperative month), by groups

		control group without platelet concentrate		platelet concentrate group by method 1		platelet concentrate group obtained by method 2		platelet concentrate obtained by method 3		group with platelet concentrate applied obtained by method 4	
		n	%	n	%	n	%	n	%	n	%
Clinical analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon (3rd postop.m.)	dense homogeneous structure	0	0,0%	2	7,7%	2	7,4%	21	75,0%	22	73,3%
	Heterogeneous structure	10	40,0%	11	42,3%	11	40,7%	6	21,4%	7	23,3%
	with few trabeculae	15	60,0%	13	50,0%	14	51,9%	1	3,6%	1	3,3%

A link between the clinical analysis of postoperative orthopantomograms and the groups ($p<0.001$) was demonstrated. The direction of the relationship is evident in Fig. 11, with increasing group numbers the proportion of patients with dense homogeneous structure increases.

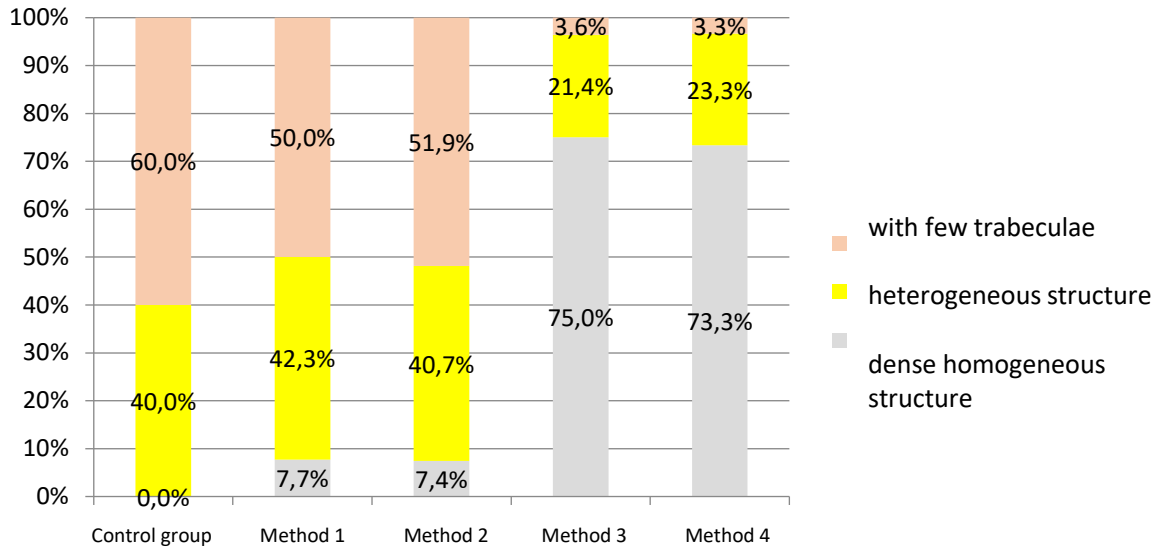
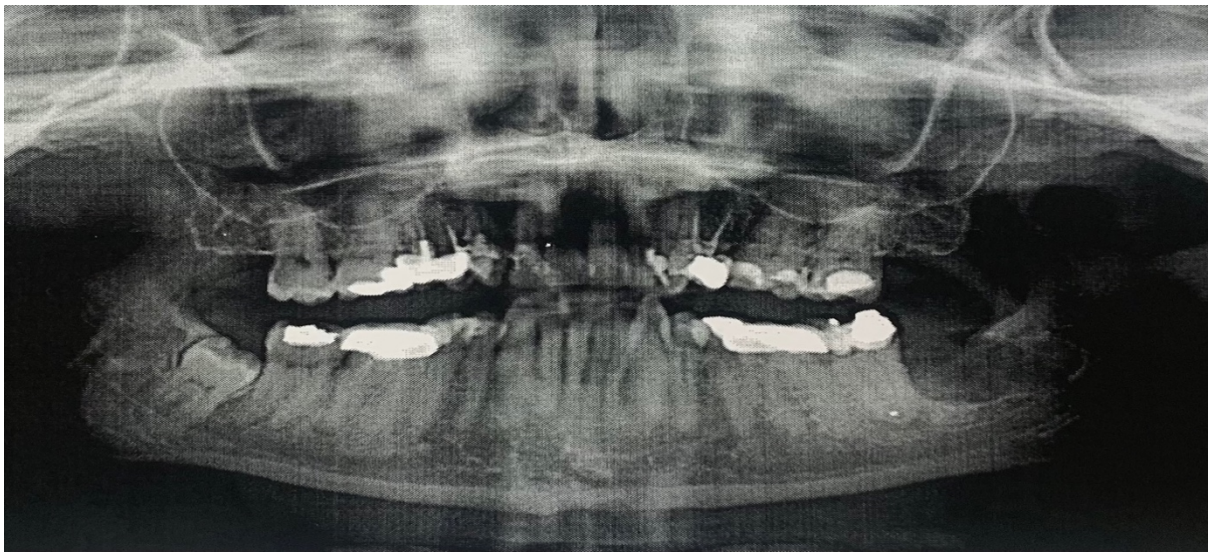
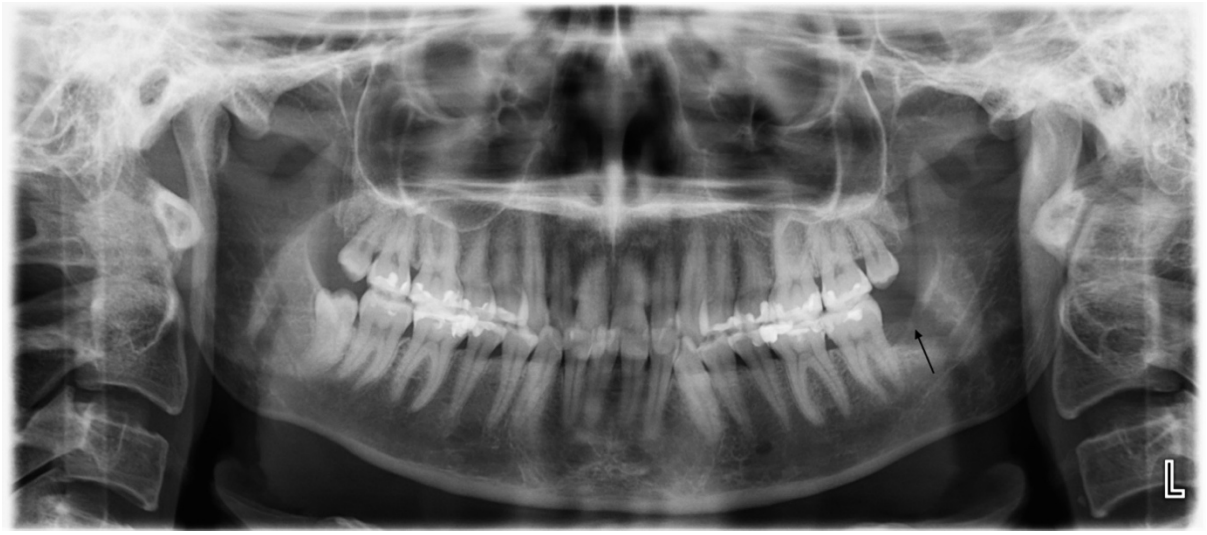


