

Clinical efficacy of injectable autologous platelet preparations in patients after arthroscopic repair of the anterior cruciate ligament

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Abstract

Background. Platelet-rich plasma (PRP) can be used in the treatment of knee joint defects, including relief of complications in the early postoperative period after arthroscopic repair of the anterior cruciate ligament. Low-pulse laser radiation (LPLR) with wavelength 635 nm

stimulates the platelet activity in PRP and increases their realization of reparative potential.

Objective. *To evaluate the clinical effect of injectable autologous platelet preparations in patients in the early postoperative period after arthroscopic repair of the anterior cruciate ligament.*

Material and methods. *The study included 45 patients, who underwent arthroscopic prosthetics of the anterior cruciate ligament or its fixation to the place of attachment. The patients were divided into 3 groups: in the 1st group (15 subjects) the operation was performed without using platelet preparations (the comparison group); in the 2nd group (15 subjects), non-activated autologous PRP was intraoperatively administered; in the 3rd group (15 subjects), LPLR-activated autologous PRP was intraoperatively administered. The PRP was exposed to LPLR with wavelength 635 nm and power 2 W for 10 minutes. Two ml of the autologous platelet-rich preparation were injected intra-articularly; additionally 1 ml of the same preparation was applied in a fan-like pattern to the surrounding soft tissues.*

Results. *After surgery patients in comparison group had moderate acute pain syndrome, while patients in main groups did not complain about severe pain in the area of surgery. The use of autologous PRP-preparations significantly reduced pain assessments by Visual Analogue Scale by 1.5 times in the 2nd group and 3 times in the 3rd group, comparing to patients without PRP treatment ($p < 0.001$). The administration of analgesics was required in 14 patients of the 1st group, 4 patients of the 2nd group, and 2 patients of the 3rd group. The formation of edema and intra-articular hematoma at surgical site in the 2nd and 3rd groups was less pronounced than in the 1st group.*

Conclusions. *Intra- and periarticular administration of non-activated and LPLR-activated PRP allowed to reduce pain and the incidence of*

hemarthrosis after knee surgery. The use of LPLR-activated PRP demonstrates more pronounced anti-inflammatory and hemostatic effects comparing to non-activated PRP.

Keywords: arthroscopic surgery, platelet-rich plasma, low-pulse laser radiation, pain syndrome, tissue edema, hematoma

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LPLR, low-pulse laser radiation

PRP, platelet rich plasma

VAS, visual analogue scale

Introduction

Currently, arthroscopy is the main diagnostic and treatment method for intra-articular injuries of the knee joint and their consequences. After arthroscopic operations on the knee joint, complications such as synovitis, hemarthrosis, paraarticular edema, and pain syndrome are often reported in the early postoperative period [1]. In this regard, the active search and developments of injectable biological drugs that stimulate reparative processes in the knee joint are underway. The use of drugs based on autologous platelet-rich plasma (PRP) is becoming increasingly popular. To obtain PRP, the patient's blood is centrifuged in order to isolate and concentrate platelets in the plasma; as a result, the concentration of platelets in PRP is 3-5 times higher than their content in blood [2]. Platelets contain various cell growth and differentiation factors, angiogenic factors, cytokines, which can stimulate many reparative

processes in tissues [3]. There are various options of using PRP in clinical practice, including for the treatment of knee joint defects [4–8]. However, so far, the methods of treating knee joint defects using PRP have not been standardized. Human platelets are highly reactive and easily damaged cells, so it is very important to optimize the methods of platelet preparation for clinical use. The preliminary platelet activation has been shown to enhance the clinical effect of PRP-based drugs in the treatment of musculoskeletal defects [5, 6]. However, in some cases, it is not recommended to use inducers widely available in clinical practice (calcium chloride, hydrogen peroxide) to activate platelets. Drugs obtained by PRP cryodestruction (freezing) can cause a significant pain effect when administered by injection [4, 8]. There is a need for preliminary platelet activation without the introducing the additional chemical agents that cause platelet damage.

The use of low-pulse laser radiation (LPLR) seems promising to obtain the PRP-based agents containing activated platelets. Under the effect of LPLR in the visible and infrared range spectrum, the proliferative activity of cells, angiogenesis, the secretion of growth factors and antioxidants increase [9]. The effect of photobiostimulation of cells under the LPLR impact is largely predetermined by the activation of intracellular signaling systems without the direct participation of surface receptors or other inducers of cellular activation [10].

The results we had obtained earlier indicated that the effect of LPLR with a wavelength of 635 nm caused partial degranulation of platelets in vitro, increased the intensity of their biological potential realization [11]. Preparations based on the autologous platelets pre-treated with LPLR could potentially be used for regenerative medicine purposes.

