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# Biochemical parameters of the blood of rats of different ages after filling the metaphyseal defect of the femur with allogeneic bone implants with local administration of blood plasma enriched with platelets

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*A promising method of regenerative medicine is the saturation of allografts with platelet-rich plasma (PRP). Objective. To evaluate the course of metabolic processes after filling a hole defect in the distal metaphysis of the femur with allogeneic bone implants in conditions of additional local administration of allogeneic PRP. Methods. In the model of a hole defect in white rats with the filling of the defect with an allograft, as well as with additional local stimulation of PRP on the 7th day; on the 3rd and 7th days and on the 1st, 3rd and 7th days in blood serum, the content of glycoproteins (GP), chondroitin sulfates (CST), total protein (TP), calcium (Ca), activity alkaline phosphatase (AlP) and acid phosphatase (AcP). The results. 14 day. In the 3-month rats, under one stimulation, an increase in TP and Ca, a decrease in AcP was observed, with two stimulations there was a 1.11 times smaller GP, 1.13 times larger TP and 1.43 times Ca compared to those in rats without stimulation. During three stimulations, the GP was 1.24 times lower than that in animals without stimulation. In the 12-month rats in comparison with the data of rats without stimulation, 1.15 times higher GP, Ca, AlP activity, and 1.44 times more AcP were noted. 28 days of the 3-month rats for one injection exceeded the data of animals without stimulation by 1.34 times for GP, and were inferior to them by 1.31 times for AcP. In the 12-month rats, compared to these animals without stimulation, with three injections, a 1.19 times greater TP was noted. 90 d. In the 3-month rats for one injection showed 1.24 times less CST with 1.28 times lower AlP compared to data from rats without stimulation. 12-month rats exceeded the data of the group without stimulation by 1.43 times for AlP. Conclusions. In rats with an alloimplant (especially at 12 months), an increase in connective tissue formation markers and a decrease in LF activity were observed. Filling the defect with an alloimplant led to an increase in inflammation indicators and an increase in markers of bone tissue formation.*

*Перспективним методом регенеративної медицини є насичення алотрансплантатів плазмою, збагаченою тромбоцитами (PRP). Мета. Оцінити перебіг метаболічних процесів після заповнення дірчастого дефекту в дистальному метафізі стегнової кістки алогенними кістковими імплантатами в умовах додаткового локального введення алогенної PRP. Методи. На моделі дірчастого дефекту в тварин із його заповненням алоімплантатом, а також із додатковою локальною стимуляцією PRP на 7-му; 3-тю та 7-му; 1-шу, 3-тю та 7-му добу в сироватці крові досліджено вміст глікопротеїнів (ГП), хондроїтинсульфатів (ХСТ), загального білка (ЗБ), кальцію (Са), активність лужної фосфатази (ЛФ) та кислої фосфатази (КФ). Результати. На 14-ту добу після відтворення дефекту в 3-міс. щурів виявили, що в разі однієї стимуляції відбулося збільшення ЗБ та Са, зниження КФ, за двох — зменшення в 1,11 разу ГП, зростання в 1,13 разу ЗБ та в 1,43 разу Са у порівнянні зі щурами без стимуляції. За умов трьох стимуляцій вміст ГП зафіксовано в 1,24 разу менше ніж у тварин без неї. У 12-міс. щурів відзначено більший у 1,15 разу вміст ГП, Са, активність ЛФ, та в 1,44 разу КФ. На 28-му добу в 3-міс. щурів за умови однієї ін'єкції маркери ГП перевищували показники тварин без стимулювання у 1,34 разу, та поступались у 1,31 разу за КФ. Дані 12–міс. щурів із трьома ін'єкціями порівняно зі значеннями тварин без стимуляції показали збільшення ЗБ у 1,19 разу. На 90-ту добу в 3-міс. щурів із однією ін'єкцією виявлено зменшення в 1,24 разу ХСТ за нижчої в 1,28 разу ЛФ порівняно з показниками щурів без стимуляції, у 12-міс. щурів перевищення склало 1,43 разу за ЛФ на відміну від інтактних тварин. Висновки. У щурів із алоімплантатом (особливо у 12-міс.) спостерігалося збільшення маркерів формування сполучної тканини та зменшення активності ЛФ. Заповнення дефекту алоімплантатом призводило до підвищення показників запалення та збільшення маркерів формування кісткової тканини. Ключові слова. Кісткова тканина, дефект, алоімплантат, терапія плазмою, збагаченою тромбоцитами, регенерація, біохімія.*

**Keywords.** Bone tissue, defect, allograft, regeneration, biochemistry

## Introduction

Platelet-rich plasma (PRP) therapy is a regenerative medicine method that has only recently attracted the interest of physicians and scientists due to its potential to improve tissue healing and regeneration [1]. PRP is obtained from the patient's blood, it contains a significant number of platelets, growth factors and biologically active proteins. The former play an important role in physiological healing processes, and PRP therapy promotes the acceleration and restoration of damaged tissues [2]. This method is potentially capable of stimulating bone regeneration [3]. Statistically significant effectiveness of PRP has been demonstrated, in particular, when used for the treatment of fractures of the lower extremities [4]. A double-blind, randomized clinical trial found a significant acceleration of complete healing of long bones during PRP treatment with a rate of 81.1 % compared to 55.3 % in the placebo group [5]. Fast fracture union is crucial to minimize treatment times, reduce negative manifestations, including chronic pain, impaired mobility, and the development of secondary complications, which is important for optimizing outcomes [6].