Fig. 11. Distribution of patients according to the clinical analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon (3rd postoperative month), by groups

From the above data (Fig. 11) it is seen that the dense homogeneous structure of the regenerated bone sought by each oral surgeon, after surgical removal of WT, has almost 3/4 prevalence in patients with PC by methods 3 and 4. In the control group, not a single case was found, and in the experimental groups with PC by method 1 and 2 – the most unwanted of surgeons rare inhomogeneous bone with few trabeculae prevailed (pp. 5-9)



Picture 5. Bone structure restored after surgical extraction of 38 teeth of a patient from the control group, without platelet concentrate



Picture 6. Bone structure in the area of 38 teeth of a patient from an experimental group with method 1

Picture 7. Bone structure in the area of 38 teeth of a patient from a group with method 2

Picture 8. Restoration of bone structure in the area of 48 tooth in a patient from group with method 3

Picture 9. Bone structure restored in the area of 38 teeth in a patient from a group with method 4

The relationship between results and groups in pairs was investigated.

Tab. 12. Study of the relationship between the clinical analysis of postoperative orthopantomograms and pairwise groups

Pairs of groups		p
control group	Method 1	0,338
control group	Method 2	<0,001
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,991
Method 1	Method 3	<0,001
Method 1	Method 4	<0,001
Method 2	Method 3	<0,001
Method 2	Method 4	<0,001
Method 3	Method 4	0,984

Clinical results at a follow-up examination by an oral surgeon – 3rd postoperative month

Tab. 13. Distribution of patients according to clinical results at a follow-up examination by an oral surgeon – 3rd postoperative month, by groups

		control group without platelet concentrate		platelet concentrate group by method 1		platelet concentrate group obtained by method 2		platelet concentrate obtained by method 3		group with platelet concentrate applied obtained by method 4	
		n	%	n	%	n	%	n	%	n	%
sensitivity distally in the second lower molars	not	1	3,3%	5	16,7%	15	50,0%	25	83,3%	26	86,7%
	yes I do	29	96,7%	25	83,3%	15	50,0%	5	16,7%	4	13,3%
depth of the periodontal pocket distally in the lower second molars >5mm	not	3	10,0%	5	16,7%	13	43,3%	28	93,3%	28	93,3%
	yes I do	27	90,0%	25	83,3%	17	56,7%	2	6,7%	2	6,7%
food retention in the extraction wound	not	3	10,0%	10	33,3%	23	76,7%	28	93,3%	28	93,3%
	yes I do	27	90,0%	20	66,7%	7	23,3%	2	6,7%	2	6,7%
halitosis despite personal oral hygiene	not	3	10,0%	10	33,3%	23	76,7%	28	93,3%	28	93,3%
	yes I do	27	90,0%	20	66,7%	7	23,3%	2	6,7%	2	6,7%

All four parameters tracked at the control examination were associated with the group, $p<0.001$. The direction of the connection is evident in Fig. 11-14, as the group number increases, the share of patients with the presence of the estimated parameters decreases.

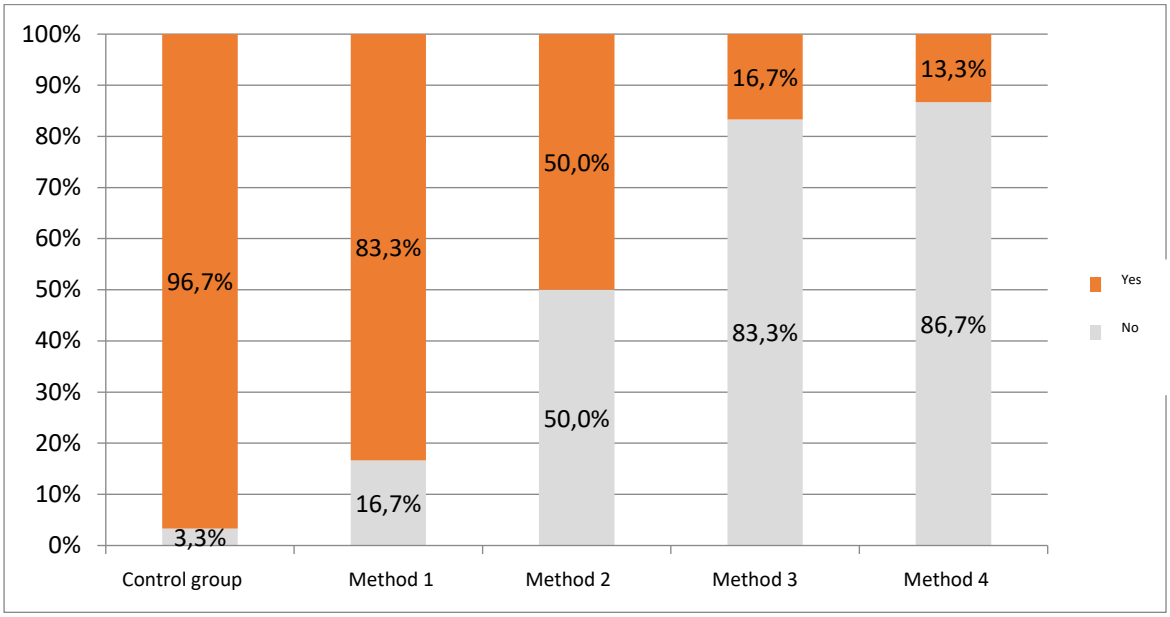


Fig. 11. Distribution of patients according to the presence of sensitivity distally in the second lower molars, by groups

Pathology distal in the 7th tooth postoperatively is common and unpleasant for patients who have undergone SE of LTM. The data of Fig. 11 point to the exceptional effectiveness of PC by method 3 and 4, as well as the absolute frequency of occurrence in patients in the control group and those with PC by method 1.