The study objective was to compare the clinical effect of injectable autologous platelet-based preparations in patients in the early

postoperative period after an arthroscopic anterior cruciate ligament reconstruction.

Material and methods

The study was approved by the Biomedical Ethics Committee of the N.V. Sklifosovsky Research Institute for Emergency Medicine, Decision No. 6-24 dated June 11, 2024, and conducted with the framework of a Scientific and Practical Project Agreement No. 1603-22/23 dated 21.04.2023. The study included 45 patients: 20 men and 25 women, aged from 19 to 45 years old. The inclusion criteria for the study were: the presence of a knee joint injury with a rupture of the anterior cruciate ligament along with a bone fragment and(or) a rupture of the anterior cruciate ligament with damage to the meniscus. After the patient examination and the diagnosis verification, all patients underwent arthroscopic surgery to replace the anterior cruciate ligament of the knee joint or fix it to the attachment site. Each patient who met the inclusion criteria for the study signed a voluntary informed consent for the administration of autologous platelet-based preparations into the knee joint.

For preparing the autologous PRP, 2 hours before surgery, patient's blood in the amount of 30–40 ml was collected from the cubital vein using a vacuum system that included a double-ended needle VISIO Plus, green standard (needle size 0.8x38 mm, 21Gx1 1/2), a disposable BD Vacutainer holder, VACUETTE 4 ml size threadless tubes for hematological studies, containing ethylenediaminetetraacetic acid preservative. Autologous PRP was isolated from blood by two-stage centrifugation: the initial blood was centrifuged for 5 minutes at 300 g, the supernatant plasma with platelets was collected and centrifuged for 17 minutes at 700 g to sediment and concentrate the platelets. Most of the platelet-poor plasma was collected from the test tube, and then the

platelet sediment was resuspended in the remaining plasma volume. As a result, PRP with a cell concentration of more than $1000 \times 10^9/L$ was obtained. The patient blood amount of 30–40 ml was needed to obtain 3.0–3.5 ml of PRP. To assess the quality of platelets, 100 μL were separated. The remaining volume was exposed to LPLR of 635 nm wavelength and a power of 2 W for 10 minutes, after which an aliquot of 100 μL was again taken to assess the activation of platelets resulted from the LPLR impact. The PRP was exposed to irradiation through the open cover of a 15 ml laboratory plastic test tube for 10 minutes under conditions of immobility of the test tube, maintaining sterility.

Platelets were examined using vital fluorochrome dyes and fluorescence microscopy [11, 12]. During the study, the total concentration of platelets (thousands/ μL) in the PRP was assessed, as well as the concentration of platelets with granules (structurally and functionally active platelets; thousands/ μL) in the PRP before and after exposure to LPLR. During the activation under the exposure to LPLR, a significant shift of platelet granules to the platelet periphery occurs, as well as the binding of granules to the cell boundary, and a partial exit to outside. This process is also accompanied by an increase in the platelet diameter.

The patients were randomly allocated into three groups. The groups were differentiated depending on the intraoperative administration of platelets: the 1st group (the comparison group) included 15 subjects in whom the surgery was performed without using platelet preparations; the 2nd group also included 15 subjects in whom non-activated PRP was administered intraoperatively; and the 3rd group of 15 subjects where LPLR-activated PRP was administered intraoperatively. The groups were comparable in terms of gender and age characteristics and the nature of pathology. The total platelet count and the count of platelets with granules

in PRP at baseline did not differ between the 2nd and 3rd groups (Table 1). On average, 24% of all PRP platelets with granules were activated via exposure to LPLR.

Table 1. Initial characteristics of platelets activated in low-pulse laser radiation, and non-activated platelet-rich plasma

Parameter	Non-activated PRP Me (Q ₁ ;Q ₃)	LPLR-activated PRP Me (Q ₁ ;Q ₃)
Total platelet concentration (TPC) in PRP, thousand/ μ L	1160 (1100;1300)	1200 (1065;1284)
Content of platelets with granules in PRP, thousand/ μ L	557 (480;685)	570 (460;703)

All patients underwent arthroscopic surgery of the knee joint. Depending on the damaged structures and the injury location, the following operations were performed: meniscus resection, meniscus suturing, anterior cruciate ligament reconstruction, anterior cruciate ligament refixation. At the final stage of the surgery, after suturing the postoperative wounds, in patients of groups 2 and 3, 2 ml of the autologous platelet preparation were injected intra-articularly (Fig. 1) into the sutured medial or lateral arthroscopic port by means of a disposable medical injection needle 0.8x40 mm 21G for Luer syringes, using a 5 ml disposable injection syringe, tip type: LUER-SLIP, with the preparation pre-filled (Fig. 1) and 1 ml was injected into the soft tissues in a fanlike fashion (Fig. 2).



