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Preliminary Report

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Possibilities of Therapeutic Angiogenesis in Patients with Critical Lower Limb Ischemia

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ABSTRACT Two clinical observations of effective treatment of patients with critical ischemia of the lower extremities using plasma-free lysate autologous platelets. Both patients were male, 73 and 53 years old. Both were diagnosed with total damage to the arteries of the infrainguinal zone. One of them had previously undergone endarterectomy from the femoral artery with subsequent thrombosis. After examination and joint discussion with x-ray endovascular surgeons, they were found inoperable. Due to the ineffectiveness of standard conservative therapy, the patient was offered local administration of plasma-free lysate autologous platelets. After receiving written informed consent, the procedure for collecting venous blood and obtaining plasma-free lysate autologous platelets and the introduction of this drug into the muscles of the leg from the side of ischemia according to the original method. The method for assessing microcirculation was three-phase scintigraphy: before treatment, both patients showed a significant decrease in the inclusion of osteotropic radiopharmaceutical in the soft tissues of the legs. For areas of radiopharmaceutical hyperfixation, relative accumulation coefficients were calculated: for one patient, the calculation results demonstrated the formation of foci of aseptic necrosis.

When patients were re-hospitalized after 6 months, there was an improvement in local status and relief of rest pain. According to scintigraphy, a significant improvement in microcirculation was noted; no foci of aseptic necrosis were identified.

Thus, the use of plasma-free lysate autologous platelets in combination with complex conservative therapy has demonstrated positive results; this method can be considered as an alternative revascularization ischemic limb.

Keywords: critical ischemia of the lower extremities, therapeutic angiogenesis, plasma-free lysate autologous platelets, conservative therapy, triphasic scintigraphy

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BP — bone phase
CAD — coronary artery disease
CLLI — critical lower limbs ischemia
DFA — deep femoral artery
EDTA — ethylenediaminetetraacetic acid
PFLAP — plasma-free lysate of autologous platelets
RAC — relative accumulation coefficient
RFP — radiopharmaceutical
SFA — superficial femoral artery
TP — tissue phase

INTRODUCTION

The optimal treatment for critical lower limb ischemia (CLLI) is revascularization, open surgical or endovascular [1, 2]. However, patients with CLLI are characterized by a high incidence of unsatisfactory condition of the distal arterial bed, and direct revascularization in such patients is feasible only in 50–75% of cases [3, 4]. Standard conservative therapy for CLLI leads to short-term improvement or is completely ineffective [5].

A breakthrough in the treatment of CLLI was the development of methods of therapeutic angiogenesis aimed at developing collateral circulation through stimulation with growth factors. To induce angiogenesis, both angiogenic factors and stem cells are used [6].

A promising method in the treatment of CLLI is the use of autoplatelet proangiogenic growth factors. It is known that platelets contain proangiogenic factors, in particular vascular endothelial growth factor (*VEGF*) and fibroblast growth factor (*FGF*) [7, 8].

However, to date, there are quite a few studies on the use of platelet-rich media in chronic lower limb ischemia (CLLI). In addition, these studies examined the effect of using autologous platelet-rich plasma in patients with intermittent claudication after revascularization [9, 10]. For patients with CLLI, good results have been reported with the local use of autoplatelet [11]. But there are no reports of special developments in the use of growth factors isolated from autologous platelets in inoperable patients with CLLI as an independent therapeutic treatment.

As part of a joint study by the Department of Vascular Surgery and the Department of Biotechnology, our institution has developed an original method for using plasma-free autologous platelet lysate (PFAPL) in inoperable patients with CLLI. This technique was used on 68 patients with CLI. Below are two clinical observations.

The aim of study is to demonstrate the effectiveness of clinical examples of the possibility of successfully carrying out therapeutic angiogenesis in patients with CLI through selective intramuscular administration of PFAPL.

MATERIAL AND METHODS

Clinical observations were carried out in 2020–2021 at the departments of emergency vascular surgery, the department of biotechnology and transfusiology, and the department of radioisotope diagnostics.

The therapeutic angiogenesis procedure was offered to patients due to the lack of results from conservative therapy and the threat of loss of the lower limb due to the progression of CLLI. The procedure was performed after obtaining voluntary informed consent.