In addition to its effect on bone healing, PRP has shown significant efficacy in improving soft tissue repair, which is a key aspect in the treatment of complex fractures [7]. A study of V. Seshan et al. (2021) determined that PRP promoted the complete closure or healing of wounds with diabetic ulcers of the lower extremities, reduced the time to complete wound closure, and reduced the area and depth of the wound [8].

PRP is a valuable therapeutic healing tool with anti-inflammatory properties. By modulating the immune response, the inflammatory cascade, usually associated with tissue damage with complex fractures, is effectively mitigated [9].

This therapy can be integrated with traditional techniques to accelerate healing. Its advantages are the safety and naturalness of the treatment, as well as the possibility of using it alone or in combination with other methods [1].

Further research is currently needed to comprehensively understand the efficacy and safety of PRP in a wider range of fracture treatments [10, 11].

*Purpose:* based on the analysis of biochemical indicators of connective tissue in the blood serum of laboratory rats, to evaluate the course of metabolic processes after filling a hole defect of a critical size in the distal metaphysis of the femur with allogeneic bone implants under conditions of additional local administration of allogeneic blood plasma enriched with platelets.

## Material and methods

The study was conducted in the certified department of transplantology, laboratory of experimental modeling with experimental biological clinic, department of laboratory diagnosis and immunology.

The study was carried out in compliance with the requirements of humane treatment of experimental animals [12–14] after the plan was approved by the Bioethics Committee at the State Establishment Professor M. I. Sytenko Institute of Spine and Joint Pathology of the National Academy of Sciences of Ukraine (Protocol No. 191 dated 22.04.2019).

The experiment involved a comparative study of the metabolic features of the regeneration of a hole defect of critical size in the distal metaphysis of the femur of laboratory rats during filling with an allogeneic bone implant in the conditions of additional stimulation of healing by local administration of PRP and without it.

At the same time, 160 white male rats were used, of which 80 were 3 months old weighing (195 ± 21) g and 80 were 12 months old (385 ± 31) g, which were randomly divided into 10 groups:

I — 15 3-month-old rats, the defect in the metaphysis of the femur was filled with a bone alloimplant without additional local stimulation;

II — 15 12-month-old animals, the defect in the metaphysis of the femur was filled with a bone alloimplant without additional local stimulation;

III — 15 3-month-old rats, the defect in the metaphysis of the femur was filled with a bone alloimplant, additional stimulation with a one-time local injection of PRP on the 7$^{th}$ day of the experiment;

IV — 15 12-month-old animals, the defect in the metaphysis of the femur was filled with a bone alloimplant, additional stimulation with a one-time local injection of PRP on the 7$^{th}$ day of the experiment;

V — 15 3-month-old rats, the defect in the metaphysis of the femur was filled with a bone alloimplant, additional stimulation with two local injections of PRP on the 3$^{rd}$ and 7$^{th}$ days of the experiment;

VI — 15 12-month-old animals, the defect in the metaphysis of the femur was filled with a bone alloimplant, additional stimulation with two local injections of PRP on the 3$^{rd}$ and 7$^{th}$ days of the experiment;

VII — 15 3-month-old individuals, the defect in the metaphysis of the femur was filled with a bone alloimplant, additional stimulation with three local injections of PRP on the 1$^{st}$, 3$^{rd}$ and 7$^{th}$ days of the experiment;

VIII — 15 12-month-old rats, the defect in the metaphysis of the femur was filled with a bone alloimplant, additional stimulation with three local injections of PRP on the 1st, 3rd and 7th days of the experiment;

IX — 20 3-month-old animals, receiving blood plasma enriched with platelets and allo-implants;

X — 20 12-month-old rats, receiving blood plasma enriched with platelets and alloimplants.

14, 28, and 90 days after the operation, respectively, 5 animals from groups I–VIII were removed from the experiment. Rats of groups IX and X were removed from the experiment on the day of creation of the defect, on the 3rd and 7th day of the experiment for extemporaneous obtaining of PRP immediately before its introduction as local stimulation.

*Surgical interventions* were performed under aseptic and antiseptic conditions under general anesthesia (ketamine, 50 mg/kg body weight, intramuscularly). After depilation on the left knee and treatment with Betadin® antiseptic, the area of the distal metaphysis of the femur was opened through an anterolateral approach, and a hole defect with a depth of 3 mm was modeled with a dental bur with a diameter of 3 mm (the critical size is considered to be the minimum size of the defect that does not heal on its own during the life of the animal or during the experiment). For the distal metaphysis of the rat femur, the minimum size of the critical defect is 2.5 mm in diameter and depth [15]. Cylindrical alloimplants with a diameter of 3 mm and a length of 3 mm were placed in the area of the defect in rats of groups I — VIII. After local treatment with an antibiotic, the muscles and the skin wound were sutured in layers, the plane of surgical intervention was treated with an antiseptic.

In rats of groups X–IX, a hollow drill with an inner diameter of 3 mm was used to extract 3.0 mm long alloimplants, packed in a polyethylene package, and sterilized by radiation using the LU-10 Accelerator device (NSC Pryskoryuvach of the National Scientific Center Kharkiv Physical Technical Institute).