Fig. 1. Intra-articular administration of platelet-rich plasma intraoperatively



Fig. 2. A fan-like PRP injections into soft tissues in the zone of the lateral arthroscopic port

On the first day after the surgical treatment, the pain level was assessed in points by a Visual Analogue Scale (VAS) and the need for additional administration of painkillers considered. The change in the limb circumference in the postoperative period was also determined. For this purpose, the sizes (circumferences) in centimeters of the thigh, knee joint and lower leg were measured before surgery and on the first day after it and the differences between them were calculated. (Fig. 3). The hematoma volume was assessed by ultrasound examination in cm^3 or by puncture in milliliters.

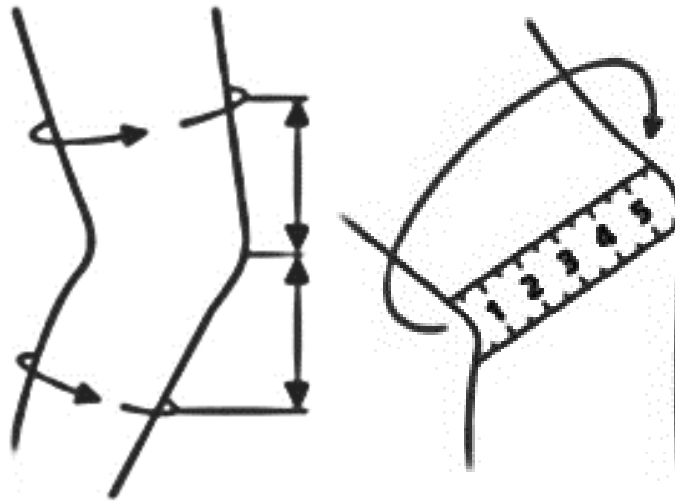


Fig. 3. The scheme of measuring anatomical zones using a measuring tape

The obtained data were processed using the variation statistics methods using the Statistica 10.0 software. The distribution was tested for normality using the Kolmogorov–Smirnov test. In case of abnormal distribution, the differences between the groups were assessed using the Mann–Whitney test, and in case of normal distribution, the Student’s t-test was used. Fisher’s exact test was used to compare qualitative variables. Differences were considered statistically significant at a significance level of over 95% ($p < 0.05$). The data are presented as median (Me), the 1st and 3rd quartiles (25%;75%), or as the mean (M) and the standard error of the mean (SEM).

Results and discussion

During the first 24 hours after surgery, patients in the comparison group experienced a moderately pronounced acute pain syndrome (mean 5–6 points by VAS) characterized by pressing pain sensations in the knee joint area. Unlike the comparison group, patients in the main groups did not complain of severe pain sensations in the surgical wound area.

Subjective assessment of pain intensity in patients in the 2nd group varied within 3–4 points by VAS, while in patients in the 3rd group this variance did not exceed 1–2 points.

To relieve postoperative pain, 14 patients in the group not receiving platelet preparations required a combination therapy (narcotic and non-narcotic analgesics) or monotherapy (narcotic analgesics). One patient received three doses of narcotic analgesics, three patients received two doses, and 10 patients received a single dose on demand. In the non-activated PRP group, a single use of narcotic analgesics was necessary in 4 patients. In the LPLR-activated PRP injection group, narcotic drugs were administered to only 2 patients.

From all of the above, we can conclude that preliminary activation of platelets using LPLR statistically significantly reduces the pain syndrome and patient's need for painkillers on the first day after surgery (Table 2).

Table 2. Pain assessment using visual analogue scale and quantitative analgesia in patients of all groups

Evaluation criteria	Comparison group (PRP-free)	Non-activated PRP (2 ml intra-articularly + 1 ml by soft tissue injection)	LPLR-activated PRP (2 ml intra articularly + 1 ml by soft tissue injection)
Quantitative pain assessment by VAS after completing arthroscopy, Me (Q ₁ ;Q ₃)	6 (5;6)	4 (3;4) p<0.001 (U-test)	2 (1;2) p<0.001 p ₁ <0.001 (U-test)
Number of patients who needed painkillers (tramadol, morphine, promedol) after administering PRP required painkillers (tramadol, morphine, promedol) after PRP administration	14 of 15 (93.33%)	4 of 15 (26.67%) p<0.001, Fisher test	2 of 15 (13.34%) p<0.001 p ₁ =0.649 Fisher test

Notes: p, significant differences compared to patients in group 1; p₁, significant differences between patients of groups 2 and 3

The results of the comparative analysis of the size (circumference) measurements obtained in three anatomical zones of the lower limb before and after surgery and the calculation of their differences are presented in Table 3.