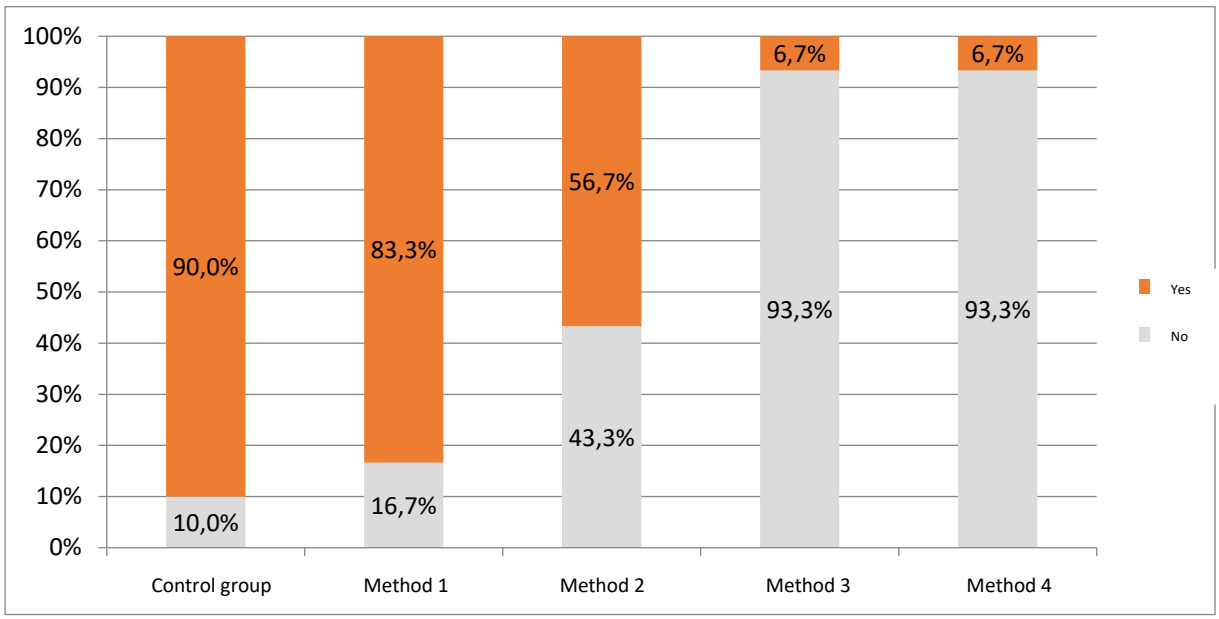


Fig. 12. Distribution of patients according to the presence of depth of the periodontal pocket distally in the lower second molars >5mm, by groups

The statistical data from our clinical results after probing in the corolla groove on the back surface of the 7th tooth, distal (Fig. 12), are identical in terms of the effectiveness of the PC obtained by the four different protocols.

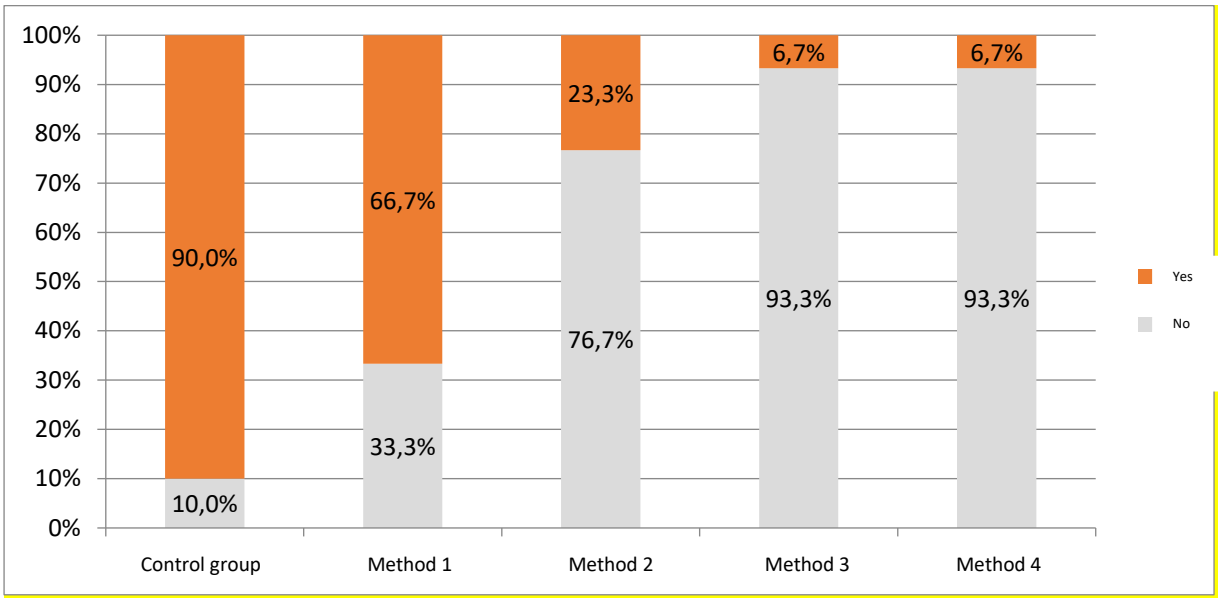


Fig. 13. Distribution of patients according to food retention in the extraction wound, by groups

The relationship between food retention in the wound due to bone resorption and insufficient regeneration is shown in Fig. 13. Again, in the PC groups by method 3 and 4, almost complete bone recovery is detected, stimulated by the applied PRP. The control group and the one with PC by method 1 resulted in inadequate bone repair.