*Obtaining blood plasma enriched with platelets.* To perform the method, blood was collected from 3- and 12-month-old rats, 20 subjects in each group. Under these circumstances, the animals were taken out of the experiment, 8 ml of venous blood was taken into an 8.5 ml vacuum tube with an anticoagulant. The test tube was centrifuged in a laboratory clinical centrifuge OPn-3.02 at 1500 rpm for 10 min (first stage), then plasma (supernatant fraction) was taken from it with a sterile pipette, which was approximately ½ of the original volume, moved to a graduated sterile test tube, samples were taken for analysis of growth factors after the first centrifugation and then centrifuged again at 3000 rpm for 10 min (second stage). Next, the PRP (surface fraction) was collected with a pipette, which was approximately ½ of the volume of the primary plasma, in which growth factors were determined, and the platelet-poor plasma left at the bottom of the test tube was disposed of.

During the removal of animals of groups I–VI from the experiment, blood was taken from them, which, after natural coagulation, was freed from formed elements by centrifugation for 15 min at 3000 rpm. The supernatant liquid was separated, and the above parameters were measured in it.

*Biochemical assay.* The selection of biochemical analysis parameters was carried out in such a way as to study the data of inflammation and metabolism of bone and connective tissues, as well as the general somatic condition of the experimental animals:

– the content of glycoproteins (GP) according to the modified method of O.P. Shtenberg and Y. N. Dotsenko [16];

– the content of chondroitin sulfates (CST) according to the Nemeth–Csoka method as modified by L. I. Slutsky [17];

– the activity of alkaline phosphatase (AL) and acid phosphatase (AC) by kinetic methods according to the instructions “Alkaline phosphatase - kinetic. Sp. L” and “Acid phosphatase - Kin. Sp. K”;

– the content of total calcium (Ca) by the potentiometric method using the AEK-01 electrolyte analyzer;

– the content of total protein (TP) by the biuret method [16].

The measurement of colorimetric indicators was performed on the electrophotocolorimeter “KFK-3” and the biochemical analyzer “GBG STAT FAX 1904 Plus”.

*Statistical processing of results.* Data analysis was performed using IBM SPSS Statistics 20 and Microsoft Office Excel 2007 software. Measurement results are given as median and quartiles (Me; 25 %; 75 %). Comparison of two groups was performed using Mann-Whitney analysis. The difference was considered statistically significant in $p < 0.05$ [18].

## Results

*14th day after the reproduction of the defect*

Activation of metabolic processes was observed in *3-month-old rats* under conditions of one local injection of PRP. At the same time, the content of GP approached the level of animals with allocyst implantation without stimulation (Table). The content of TP and Ca exceeded the values of the indicators of the comparison group by 1.30 times ($p < 0.01$)

and 1.15 times ($p < 0.01$), respectively. A two-fold decrease in AC activity was recorded compared to the data of animals without additional stimulation.

After two injections of PRP, 3-month-old rats also had a 1.11-fold lower GP level ($p < 0.01$) than animals without stimulation. Greater values were observed for the level of TP by 1.13 times ($p < 0.01$) and Ca by 1.43 times ($p < 0.01$). During three local injections of PRP, the content of GP was 1.24 times lower than that in the group of animals without stimulation ($p < 0.01$) (Table).

*In 12-month-old rats*, a 1.22-fold lower GP level ($p < 0.01$), a 1.21-fold lower AL activity ($p < 0.01$) and a 1.20 times AC ($p < 0.01$), as well as 1.34 times higher CST content ($p < 0.01$).

After two-time stimulation, 1.10 times less GP content ($p < 0.01$), 1.14 times less TP ($p < 0.01$) and 1.22 times lower AL activity ($p < 0.01$) than in 3-month-old animals were recorded. Compared to the indicators of rats with an alloimplant without stimulation, a 1.15 times higher content of GP ($p < 0.01$), Ca ($p < 0.01$), AL activity ($p < 0.01$), and in 1.44 times of AC ($p < 0.01$). After three-timenstimulation, 12-month-old rats were inferior to 3-month-old rats in terms of CST level by 1.27 times ($p < 0.008$), AL activity by 1.47 times ($p < 0.01$).

*The 28th day after the reproduction of the defect*

After a single stimulation, *3-month-old rats* exceeded animals without stimulation by the level of GP 1.34 times ($p < 0.05$) and inferior to the latter by 1.31 times in AC activity ($p < 0.01$) (Table). Compared to the data on the 14th day, a 1.74-fold increase in AC activity was found ($p < 0.01$) (Table).

In *12-month-old rats* with a single stimulation, AL activity was 1.50 times less ($p < 0.01$) compared to 3-month-old animals and 1.21 times less than that on the 14th day ($p < 0.01$) (Table).

Under two-time local stimulation of PRP, 12-month-old rats with alloimplants without saturation with stimulants showed no statistical significance of discrepancies with the data of the comparison groups.

Under the conditions of triple stimulation, an excess of 1.27 times ($p < 0.01$) was recorded in the level of GP in 3-month-old rats (Table). In comparison to the indicators of animals without stimulation, a 1.19 times higher level of TP was noted ($p < 0.01$), on the 14th day an excess of the level of GP was found by 1.54 times ($p < 0.01$), TP by 1.14 times ($p < 0.01$), Ca by 1.19 times ($p < 0.01$), CST by 1.37 times ($p < 0.01$) (Table).

*The 90th day after the reproduction of the defect*

In *3-month-old rats* with a single stimulation, 1.24 times less CST was recorded with 1.28 times lower AL activity ($p < 0.01$) in comparison with the data of animals without additional stimulation (Table). Comparison with the indicators of rats on the 28th day showed 1.26 times less activity of AL ($p < 0.01$), and 1.56 times less activity of AC ($p < 0.01$).