To calculate the dose of the drug and ensure the possibility of its administration directly into ischemic tissues (selectively), patients were examined by three-phase scintigraphy with an osteotropic radiopharmaceutical (RP) 99 *m Tc-pyrfotech (dose 500 MBq, radiation dose 2.85 mSv) on a Discovery* 670 hybrid scanner *NM/CT* (*GE*, USA). Processing of the results with assessment of the nature, location and volume of the lesion (according to the degree of accumulation of radiopharmaceuticals) was carried out on the *XELERIS* workstation.

Signs of microcirculation disorders included: a decrease or absence of accumulation of radiopharmaceuticals in the phase of tissue blood flow, zones of hyperfixation of radiopharmaceuticals in the tissue phase with an increase in uptake in the bone phase, corresponding to areas of developing aseptic necrosis (this sign was assessed visually and by the values of the relative accumulation coefficient (RAC) of radiopharmaceuticals in both phases of the study).

The calculation of the lesion volume was carried out according to the method outlined in patent RU 2759478 C1 dated November 15, 2021. In the case of diffuse ischemic lesions, the truncated cone formula was used, and in the case of focal microcirculation disorders, the ellipsoid volume formula was used.

Calculation of the volume of platelet suspension (with a concentration of at least 1000×10^9 cells/l and the proportion of functional cells of at least 38%) for the preparation of an autologous cell-free preparation was made according to the formula V_{drug} = "volume of the ischemic zone" × 0.05, where 0.05 is optimal dose of the drug (ml) for every 1 cm^3 of the volume of the ischemic zone (data obtained from a study on cell culture of the growth-stimulating effect of plasma-free platelet lysate). Then the amount of venous blood of the patient (V_{blood}, ml) required to form a suspension of the previously calculated volume was calculated using the empirical formula $V_{blood} = V_{drug} \times 10$.

Plasma-free lysate of autologous platelets was prepared by the method presented in the materials of the invention according to patent RU2739515. To do this, blood was taken from the patient's cubital vein into sterile tubes with ethylenediaminetetraacetic acid (EDTA) in a previously calculated volume (V_{blood}). Platelets were isolated using standard methods with two centrifugations at 300 and 700 g, respectively. After the formation of a platelet sediment, the depleted plasma was completely removed from the test tube and a sterile physiological solution of 0.9% NaCl was added in an amount equivalent to the calculated dose of the drug (V_{drug}). The contents of the tube were thoroughly mixed and frozen at –40°C. Thawing of platelets in a plasma-free environment was carried out at +4°C for 12 hours. After thawing, the tube was centrifuged at 3000 g for 20 minutes. The cell-free supernatant was collected for intramuscular injection.

The drug was administered in a clean manipulation room, with patients lying on their back or stomach. Areas corresponding to microcirculation disorders were previously marked on the skin using scintigraphy data. The drug was injected so that the drug reached both ischemic and border tissues (Fig. 1).

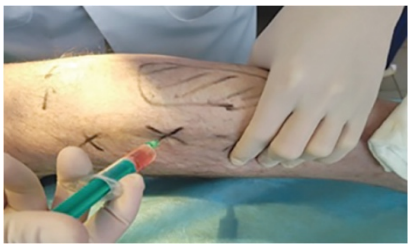

Fig. 1. Preliminary marking of the greatest microcirculation disturbance based on scintigram data for selective administration of plasma-free lysate autologous platelets

Six months after the procedure, patients were re-examined using three-phase scintigraphy with osteotropic radiopharmaceuticals, and the nature, location and volume of the lesion were also assessed. Based on the difference between the original and new data, the dynamics of the process and the effectiveness of the therapeutic angiogenesis procedure were assessed.

To demonstrate the effectiveness of PFAPL use, we present two clinical observations.

Clinical observation 1

Patient S., 73 years old, diagnosed with: "Atherosclerosis. Occlusion of the superficial femoral artery, popliteal artery, and left leg arteries. Critical ischemia of the left lower limb."

Complaints: pain at rest in the left lower limb, decreased sensitivity in the toes of the left foot.

Medical history: suffered from intermittent claudication for a long time, pain at rest in the left lower limb was noted during the last 6 months before admission. Repeatedly underwent courses of conservative therapy without significant improvement. Concomitant diseases: coronary artery disease (CAD): post-infarction cardiosclerosis; smoker's chronic bronchitis.