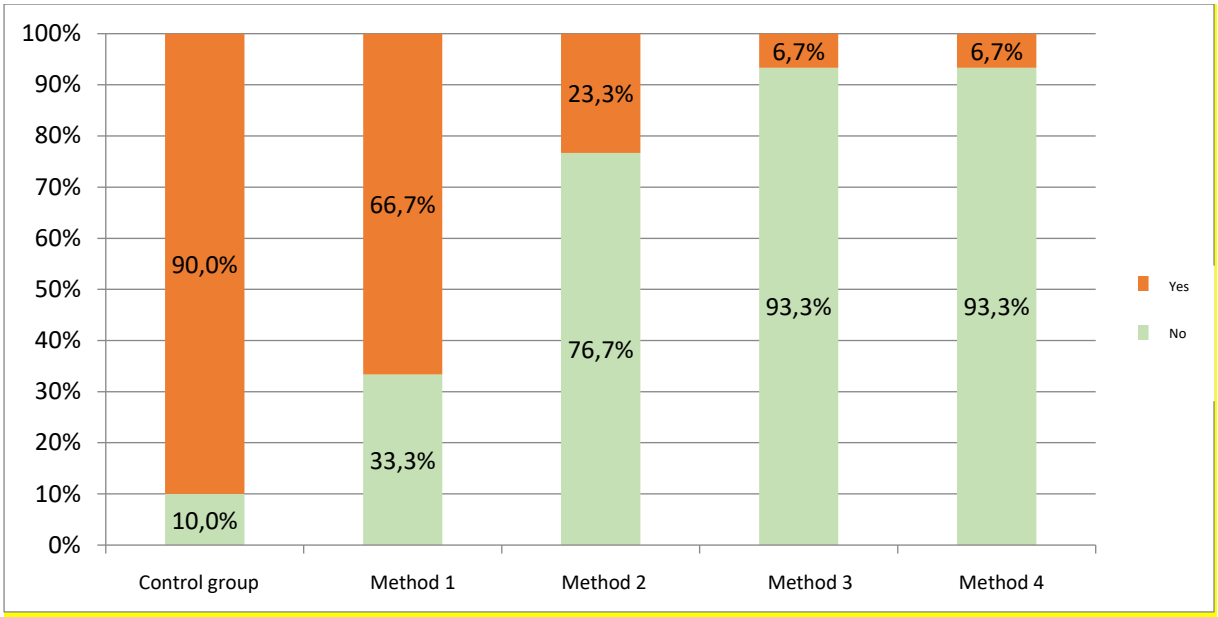


Fig. 14. Distribution of patients according to the presence of halitosis despite personal oral hygiene, by groups

The relationship between halitosis and food retention is directly proportional. The results were identical in these indicators and showed inferiority in the control group and the one with method 1.

The relationship between results and groups in pairs was investigated.

Tab. 14. Study of the relationship between the presence of sensitivity distally in the second lower molars and the groups in pairs

Pairs of groups		p
control group	Method 1	0,085
control group	Method 2	<0,001
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,006

Method 1	Method 3	<0,001
Method 1	Method 4	<0,001
Method 2	Method 3	0,006
Method 2	Method 4	0,002
Method 3	Method 4	0,718

Tab. 15. Study of the relationship between the presence of periodontal pocket depth distally in the lower second molars >5mm and the groups in pairs

Deuces of the group		p
Control Group	Method 1	0,448
Control Group	Method 2	0,004
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,024
Method 1	Method 3	<0,001
Method 1	Method 4	<0,001
Method 2	Method 3	<0,001
Method 2	Method 4	<0,001
Method 3	Method 4	0,999

Tab. 16. Investigation of the relationship between the presence of food retention in the extraction wound and the groups in pairs

Deuces of the group		p
Control Group	Method 1	0,028
Control Group	Method 2	<0,001
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,001
Method 1	Method 3	<0,001
Method 1	Method 4	<0,001
Method 2	Method 3	0,071
Method 2	Method 4	0,071
Method 3	Method 4	0,999

Tab. 17. Investigation of the relationship between halitosis despite personal oral hygiene and paired groups

Deuces of the group		p
Control Group	Method 1	0,028
Control Group	Method 2	<0,001
Control Group	Method 3	<0,001
Control Group	Method 4	<0,001
Method 1	Method 2	0,001
Method 1	Method 3	<0,001

Method 1	Method 4	<0,001
Method 2	Method 3	0,071
Method 2	Method 4	0,071
Method 3	Method 4	0,999

Summary of pairwise group comparison

Method 1 showed better results than the control group only in terms of 3 indicators: postoperative bleeding, food retention in the extraction wound and halitosis despite personal oral hygiene.

Method 2 outperformed the control group on 10 indicators – these were all but the first one, the index of Landry et al.

Methods 3 and 4 showed better results than the control group, method 1 and 2 on all indicators.

Method 2 outperforms method 1 in 7 indicators, method 3 and 4 outperforms 1 in 10 indicators, and method 4 in 9 indicators.

Methods 3 and 4 are better than 2 by 5 indicators.

Method 4 has not proven to be better than 3 on any indicator.

Tab. 18. Summary of pairwise group comparison

Pairs of groups		Determination of the extent of the healing process through the index of Landry et all (7th postoperative day)	alveolar osteoitis - throbbing pain, swelling, fever	alveolitis - pain, without swelling and exudation, without fever	postoperative bleeding	persistent trismus after 48 hours	difficulty eating, speaking, working process after 48 hours	Clinical analysis of postoperative orthopantomograms by subjective visual assessment by oral surgeon (12 weeks)	sensitivity distally in the second lower molars	depth of the periodontal pocket distally in the lower second molars >5mm	food retention in the extraction wound	halitosis despite personal oral hygiene
		p										
control group	Method 1	0,547	0,248	0,605	0,005	0,584	0,067	0,338	0,085	0,448	0,028	0,028
control group	Method 2	0,14	0,02	<0,001	<0,001	0,002	<0,001	<0,001	<0,001	0,004	<0,001	<0,001
control group	Method 3	<0,001	0,02	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
control group	Method 4	<0,001	0,02	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
Method 1	Method 2	0,391	0,15	0,002	0,389	0,01	0,01	0,991	0,006	0,024	0,001	0,001

Method 1	Method 3	0,001	0,15	<0,001	0,038	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
Method 1	Method 4	0,001	0,15	<0,001	0,161	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001	<0,001
Method 2	Method 3	0,004		0,389	0,15	0,02	0,067	<0,001	0,006	<0,001	0,071	0,071
Method 2	Method 4	0,002		0,389	0,554	0,02	0,067	<0,001	0,002	<0,001	0,071	0,071
Method 3	Method 4	0,558		0,999	0,313	0,999	0,999	0,984	0,718	0,999	0,999	0,999

1.3. Comparative analysis in study groups of patients – third follow-up examination, 6th postoperative month

Statistical methods: results were presented as absolute frequencies (n) and relative rates (%) of patients in individual groups. The comparison between groups was made with chi square analysis (exacter test of Fischer when applicable).