During two-time stimulation, 1.31 times lower CST content ($p < 0.01$) and 1.46 times lower AL activity ($p < 0.01$) than on the 14th day were found. Comparison with the data on the 28th day of the experiment showed a 1.26-fold decrease in AL activity and a 1.56-fold decrease in AC activity ($p < 0.01$) (Table).

Under the conditions of triple stimulation, relative to the indicators on the 14th day, the concentration of GP was 1.15 times higher ($p < 0.01$), TP was 1.16 times higher ($p < 0.01$), and Ca was 1.07 times higher ($p < 0.01$), AL activity 1.76 times ($p < 0.01$) by 1.55 times lower AC ($p < 0.01$). In comparison with the data on the 28th day, a 1.47-fold decrease in AC activity was recorded ($p < 0.01$).

*12-month-old rats.* Under conditions of single stimulation, 1.23 times lower GP concentration ($p < 0.01$) and 1.65 times lower AL activity ($p < 0.01$) were observed compared to data in 3-month-old rats. On the 14th day, a 1.13-fold increase in the concentration of TP ($p < 0.01$), a 1.08-fold increase in Ca ($p < 0.01$) against a background of a 1.37-fold decrease in AL activity ($p < 0, 01$). Under the conditions of single stimulation, in comparison with the indicators on the 28th day, an increase of 1.15 times of GP ($p < 0.01$), 1.16 times of TP ($p < 0.01$), 1.07 times of Ca ($p < 0.01$) and 1.33 times the activity of AL ($p < 0.01$).

During two-time stimulation, a 1.29 times higher level of CST was determined compared to that in 3-month-old rats ($p < 0.01$). Analysis of the data of the group without stimulation revealed a 1.43-fold increase in AL activity ($p < 0.01$). On the 14th day, a 1.15-fold increase in the level of TP ($p < 0.01$), a 1.24-fold decrease in CRT concentration ($p < 0.01$), and a 1.62-fold decrease in AL activity ($p < 0.01$).

Comparison of indicators in 12-month-old rats with triple stimulation with such in 3-month-old animals showed a 1.25-fold increase in CST content ($p < 0.01$).

Compared to the data on the 14th day, a higher content of glycoproteins was recorded by 1.28 times ($p < 0.01$) with a decrease in AL activity by 1.54 times ($p < 0.01$). On the 28th day, GP was found to be 1.18 times less ($p < 0.01$), chondroitin sulfates — 1.15 times ($p < 0.01$), AL activity — 1.66 times ($p < 0.01$).

*Table*

**Biochemical parameters of the blood serum of white rats of different ages after filling a critical size defect in the metaphysis of the femur with an allogeneic bone implant, in particular under the conditions of local stimulation of the healing of the defect by the introduction of blood plasma enriched with platelets (Me, 25%; 75%)**