Table 3. Assessing the knee joint volume and the intra-articular hematoma formation on the 1st postoperative day in patients of all groups

Evaluation criteria		Comparison group (PRP-free)	Non-activated PRP (2 ml intra-articularly + 1 ml by soft tissue injection) M±SEM	LPLR-activated PRP (2 ml intra-articularly + 1 ml by soft tissue injection) M±SEM
Difference in coverage of the anatomical zone before and after surgery, cm	Hips	1.87±0.08	1.38±0.07 p<0.001	1.19±0.07 p<0.001 p ₁ = 0.073
	Knee joint	1.67±0.04	1.34±0.06 p<0.001	1.19±0.03 p<0.001 p ₁ =0.037
	Shins	1.69±0.10	1.21±0.03 p< 0.001	1.11±0.03 p<0.001 p ₁ =0.029
Volume of evacuated hemorrhagic contents during knee joint puncture, ml		In 7 patients from 70 to 90 ml	In 4 patients from 50 to 80 ml	In 2 patients 50 ml

Notes: p, statistically significant differences compared with data from patients in group 1 (p<0.05); p₁, statistically significant differences between the data of patients in groups 2 and 3 (p<0.05)

Table 3 shows that on the first day the patients developed post-traumatic edema at the site of surgery. At the same time, in the groups where platelet preparations were used (Groups 2 and 3), edema in the area of surgery in patients was significantly less pronounced compared to the data in the comparison group. Meanwhile, the most pronounced anti-inflammatory effect was observed in patients of Group 3, where LPLR platelet activation had additionally been used.

The presence of fluid collection in patients in the postoperative period was verified by diagnostic ultrasound examination (Fig. 4). Analysis of the

hemarthrosis incidence demonstrated differences between the groups. In the comparison group, hemarthrosis was diagnosed in 7 patients (46.6%), they required puncture of hemorrhagic contents (the volume of evacuated fluid was 70–90 ml). In the group of patients who received non-activated PRP, fluid collection requiring puncture was recorded in 4 patients (26.6%). The lowest incidence of hemarthrosis (in a volume of 50 ml) was noted in 2 patients (13.3%) in the 3rd group (Table 3).



Fig. 4. Ultrasound examination of the upper knee joint to visualize fluid collections: A, without platelet-rich plasma; B, after administering non-activated platelet-rich plasma; C, after administering platelet-rich plasma activated by low-pulse laser radiation

Thus, the obtained data have demonstrates a pronounced anti-inflammatory and hemostatic effect of platelet preparations, especially with their preliminary activation by LPLR. The relief of the inflammatory reaction can be largely predisposed by the action of anti-inflammatory cytokines, which are formed during the platelet activation [3]. It should be emphasized that many anti-inflammatory cytokines are initially absent in platelet granules and are formed during changes in platelet morphology associated with the effect of activating signals [13, 14]. Preliminary LPLR-induced PRP activation enhances the reparative effect. It can be

assumed that LPLR stimulates not only the platelet activation in vitro in the absence of chemical or biological inducers of activation, but also stimulates the production of anti-inflammatory cytokines in platelets. There are different ways of launching platelet activation, in which structural changes in platelets occur differently, which can significantly affect the rate and nature of the implementation of platelet reparative and regenerative potential [2, 13]. The components of platelet granules have a broad and multidirectional effect, and very often their positive effect is synergistic [3, 15], i.e., to potentiate reparative regeneration, the combination of factors is needed rather than individual factors. Provided that the quality of platelets is normal, the autologous PRP is potentially a very valuable reservoir of biologically active substances [2, 11-15].

Conclusions

1. Intra- and periarticular administration of non-activated platelet-rich plasma helps to reduce the severity of pain syndrome by mean of 33% (1.5 times) as assessed by a visual analogue scale, whereas the use of low-pulse laser-radiation-activated platelet-rich plasma provides a more pronounced effect, namely, a 3-fold pain reduction ($p < 0.001$, statistically significant).

2. In 73% of patients who received non-activated platelet-rich plasma and in 86% of patients after administration of low-pulse laser-radiation-activated platelet preparation, there was no need to use narcotic analgesics in the early postoperative period.

3. Postoperative swelling in the knee joint area in patients in the group of low-pulse laser-radiation-activated platelet-rich plasma was 1.7 times less pronounced compared to the data of the non-platelet-rich plasma group/treatment group without platelet-rich plasma ($p < 0.001$, statistically significant). The hemarthrosis incidence, decreased by 1.75

times when using non-activated platelet-rich plasma, and by 3 times when using low-pulse laser-radiation-activated platelet-rich plasma ($p < 0.01$, Fisher's exact test, statistically significant).

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