150 patients were followed, divided into 5 groups as follows: control group with extracted lower third molar teeth without platelet concentrate applied - 25 patients; a group of patients with extracted lower third molar teeth and platelet concentrate obtained by method 1 - 26

patients; a group of patients with extracted lower third molar teeth and platelet concentrate obtained by method 2 - 27 patients; a group of patients with extracted lower third molar teeth and platelet concentrate obtained by method 3 - 28 patients; group of patients with extracted lower third molar teeth and platelet concentrate obtained by method 4 - 30 patients.

Patients were followed at 6 months post-intervention. The results were compared on two indicators: analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon and depth of the periodontal pocket when probing behind the lower second molars.

There were no significant differences between groups neither for the whole sample nor in pairs in terms of the results of the analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon ($p>0.05$), Tab. 19 and Tab. 20. The dense homogeneous bone structure sought as a normal healing process after LTM SE, is present in about 80% of clinical cases. We assume that it is impossible its theoretical spread of 100% - 6 months after the operation, due to individual biological characteristics of each patient related to both genetics and diet and professional harmfulness.

Tab. 19. Distribution of patients from the five follow-up groups according to the analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon at 6 months after surgery

	Clinical analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon - 6 months after dental extractions					
	dense homogeneous structure		Heterogeneous structure		with few trabeculae	
p=0,988	n	%	n	%	n	%
control group without platelet concentrate administered	20	80,0%	5	20,0%	0	0,0%

platelet concentrate group by method 1	21	80,8%	5	19,2%	0	0,0%
Method 2 platelet concentrate group	21	77,8%	6	22,2%	0	0,0%
platelet concentrate group by method 3	23	82,1%	5	17,9%	0	0,0%
group with platelet concentrate applied by method 4	25	83,3%	5	16,7%	0	0,0%

Tab. 20. Results of the pair-group comparison of the analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon at 6 months after surgery

	p
Control group-method1	0,945
Control group-method2	0,945
Control group-method3	0,942
Control group-method4	0,750
Method1-Method2	0,788
Method1-Method3	0,897
Method1-Method4	0,803
Method2-Method3	0,686
Method2-Method4	0,740
Method3-Method4	0,999

There were no significant differences between groups neither for the whole sample nor in pairs in the results of the depth of the periodontal pocket when probing behind the lower second molars, 6th postoperative month ($p>0.05$), Tab. 21 and Tab. 22. The diagnosis of pathological periodontal pocket at the distal root of the 7th tooth >5mm was established in sporadic cases

by all studied groups, which we could explain again with the hereditary factors of each patient, as well as with their lifestyle. Finding over 95% lack of such a gum pocket among all study participants, 6 months postoperatively meets the requirements for normal bone and soft tissue healing process.

Tab. 21. Distribution of patients from the five tracked groups according to the depth of the periodontal pocket when probing behind the lower second molars, 6th postoperative month

p=0,947	Periodontal pocket depth at probing behind lower second molars, 6th postoperative month			
	presence of periodontal pocket < 5mm		presence of periodontal pocket > 5mm	
	n	%	n	%
control group without platelet concentrate administered	24	96,0%	1	4,0%
platelet concentrate group by method 1	25	96,2%	1	3,8%
Method 2 platelet concentrate group	25	92,6%	2	7,4%
platelet concentrate group by method 3	27	96,4%	1	3,6%
group with platelet concentrate applied by method 4	29	96,7%	1	3,3%

Tab. 22. Results of pairwise group comparison of the depth of the periodontal pocket when probing behind the lower second molars, 6th postoperative month

	p
Control group-method1	0,999
Control group-method2	0,999
Control group-method3	0,999

Control group-method4	0,999
Method1-Method2	0,999
Method1-Method3	0,999
Method1-Method4	0,999
Method2-Method3	0,611
Method2-Method4	0,599
Method3-Method4	0,999

1.4.Comparative analysis in the study patients of the results between 3rd and 6th postoperative month

The comparison between the results at 3 and 6 postoperative month was made with the tests of Wilcoxon (in three categories of the compared variable) and McNemar (in two categories). Comparisons were made for the whole sample as well as for each group separately: controls, patients with platelet concentrate by methods 1, 2, 3 and 4. Since some of the patients were not

followed at the 6th postoperative month, they were excluded from the analysis. The results at values of $p<0.05$ were assumed to be significant.

With regard to postoperative orthopantomograms, a significant change in the direction of improvement of the entire sample was demonstrated, as well as among controls and patients with platelet concentrate obtained by methods 1 and 2 ($p<0.001$), Table 21. For patients using method 3, no significant change ($p>0.05$) was demonstrated, probably because a large number of them showed very good results as early as the 3rd postoperative month. Patients who received platelet concentrate by method 4 showed significant improvement, but its significance is close to the borderline ($p=0.046$) because they also had significant improvement as early as the 3rd postoperative month.

Tab. 21. Distribution of patients – total and in groups, according to the results of the clinical analysis of postoperative orthopantomograms by subjective visual assessment by an oral surgeon at the 3rd and 6th postoperative month

Group	Results	3 m		6 m		p
		n	%	n	%	
for the whole sample	dense homogeneous structure	47	34,6%	110	80,9%	<0,001
	Heterogeneous structure	45	33,1%	26	19,1%	
	with few trabeculae	44	32,4%	0	0,0%	
control group	dense homogeneous structure	0	0,0%	20	80,0%	<0,001
	Heterogeneous structure	10	40,0%	5	20,0%	
	with few trabeculae	15	60,0%	0	0,0%	
Method 1	dense homogeneous structure	2	7,7%	21	80,8%	<0,001
	Heterogeneous structure	11	42,3%	5	19,2%	
	with few trabeculae	13	50,0%	0	0,0%	
Method 2	dense homogeneous structure	2	7,4%	21	77,8%	<0,001
	Heterogeneous structure	11	40,7%	6	22,2%	

	with few trabeculae	14	51,9%	0	0,0%	
Method 3	dense homogeneous structure	21	75,0%	23	82,1%	0,083
	Heterogeneous structure	6	21,4%	5	17,9%	
	with few trabeculae	1	3,6%	0	0,0%	
Method 4	dense homogeneous structure	22	73,3%	25	83,3%	0,046
	Heterogeneous structure	7	23,3%	5	16,7%	
	with few trabeculae	1	3,3%	0	0,0%	

The presence of a periodontal pocket >5 mm showed a significant change in the direction of improvement when comparing the 3rd and 6th postoperative months: for the entire sample, for the control group and for patients receiving platelet concentrate by methods 1 and 2 ($p<0.001$), Table 22. For the groups of patients given platelet concentrate using methods 3 and 4, no significant change was demonstrated ($p>0.05$), Because in almost all of them the depth of the periodontal pocket was less than 5 mm as early as the 3rd postoperative month.