| Indicator, unit of measurement | Without additional stimulation | | 1 injection of blood plasma enriched with platelets | | 2 injections of blood plasma enriched with platelets | | 3 injections of blood plasma enriched with platelets | |
|---|---|---|---|---|---|---|---|---|
| | 3-month-old rats, n = 5 | 12-month-old rats, n = 5 | 3-month-old rats, n = 5 | 12-month-old rats, n = 5 | 3-month-old rats, n = 5 | 12-month-old rats, n = 5 | 3-month-old rats, n = 5 | 12-month-old rats, n = 5 |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 14-th day | | | | | | | | |
| glycoproteins, g/l | 0.92; 0.88; 0.97 | 0.81; 0.78; 0.84 $p_1 < 0.01$ | 0.92; 0.83; 0.96 $p_2 > 0.05$ | 0.73; 0.70; 0.78 $p_1 < 0.01$ $p_2 > 0.05$ | 0.82; 0.72; 0.89 $p_2 < 0.01$ | 0.76; 0.62; 0.86 $p_1 > 0.05$ $p_2 > 0.05$ | 0.74; 0.63; 0.89 $p_2 > 0.05$ | 0.65; 0.56; 0.79 $p_1 > 0.05$ $p_2 > 0.05$ |
| total protein, g/l | 56.7; 54.9; 63.4 | 65.7; 60.4; 67.3 $p_1 > 0.05$ | 73.8; 69.2; 79.7 $p_2 < 0.01$ | 71.2; 63.9; 76.8 $p_1 > 0.05$ $p_2 > 0.05$ | 77.1; 71.6; 80.0 $p_2 < 0.01$ | 62.1; 56.7; 69.6 $p_1 < 0.05$ $p_2 > 0.05$ | 65.7; 60.4; 69.4 $p_2 > 0.05$ | 65.5; 61.0; 73.0 $p_1 > 0.05$ $p_2 > 0.05$ |
| Ca, mmol/l | 2.02; 2.01; 2.23 | 2.30; 2.15; 2.35 $p_1 > 0.05$ | 2.38; 2.23; 2.60 $p_2 < 0.05$ | 2.25; 2.14; 2.42 $p_1 > 0.05$ $p_2 > 0.05$ | 2.44; 2.30; 2.56 $p_2 < 0.01$ | 2.31; 2.16; 2.48 $p_1 > 0.05$ $p_2 > 0.05$ | 2.29; 2.12; 2.44 $p_2 > 0.05$ | 2.14; 2.02; 2.25 $p_1 > 0.05$ $p_2 > 0.05$ |
| chondroitin-sulfates, g/l | 0.300; 0.286; 0.350 | 0.384; 0.374; 0.399 $p_1 < 0.01$ | 0.259; 0.227; 0.288 $p_2 > 0.05$ | 0.347; 0.328; 0.361 $p_1 < 0.01$ $p_2 > 0.05$ | 0.287; 0.254; 0.315 $p_2 > 0.05$ | 0.353; 0.331; 0.386 $p_1 < 0.01$ $p_2 > 0.05$ | 0.285; 0.222; 0.326 $p_2 > 0.05$ | 0.362; 0.331; 0.388 $p_1 < 0.01$ $p_2 > 0.05$ |
| alkaline phosphatase activity, U/l | 343.0; 296.0; 408.0 | 295.0; 276.0; 416.0 $p_1 < 0.05$ | 429.0; 389.0; 443.5 $p_2 < 0.01$ | 366.5; 345.0; 385.0 $p_1 < 0.01$ $p_2 > 0.05$ | 485.0; 435.5; 520.0 $p_2 > 0.05$ | 356.8; 331.0; 378.9 $p_1 < 0.01$ $p_2 < 0.01$ | 449.0; 390.0; 529.0 $p_2 > 0.05$ | 301.6; 268.4; 362.0 $p_1 < 0.01$ $p_2 < 0.01$ |
| acid phosphatase activity, U/l | 35.5; 32.1; 37.2 | 28.5; 23.9; 33.0 $p_1 > 0.05$ | 18.8; 15.8; 22.9 $p_2 < 0.01$ | 19.2; 16.0; 25.1 $p_1 > 0.05$ $p_2 > 0.05$ | 28.4; 24.8; 33.4 $p_2 > 0.05$ | 26.5; 22.8; 31.3 $p_1 > 0.05$ $p_2 < 0.05$ | 33.3; 30.5; 35.7 $p_2 > 0.05$ | 28.6; 23.6; 33.0 $p_1 > 0.05$ $p_2 < 0.01$ |
| 28-th day | | | | | | | | |
| glycoproteins, g/l | 0.67; 0.64; 0.71 $p_3 < 0.01$ | 0.95 0.87; 1.07 $p_1 < 0.01$ $p_3 < 0.018$ | 0.75; 0.68; 0.82 $p_2 < 0.05$ $p_3 < 0.01$ | 0,83; 0.75; 0.90 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 0.73; 0.65; 0.79 $p_2 > 0.05$ $p_3 > 0.05$ | 0.1; 0.72; 0.91 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 0.77; 0.71; 0.83 $p_2 < 0.01$ $p_3 > 0.05$ | 0.98; 0.91; 1.08 $p_1 < 0.01$ $p_2 > 0.05$ $p_3 < 0.01$ |
| total protein, g/l | 64.1; 61.0; 70.1 $p_3 > 0.05$ | 68,4 60.8; 70.4 $p_1 > 0.05$ $p_3 > 0.05$ | 71.4; 65.5; 76.4 $p_2 > 0.05$ $p_3 > 0.05$ | 67.6; 61.2; 73.0 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 63.2; 56.9; 68.8 $p_2 > 0.05$ $p_3 < 0.01$ | 68.4; 61.3; 75.0 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 73.4; 68.2; 79.1 $p_2 < 0.01$ $p_3 < 0.01$ | 74.6; 71.2; 81.2 $p_1 > 0.05$ $p_2 < 0.01$ $p_3 < 0.01$ |
| Ca, mmol/l | 2.27; 2.18; 2.36 $p_3 < 0.05$ | 2.37 2.16; 2.38 $p_1 > 0.05$ $p_3 < 0.05$ | 2.37; 2.37; 2.38 $p_2 > 0.05$ $p_3 > 0.05$ | 2.29; 2.14; 2.35 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 2.25; 2.21; 2.31 $p_2 > 0.05$ $p_3 < 0.01$ | 2.31; 2.30; 2.37 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 2.39; 2.38; 2.41 $p_2 < 0.05$ $p_3 < 0.01$ | 2.40; 2.36; 2.41 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ |
| chondroitin-sulfates, g/l | 0.253; 0.218; 0.304 $p_3 < 0.01$ | 0.274; 0.241; 0.305 $p_1 > 0.05$ $p_3 < 0.01$ | 0.274; 0.254; 0.290 $p_2 > 0.05$ $p_3 > 0.05$ | 0.315; 0.296; 0.331 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 0.265; 0.230; 0.290 $p_2 > 0.05$ $p_3 < 0.05$ | 0.325; 0.285; 0.344 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 0.272; 0.250; 0.290 $p_2 > 0.05$ $p_3 > 0.05$ | 0.350; 0.335; 0.373 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ |
| alkaline phosphatase activity, U/l | 348.0; 339.5; 426.0 $p_3 > 0.05$ | 302.0; 269.0; 344.5 $p_1 < 0.05$ $p_3 > 0.05$ | 438.0; 391.0; 467.5 $p_2 > 0.05$ $p_3 > 0.05$ | 301.6; 255.9; 331.8 $p_1 < 0.01$ $p_2 > 0.05$ $p_3 < 0.01$ | 418.0; 341.0; 460.5 $p_2 > 0.05$ $p_3 > 0.05$ | 314.6; 286.4; 350.0 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 416.0; 394.5; 471.0 $p_2 > 0.05$ $p_3 > 0.05$ | 325.0; 291.6; 343.5 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ |

*Continuation of the Table*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| acid phosphatase activity, U/l | 26.8; 23.9; 30.7 $p_3 < 0.05$ | 22.8; 19.1; 25.4 $p_1 > 0.05$ $p_3 > 0.05$ | 32.7; 29.2; 36.0 $p_2 < 0.05$ $p_3 < 0.05$ | 26.4; 22.1; 31.2 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ | 34.9; 28.5; 39.9 $p_2 < 0.05$ $p_3 > 0.05$ | 25.0; 20.2; 29.3 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ | 31.5; 27.3; 35.9 $p_2 > 0.05$ $p_3 > 0.05$ | 24.6; 20.3; 29.6 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.690$ |
| 90-th day | | | | | | | | |
| glycoproteins, g/l | 0.80; 0.77; 0.85 $p_3 < 0.01$ $p_4 < 0.01$ | 0.93; 0.86; 1.14 $p_1 < 0.05$ $p_3 < 0.05$ $p_4 > 0.05$ | 1.02; 0.96; 1.11 $p_2 < 0.01$ $p_3 < 0.01$ $p_4 < 0.01$ | 0.83; 0.76; 0.89 $p_1 > 0.05$ $p_2 < 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 0,79 0.77; 0.85 $p_2 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 0.91; 0.84; 0.96 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.05$ $p_4 > 0.05$ | 0.85; 0.81; 0.90 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 0,83; 0.76; 0.89 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 < 0.01$ |
| total protein, g/l | 76.7; 69.9; 93.0 $p_3 < 0.01$ $p_4 < 0.05$ | 75.6; 68.8; 96.4 $p_1 > 0.05$ $p_3 < 0.05$ $p_4 > 0.05$ | 87.7; 82.4; 93.9 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 81.0; 74.3; 86.8 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 67.8; 62.1; 75.2 $p_2 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 71.2; 66.5; 79.2 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 76.0; 71.6; 81.1 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 78.3; 72.6; 82.3 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ |
| Ca, mmol/l | 2.41; 2.38; 2.55 $p_3 < 0.05$ $p_4 > 0.05$ | 2.38; 2.33; 2.42 $p_1 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 2.58; 2.50; 2.64 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 < 0.01$ | 2.44; 2.36; 2.50 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 < 0.01$ | 2.35; 2.29; 2.40 $p_2 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 2.41; 2.35; 2.47 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 2.42; 2.33; 2.48 $p_2 > 0.05$ $p_3 < 0.05$ $p_4 > 0.05$ | 2.36; 2.28; 2.47 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.05$ $p_4 > 0.05$ |
| chondroitin-sulfates, g/l | 0.255; 0.241; 0.288 $p_3 < 0.05$ $p_4 > 0.05$ | 0.273; 0.267; 0.296 $p_1 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 0.330; 0.300; 0.360 $p_2 < 0.01$ $p_3 < 0.01$ $p_4 < 0.01$ | 0.364; 0.331; 0.401 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 0.220; 0.180; 0.245 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 0.285; 0.250; 0.319 $p_1 < 0.01$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 0.244; 0.223; 0.272 $p_2 < 0.01$ $p_3 > 0.05$ $p_4 > 0.05$ | 0.305; 0.271; 0.332 $p_1 < 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 < 0.05$ |
| alkaline phosphatase activity, U/l | 278.0; 244.5; 308.5 $p_3 < 0.01$ $p_4 < 0.01$ | 245.0; 210.5; 292.5 $p_1 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 373.0; 324.0; 406.0 $p_2 < 0.01$ $p_3 > 0.05$ $p_4 < 0.01$ | 226.8; 202.0; 251.3 $p_1 < 0.01$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 332.0; 291.5; 374.5 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 < 0.01$ | 220.4; 194.3; 248.6 $p_1 < 0.01$ $p_2 < 0.01$ $p_3 < 0.01$ $p_4 > 0.05$ | 255.5; 219.2; 307.5 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 195.6; 176.0; 226.5 $p_1 > 0.05$ $p_2 < 0.01$ $p_3 < 0.01$ $p_4 < 0.01$ |
| acid phosphatase activity, U/l | 26.5; 19.9; 35.6 $p_3 < 0.01$ $p_4 < 0.01$ | 28.2; 21.8; 34.3 $p_1 < 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 0.85; 0.78; 0.92 $p_2 > 0.05$ $p_3 > 0.05$ $p_4 < 0.05$ | 1.03; 0.92; 1.20 $p_1 < 0.01$ $p_2 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 0.70; 0.64; 0.77 $p_2 > 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 0.87; 0.81; 0.95 $p_1 < 0.01$ $p_2 < 0.05$ $p_3 > 0.05$ $p_4 > 0.05$ | 0.85; 0.80; 0.92 $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ | 0.96; 0.85; 1.04 $p_1 > 0.05$ $p_2 > 0.05$ $p_3 < 0.01$ $p_4 > 0.05$ |

*Notes:* $p_1$ — comparison of indicators in rats of different ages with the same type of defect filling at the same time after the intervention; $p_2$ — comparison of the indicators of rats of the same age for the same period after the intervention with the alloimplant group without injections of blood plasma enriched with platelets; $p_3$ — comparison of indicators of groups of laboratory rats on the 90$^{th}$ day with indicators of groups of animals of the same treatment conditions on the 14$^{th}$ day; $p_4$ — comparison of indicators of groups of laboratory rats on the 90$^{th}$ day with indicators of groups of animals of the same treatment conditions on the 28$^{th}$ day.

## Discussion

During the experiment, the peculiarities of the course of the reparative process were studied in laboratory rats with hole defects of the metaphysis of the femur, which were filled with alloimplants, accompanying their use with local injections of PRP on the 7$^{th}$ day (single injection); on the 3$^{rd}$ and 7$^{th}$ days (two-time administration); and on the 1$^{st}$, 3$^{rd}$ and 7$^{th}$ days (triple administration).

The results of a biochemical study of the blood serum of white rats with the filling of transcortical bone defects of the femur of a critical size with an alloimplant under the conditions of local administration of PRP showed the effectiveness of additional stimulation, which was reflected in an increase in markers of anabolism and a decrease in indicators of bone tissue catabolism. At the same time, biochemical signs of moderate inflammation were observed, which could slow down the regeneration of bone tis-

sue. This corresponds to the data of Y. Zhang et al. (2021), according to which the general effect of PRP on the treatment of bone fractures remains controversial and is discussed in scientific circles [3]. In particular, PRP administration is considered to be effective, especially for the treatment of bone fractures of the lower extremities [19]. Other authors indicate that the use of PRP did not significantly accelerate the healing of closed long bone fractures [20].

Activation of metabolic processes was observed in rats within 3 months after one PRP injection.

A better response of biochemical parameters to local PRP stimulation was found in 3-month-old animals compared to 12-month-old animals. The introduction of PRP on the 1st day was ineffective and caused the activation of inflammation with a slowdown in regeneration. No significant changes in indicators were recorded on the 3rd day. According to the data of biochemical studies, it can be assumed that the stimulating effect was mostly achieved by the introduction of PRP on the 7th day.

Additional stimulation by local injection of PRP led to a moderate activation of bone healing with the formation of bone tissue. This indicates manifestations of limited reparative capabilities of bone tissue in the studied conditions, possibly because of active inflammation.

In rats with an alloimplant, biochemical signs of moderate activity of bone tissue regeneration were found only in the early stages of the experiment, they decreased on the 28th day and even more on the 90th day. Since under the conditions of additional local administration of PRP to animals with an alloimplant, the expressiveness of biochemical signs of bone tissue regeneration was only slightly greater than in animals with an implant without additional local stimulation, it was determined that it was appropriate to intensify the stimulation of the remodeling of the alloimplant into its own bone tissue by saturating with stimulants, for example, MSC or PRP. This is consistent with the results of a recently published meta-analysis (with the participation of 420 patients), which did not confirm a higher rate of bone recovery according to radiological studies in individuals who had PRP injected into the bone tissue defect simultaneously with an auto-implant, compared to those in the group where only autologous graft was used bone plastic, however, a shorter healing time of a bone fracture by 1.35 months was recorded on average [21].

Additional studies are also needed to fully understand the effectiveness of PRP for the treatment of fractures [3, 22].

## Conclusions

Based on the results of a biochemical study of blood serum of 3- and 12-month-old experimental rats with a critical size defect in the metaphysis of the femur with its filling with bone alloimplants under the conditions of additional stimulation of healing by local administration of platelet-rich blood plasma and without stimulation, it was determined that in animals with an alloimplant without additional stimulation, markers of connective tissue formation increased in the early stages of the experiment (especially in 12- month-old rats), under the conditions of a decrease in the activity of alkaline phosphatase in blood serum. This may indicate a slowdown in the formation of bone tissue. Filling the defect with an alloimplant without additional stimulation led to an increase in biochemical indicators of inflammation, which may be related to the reaction to the implant as a foreign body and to the process of its reconstruction. In animals of both age groups, there was an increase in the values of markers of bone tissue formation, in particular the activity of alkaline phosphatase, the maximum of which was recorded on the 28th day of the experiment with a further decrease, which is a sign of exhaustion of the reparative potential of bone tissue.

With local administration of PRP in rats, a moderate effectiveness of local stimulation was observed, which was reflected in a slight increase in anabolism markers, in particular the activity of alkaline phosphatase, and a decrease in the value of indicators of bone tissue catabolism, and even acid phosphatase activity.

The results of a biochemical study of the blood serum of 3- and 12-month-old white rats with a critical-sized defect in the metaphysis of the femur with filling of the defect with a bone alloimplant showed that additional stimulation of the healing of the defect by local administration of platelet-rich blood plasma is a valid course of action, but expressiveness — insufficient for practical use in the treatment of bone tissue lesions and needs to be strengthened.

**Conflict of interest.** The authors declare no conflict ofinterest.

## References

1. Kale, P., Shrivastava, S., Pundkar, A., & Balusani, P. (2024). Harnessing healing power: A comprehensive review on platelet-rich plasma in compound fracture care. *Cureus.* https://doi.org/10.7759/cureus.52722.
2. Everts, P., Onishi, K., Jayaram, P., Lana, J. F., & Mautner, K. (2020). Platelet-rich plasma: New performance understandings and therapeutic considerations in 2020. *International Journal of Molecular Sciences, 21*(20), 7794. https://doi.org/10.3390/ijms21207794
3. Zhang, Y., Xing, F., Luo, R., & Duan, X. (2021). Platelet-rich