Tab. 22. Distribution of patients – total and in groups, according to the results of the measured depth of the periodontal pocket distally in the lower second molars (below or above 5mm) at the 3rd and 6th postoperative months.

Group	Results	3 m		6 m		p
		n	%	n	%	
the whole sample	presence of periodontal pocket < 5mm	65	47,8%	130	95,6%	<0,001

	presence of periodontal pocket > 5mm	71	52,2%	6	4,4%	
control group	presence of periodontal pocket < 5mm	0	0,0%	24	96,0%	<0,001
	presence of periodontal pocket > 5mm	25	100,0%	1	4,0%	
Method 1	presence of periodontal pocket < 5mm	1	3,8%	25	96,2%	<0,001
	presence of periodontal pocket > 5mm	25	96,2%	1	3,8%	
Method 2	presence of periodontal pocket < 5mm	10	37,0%	25	92,6%	<0,001
	presence of periodontal pocket > 5mm	17	63,0%	2	7,4%	
Method 3	presence of periodontal pocket < 5mm	26	92,9%	27	96,4%	0,999
	presence of periodontal pocket > 5mm	2	7,1%	1	3,6%	
Method 4	presence of periodontal pocket < 5mm	28	93,3%	29	96,7%	0,999
	presence of periodontal pocket > 5mm	2	6,7%	1	3,3%	

DISCUSSION

The aim of this dissertation is to study the effect of PRP (PRP, PC), obtained under 4 new approved protocols in our country, in connection with the healing process after surgical extraction of lower third molar tooth. This is one of the main surgical interventions in the field of oral surgery, which almost every person needs earlier or later in life. It is associated with some traumatism, postoperative, which in most cases causes early and late negative consequences. There are persistent symptoms such as bleeding, pain, restriction in the opening of the mouth, swelling, the development of inflammatory complications – alveolar osteitis and

dry alveolitis, with or without an increase in body temperature. They are of an individual quantitative and qualitative nature, but in some cases, lead to impaired eating and speech within 7 days after the removal of WT, sometimes affecting the normal daily activities of patients. There are also late complications in this pathology, damaging the second lower molar, located adjacent to the operative wound, as well as the formation of a periodontal pocket at its distal root, leading to food retention and bad breath – daily discomfort disturbing social contacts. The methodology with APLICATION of PRP in post-extraction areas when removing WT, intraoperatively, aims to provide a more comfortable early postoperative period without possible complications and better dental health in a shorter period - respectively to improve the quality of life of patients and their timely return to daily activities. This thesis has become a working hypothesis of our research.

Due to the many discussions on PRP outlined above, we chose to study the clinical results of platelet concentrates obtained under 4 new protocols proposed for our country (Ivanova et al.), applied in the post-extraction wounds of lower third molar teeth removed by us and to make possible comparisons of the healing effect of PC between different groups, as well as with a control group. in which patients with WT extracted did not receive PRP, intraalveolar.

The results indicated by Ivanova et al. for the extraction of platelet-enriched plasma on all four experimental protocols have constant values of initially withdrawn venous blood of 8 ml and a final obtained final product PRP – 3 ml. As mentioned, calcium gluconate, easy to obtain as a pharmaceutical product and a reliable source of Ca ions, is accepted for the selection of an exogenous activator, which results in a gel-like platelet-enriched plasma in about 20 minutes.

As noted, Marx (2004) proposes to set a threshold concentration of 1×10^6 platelet/μL or of 3-5 times platelet saturation to provide a therapeutically effective amount of growth factors in PRP products. In the present scientific study, the enriched plasma obtained under the conditions of **Method 1** (single centrifugation of blood in monovets without separating gel), had the lowest amount of platelet saturation – 1.92 times the control value. In the plasma, Received along **protocol 2** (single centrifugation in vacutainers with separating gel), platelet saturation was 4.37 times the platelet content of the whole blood control. In the plasma obtained by experimental **Method 3** (double centrifugation in monovetes without separating gel) – 4.52 times, in the product obtained by **Protocol 4** (double centrifugation in vacutainers with separating gel), platelet saturation was 4.9 times. Adhering to the definition

proposed by Marx, the product obtained by Ivanova et al. method 1 cannot be defined as a true type of PRP, the PRP products indicated in the literature have platelet saturation more than 2 times the control comparable values.

Autologous enriched plasma, containing high values of concentrated platelets, has an abundance of growth factors allowing healing and regeneration of tissues. Lee et al. (2013) proved that growth factors are contained in the highest concentration in platelets (in their α-granules) and play an essential role in the process of wound healing and tissue regeneration – the main ones with proven clinical action are PDGF-AB and TGF beta 1 (180). A number of authors have considered in depth the dependence of platelet counts in PRP and the level of growth factors Anitua et al. (2009), Eppley et al. (2004), Magalon et al. (2014), McCarrel & Fortier (2009), Sanchez et al. (2007) (16, 98, 192, 211, 269).

PDGF-AB is separated from platelets and affects multiple cells. In the study conducted by Ivanova et al., after a T-test for correlated samples, a statistically significant difference was found between the mean values of PDGF-AB before and after activation by methods ($p<0.05$). For **method 1,** the mean values of PDGFAB in activated plasma increased by 1.18-fold compared to the non-activated plasma, obtained under the same conditions ($p<0.05$). For **Method 2, the mean PDGF-AB values in the activated plasma increased by 1.20-fold relative to the non-activated plasma obtained under the same conditions ($p<0.05$). In Method 3, the** PDGF-AB values in the activated plasma increased by 1.13-fold, relative to the non-activated plasma obtained under the same conditions ($p<0.05$), and in Method **4** the increase in PDGF-AB in the activated plasma was 1.08-fold relative to the unactivated plasma. From the results obtained, it is clearly seen that PDGF-AB positively correlates with platelet counts from the control rupa ($p<0.05$). However, the positive correlation was also found between the number of leukocytes obtained after extraction of the enriched plasma by methods versus PDGF-AB($p<0.05$). The highest value of PDGF-AB in the samples of method 2 is impressive. This is the autologous platelet-enriched product, in which both the platelet content and the leukocytes content at one place are presented with high values. As a conclusion, it should be noted that the amount of PDGF-AB also depends on the content of leukocytes in the final product.

Another of the main growth factors in autologous platelet products with clinical results for medical practice is TGF beta-1. It is a multifunctional cytokine involved in the control of cell growth and stimulation of matrix production. In the study of Ivanova et al. we have the following data (by T-test for correlated samples): in PCs prepared by method 1 and 2, there is a minimal decrease in TGF beta 1 values after addition of activator. In the PCs prepared by Method 3 and Method 4, there was an increase in the mean TGF beta 1 in the activated samples, the highest mean TGF beta 1 values being in the samples of Method 4. The lowest mean TGF beta 1 is found in the samples of Method 1.

With regard to the classification of the resulting PCs according to those presented above, the following should be considered: according to the erythrocyte content, the samples of Method 1 and 2 are rich in erythrocytes and the samples of Method 3 and 4 are poor in erythrocyte residue (content 15 times lower than the control values). With regard to platelet saturation, the product obtained by Method 1 cannot be classified against the indicated minimum saturations within a limit of 2-3 times the base control values (mean 1,92 times the mean of the control group). The platelet concentrates obtained by method 2, 3 and 4 were within the range of saturation between 4-6 times the mean of the control group. The products obtained by Method 1 and 2 were rich in leukocyte content (mean of samples above the mean of the control group) and the products obtained by Method 3 and 4 were poor in leukocytes (mean of samples lower than the mean value of the control group).

Table 23 presents the abbreviations of the abbreviations of those received by Ivanova et al. autologous platelet concentrates classified against PAW and MARSPILL systems.

Tab. 23. Classification of those received by Ivanova et al. four products compared to the PAW and MARSPILL systems

Classification tion system	**Autologous platelet enriched product**			
	Method 1	Method 2	Method 3	Method 4
PAW	P2-Aα P2-x-Aα	P4-Aα P4-x-Aα	P4-BB P4-xBB	P4-BB P4-x-BB

MARSPILL	**M**(H) **A**(A–) **R**(RBCR) **S**(Sp1) **P**(PL-) **I**(G–) **L(LR)** L(L–) **M**(H) **A**(A+) **R**(RBCR) **S**(Sp1) **P**(PL-) **I**(G–) L(**LR**) L(L–)	**M**(H) **A**(A–) **R**(RBCR) **S**(Sp1) **P**(PL 4-6) **I**(G–) **L (LR)** L(L–) **M**(H) **A**(A+) **R**(RBCR) **S**(Sp1) **P**(PL 2-3) **I**(G–) **L (LR)** L(L–)	**M**(H) **A**(A–) **R**(RBC-P) **S**(Sp2) **P**(PL 4-6) **I**(G–) **L (P)** L(L–) **M**(H) **A**(A+) **R**(RBC-P) **S**(Sp2) **P**(PL 4-6) **I**(G–) **L (P)** L(L–)	**M**(H) **A**(A–) **R**(RBC-P) **S**(Sp2) **P**(PL 4-6) **I**(G–) **L (P)** L(L–) **M**(H) **A**(A+) **R**(RBC-P) **S**(Sp2) **P**(PL 4-6) **I**(G–) **L (P)** L(L–)

Conclusions on the preparation of autologous platelet concentrates under 4 new own protocols for extraction of PC (Ivanova et al.)

According to the literature studied and accumulated data on PRP species and their platelet saturation, the product obtained under the conditions of method 1 cannot be called a true platelet-enriched plasma due to platelet saturation in it lower than twice the control values. The platelet-enriched plasma obtained under the conditions of method 2 is, according to the classifications discussed above, referred to as LR-PRP. This type of platelet concentrate is also characterized by an increase in the amount of PDGF-AB I IL-8 protein (growth factors). A product with such characteristics may affect the healing of soft tissues and persistent soft tissue inflammation due to its antibacterial activity. The platelet-enriched plasma obtained by methods 3 and 4 is similar in its characteristics for the quantitative presence of the cellular components with curative action. These are inherently "purified" products – mostly platelet-rich, labeled LP-PRP, with a proposal for clinical application as products with a recognized absence of leukocytes. Platelet concentrates obtained by methods 2, 3 and 4 may find their application after application of an activator or not, depending on the state in which they are to be applied. In the case of PC obtained by Method 1 and 2, it was found that after activation, depending on a slight increase, TGF beta 1 and PDGF-AB values may have a volume replacement effect and, if necessary, be used to fill cavities or bone defects.

Algorithm of our study

In the present study, we covered a total of 150 patients with surgically removed WT for the period October 2022 – March 2023, divided into five groups of 30. We formed a control group in which we did not use TK. In the other 4 groups, we applied autologous PC, in the bone wound of the removed WT, which we extracted preoperatively through the four new protocols of receipt according to Ivanova et al. (discussed above). All 30 patients from one experimental group received PC by method 1, 2, 3 or 4, on the basis of which we did the grouping. After extraction of PC (final product about 3ml), an exogenous activation process followed with 10% p-p of calcium gluconate, in a ratio of 3:1, at room temperature for about 20 minutes. The process-derived gel-like autologous biological substrate, we placed in the post-extraction bone defects of LTM.

The participants in the study are military personnel in the system of the Ministry of Defense, treated in our Department of Oral Surgery at the Military Medical Academy in Sofia, to whom the purpose of this study was explained in detail. They signed an informed consent regarding their participation in this study. For them, especially those serving in the Air Force Aviation Detachment, it is extremely important that the postoperative period is easy, quickly and without complications. This allows them to quickly resume their duties.

We performed 3 control examinations - 7 days after their surgical intervention, at the 3rd and 6th postoperative months. The first of them was attended by all 150 operated patients. With the help of colleagues from our team, the servicemen filled in anonymous questionnaires prepared by us in advance. Objectively, the condition of soft tissues in the oral cavity, in the area of the surgical wound, as well as the presence of possible postoperative complications – alveolar osteitis / dry alveolitis was assessed. The second check-up was performed on the 3rd postoperative month, when we requested from the patients control X-ray panoramic dental pictures taken on the same device as the preoperative ones. We analyzed them by subjective visual assessment (by members of our team together with a radiologist) of the density of the restored bone structure in the area of the removed WT. Also, we diagnosed by probing the intraoral local status in terms of manifest pathology in the lower second molar on the side of

surgical extraction – sensitivity and periodontal dtsob at the distal root of the 7th tooth, food retention at the extraction site and halitosis (new questionnaires were completed). All patients tested (150) reappeared, but only 136 of them had the OPG we requested. The other 14 had taken dental pictures in an X-ray laboratory other than the original one, which we did not include in the study. On the 6th postoperative month we conducted a third check-up. To analyze the results at that time, we needed from each patient an up-to-date postoperative OPG (made on the same device as the previous two), as well as determining the depth of a permanently formed, if any, periodontal pocket at the distal root of the 7th tooth. There were 136 patients – the same patients who were observed in the second check-up.