plasma for bone fracture treatment: A systematic review of current evidence in preclinical and clinical studies. *Frontiers in Medicine, 8*. https://doi.org/10.3389/fmed.2021.676033
4. Bezuglov, E., Zholinsky, A., Chernov, G., Khaitin, V., Goncharov, E., Waskiewicz, Z., Barskova, E., & Lazarev, A. (2021). Conservative treatment of the fifth metatarsal bone fractures in professional football players using platelet-rich plasma. *Foot & Ankle Specialist, 15*(1), 62–66. https://doi.org/10.1177/19386400211017368
5. Ghaffarpasand, F, Shahrezaei, M, & Dehghankhalili, M., (2016). Effects of platelet rich plasma on healing rate of long bone non -union fractures: a randomized double-blind placebo controlled clinical trial. *Bulletin of Emergency And Trauma, 4*, 134–140.
6. Coles, C. P. (2020). Open fractures with soft-tissue loss. OTA International: The Open Access *Journal of Orthopaedic Trauma, 3*(1), e053. https://doi.org/10.1097/oi9.0000000000000053
7. Ganji, E., & Killian, M. L. (2018). Tendon healing in the context of complex fractures. *Clinical Reviews in Bone and Mineral Metabolism, 16*(4), 131–141. https://doi.org/10.1007/s12018-018-9254-z
8. Seshan, V., Matua, G. A., Raghavan, D., Arulappan, J., Al Hashmi, I., Roach, E. J., Sunderraj, S. E., & Prince, E. J. (2021). Case study analysis as an effective teaching strategy: Perceptions of undergraduate nursing students from a Middle Eastern country. *SAGE Open Nursing, 7*, 237796082110592. https://doi.org/10.1177/23779608211059265
9. Ehnert, S., Relja, B., Schmidt-Bleek, K., Fischer, V., Ignatius, A., Linnemann, C., Rinderknecht, H., Huber-Lang, M., Kalbitz, M., Histing, T., & Nussler, A. K. (2021). Effects of immune cells on mesenchymal stem cells during fracture healing. *World Journal of Stem Cells, 13*(11), 1667–1695. https://doi.org/10.4252/wjsc.v13.i11.1667
10. Li, S., Xing, F., Luo, R., & Liu, M. (2022). Clinical effectiveness of platelet-rich plasma for long-bone delayed union and Nonunion: A systematic review and meta-analysis. *Frontiers in Medicine, 8*. https://doi.org/10.3389/fmed.2021.771252
11. Jamal, M., Hurley, E., Asad, H., Asad, A., & Taneja, T. (2022). The role of platelet rich plasma and other orthobiologics in bone healing and fracture management: A systematic review. *Journal of Clinical Orthopaedics and Trauma, 25*, 101759. https://doi.org/10.1016/j.jcot.2021.101759
12. Law of Ukraine No. 3447-IV, article 26. On the protection of animals from cruel treatment. Kyiv, 21 February, 2006. (In Ukrainian).
13. European convention for the protection of vertebrate animals used for experimental and other scientific purposes: Strasbourg, 18 March 1986. (2000)
14. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes (Text with EEA relevance). (2010). *Official Journal of the European Union*
15. Tao, Z., Wu, X., Zhou, W., Wu, X., Liao, W., Yang, M., Xu, H., & Yang, L. (2019). Local administration of aspirin with β-tricalcium phosphate/poly-lactic-co-glycolic acid (β-TCP/PLGA) could enhance osteoporotic bone regeneration. *Journal of Bone and Mineral Metabolism, 37*(6), 1026–1035. https://doi.org/10.1007/s00774-019-01008-w.
16. VH, T. (2013). Practical Textbook laboratory medicine: Practical textbook of laboratory medicine.
17. Morozenko, D. V, & Leontjeva, F. S., (2016). Methods of researching markers of connective tissue metabolism in clinical and experimental medicine. *Molodiy vcheniy, 2*(29),168–172. (In Ukrainian)
18. Medical statistics: A textbook for the health sciences, 4th edition by Campbell, M. J., Machin, D., & Walters, S. J. (2008). *Biometrics, 64*(4), 1309-1310. https://doi.org/10.1111/j.1541-0420.2008.01138_14.x
19. SK, S. (2019). Fracture non-union: A review of clinical challenges and future research needs. *Malaysian Orthopaedic Journal, 13*(2), 1–10. https://doi.org/10.5704/moj.1907.001
20. Xu, Z., Hu, H., Wu, B., Huang, C., Liu, Q., & Chen, B. (2022). Efficacy of platelet-rich plasma in the treatment of fractures: *A meta-analysis. computational and mathematical methods in medicine*, 2022, 1–8. https://doi.org/10.1155/2022/5105725
21. An, W., Ye, P., Zhu, T., Li, Z., & Sun, J. (2021). Platelet-rich plasma combined with autologous grafting in the treatment of long bone delayed union or non-union: *A meta-analysis. frontiers in surgery, 8*. https://doi.org/10.3389/fsurg.2021.621559
22. Yaman, R., & Kinard, T. N. (2022). Platelet rich plasma: Hope or hype? *Annals of blood, 7*, 6-6. https://doi.org/10.21037/aob-21-57

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# BIOCHEMICAL PARAMETERS OF THE BLOOD OF RATS OF DIFFERENT AGES AFTER FILLING THE METAPHYSEAL DEFECT OF THE FEMUR WITH ALLOGENEIC BONE IMPLANTS WITH LOCAL ADMINISTRATION OF BLOOD PLASMA ENRICHED WITH PLATELETS

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