The clinical and statistical data from our study confirmed the facts about the relationship between the way of extracting PRP and the desired curative / prophylactic effect. Our team found that for the needs of our practice related to surgical extraction of lower third molar tooth, the added PC needs to be obtained by method 3 or 4.

CONCLUSIONS

Autologous PRP products have been widely promoted as an option to treat and improve the healing of bone as well as soft tissue atonic wounds by promoting granulation tissue formation.

Platelet-rich plasma is increasingly used as an effective means of restoring bones and tissues in all areas – traditional and avant-garde, dental surgery, as well as dental implant placement.

PRP products are gaining increasing popularity and it is generally accepted that the correct strategy in wound treatment is the use of autologous platelet-rich plasma containing growth factors, which is applied directly (locally) to sites with surgical interventions, bone wounds or soft tissue injuries.

We drew the following conclusions from the conducted study:

1. The application of PRP/PC (PRP) in bone defects of the jaws can be applied in addition to some of the standard surgical methods in oral surgery, the most common of which is surgical extraction of the lower third molar tooth.

2. The use of PC in post-extraction defects of the lower wisdom tooth stimulates and optimizes the bone and soft tissue healing process.

3. After the comparative analyses in the five groups we studied, between the control/experimental patients, we found a healing and prophylactic effect of PC.

4. The existing literature data on the clinical results of PC were confirmed, depending on the way they are obtained – the numerous protocols for extraction of BCP lead to different consequences on healing processes, some of which have almost no clinical significance.

5. The 4 methods used by us for the extraction of PC (after Ivanova et al.) are the subject of previous scientific and practical work, where the obtained biological substrates – the amount of formed elements and main acting proteins (PDGF-AB, TGF-beta1, ePC.) have been analyzed in detail, depending on the required clinical application.

6. Our clinical results, depending on the 4 protocols used to obtain PRP, confirm the differences between them and show which of them are preferable in our surgical removal and inferior wisdom tooth.

7. The application of PC improves the comfort of the patient in the early postoperative period, up to 7 days after surgery, as well as prevents the occurrence of post-surgical complications. Prevention is due to the fact that BCP is a physiological antibiotic – pH 6.6.

8. Added, post-extractive, PC also protects the spread of late postoperative complications associated with pathology of the second lower molar.

9. Patients in whom we administered PRP reported faster local and general recovery postoperatively, associated with timely return to their normal routine duties.

10. The methodology for extraction and use of PRP is safe, effective (the extraction of autologous PCs was implemented by medical professionals available in our structure in a hospital hospital) with the possibility of an 8-hour duration of the surgical intervention due to the vitality of the activated platelets for this period of time.

11. The financial analysis of PRP alone is for the benefit of the patient.

12. The methodology using extracted autogenic PC in lower third molar surgery does not require a prolonged hospital stay and does not prolong the time of the operative activity due to its preliminary preparation.

CONCLUSION

This study presents a variation of the traumatic surgical method of removal of lower third molar teeth, which in a large percentage of cases is associated with a violation of the quality of life of patients. We propose the addition of autologous platelet concentrate, intraalveolar in post-extraction defects, extracted by some of the new standardized protocols for our country, which leads to stimulation of the usually severe post-extraction period without the development of complications. We dare say that this rationalization is to the absolute benefit of the modern patient and does not disturb his daily activities.

**Note: The numbers of figures, tables and photos in this autoabstract does not follow the numbering of the same in the dissertation.*

OFFERINGS

Of original character:

1. For the first time in Bulgaria, at the Military Medical Academy, a proposal for an avant-garde algorithm of behavior has been prepared for military pilots who need surgical extraction of a lower third molar tooth, namely – the addition of autologous PRP to the post-extraction defect. The main objective – as quickly and fully as possible to restore the official commitments in the flight space of the Republic of Bulgaria.

With attached character:

2. The difficulties of the most commonly used surgical method in our department of oral surgery – surgical extraction of lower third molar teeth among servicemen are analyzed.

3. The necessary optimization measures are derived, needing stimulation of the bone healing process after surgical extraction of inferior wisdom teeth, by using autologous platelet concentrates.

4. An algorithm of behavior has been created in the Department of Oral Surgery at the Military Medical Academy in Sofia. Sofia - in surgical extraction of lower third molars in servicemen to apply PRP in post-extraction defects standardized for our country protocol 3 and 4 - according to Ivanova et al.

Confirmatory in nature:

5. The stimulating effect of PC in relation to the early recovery period after surgery of the lower wisdom tooth has been proven.

6. Optimized bone regeneration in the surgical wound and prevention of subsequent pathology in the second lower molar as a result of the actions of the platelet concentrate, 3 months after the surgical intervention, has been established.

7. It has been confirmed that clinical postoperative results in surgery of lower wisdom teeth correlate with the technique of obtaining platelet concentrate, that is, there is a direct dependence on the quantity, concentration of cellular components and acting proteins in them.

8. It has been confirmed that the yield of platelet-enriched plasma is reliable using manual methods with a standard small laboratory centrifuge, without the aid of closed commercial kits and kits, making the methodology easily feasible and reliable under standard conditions, even in outpatient dental surgical practice.

SCIENTIFIC PUBLICATIONS ON THE TOPIC

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THANKS:

In conclusion, I would like to thank:

- *My supervisor prof. Rosen Gospodinov Kolarov, MD, PhD – for the support and assistance in the development, analysis and preparation of the dissertation*
- *Prof. Stefan Vasilev Peev, MD, PhD – for Shared Professional Experience on PRP Acquisition and Application*
- *Faculty of Dental Medicine at MU-Varna, Department of Oral Surgery, where the dissertation is presented and defended*
- *The management of the Military Medical Academy in Sofia, where the practical part of the scientific research was conducted;*
- *All my colleagues for the shared experience, professional and technical assistance in carrying out the experimental part; on the subsequent statistical and technological layout of the dissertation*
- *of Nikoleta Ivanova et al. for the use of their scientific and practical results for the purposes of their own research*