Locally: the left lower limb is pale in color, the foot is with congestive hyperemia, cool to the touch. Movements are preserved, sensitivity in the foot is reduced. The calf muscles are soft, somewhat painful on palpation. The pulsation of the main arteries is determined at the level of the inguinal fold, but is not determined distally. The right lower limb is normal in color and warm to the touch. Movement and sensation are preserved. The calf muscles are painless on palpation. The pulsation of the main arteries is determined in the popliteal fossa, but not more distally.

CT angiography: on the left revealed occlusion from the level of the distal part of the common femoral artery with a transition to the initial part of the deep femoral artery (DFA), the superficial femoral artery (SFA) to the left of the mouth along its entire length, the popliteal artery, lack of contrast of the arteries of the leg on the left, the anterior tibial arteries (ATA) on the right, signs of calcification of the arteries of the lower extremities (Fig. 2).

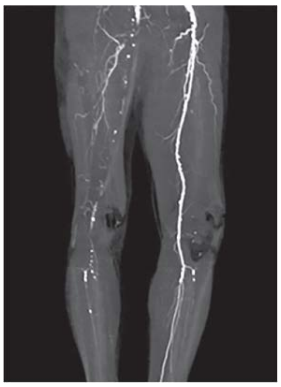

Fig. 2. CT-angio scan of patient 1

The patient was examined together with x-ray endovascular surgeons: given the extensive total damage to the arterial bed of the left lower limb, there are no conditions for performing reconstructive vascular and endovascular surgery. The patient was offered a therapeutic angiogenesis procedure. After obtaining voluntary informed consent, in preparation for receiving PFAPL, three-phase scintigraphy of the soft tissues and bones of the lower extremities was performed with osteotropic radiopharmaceutical ^{99m}Tc-pyrfotech.

Study protocol. In the tissue and bone phases, both lower limbs are visualized along their entire length in both phases. At the level of the hips, the blood flow is symmetrical. On the left, in both phases of the study, diffusely increased accumulation of radiopharmaceuticals is noted (RAC = 1.3 in the tissue phase and 2.0 in the bone phase), against the background of which, at the level of the upper third of the left leg in the projection of the posterior group of muscles, a zone of focal hyperfixation of radiopharmaceuticals with RAC is recorded 1.9 in the tissue phase with an increase to 5.7 in the bone phase (forming necrotic changes) (Fig. 3).

The volume of radiopharmaceutical hyperfixation foci was calculated using the ellipsoid volume formula. The total volume of ischemic foci was 80 cm^3. The dose of PFAPL was determined and was 80×0.05 ml = 4 ml.

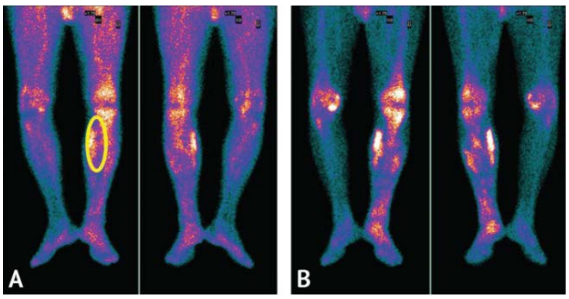

Fig. 3. Patient S. A – tissue phase, B – bone phase. Zone of focal hyperfixation radiopharmaceutical is outlined in an ellipse

The amount of patient's venous blood required to form the drug was 40 ml. Next, PFAPL was obtained using standard methods.

The procedure for administering the drug: the patient was lying on his stomach, the skin of the left leg in the projection of the posterior muscle group was extensively processed in accordance with the rules of asepsis and antisepsis. Previously, the points for drug administration, especially in the projection of developing necrosis, were marked with a skin marker. The drug was administered with a syringe through a needle to a depth of 5 cm. After all injections, an aseptic dressing was applied. No local complications were noted.

The patient was discharged in satisfactory condition after 5 days. Repeated hospitalization after 6 months.

Complaints: pain in the left lower limb when walking 200 m.

Locally: the left lower limb is of normal color, warm to the touch. Movement and sensation are preserved. The calf muscles are painless on palpation. The pulsation of the main arteries is determined at the level of the inguinal fold, but is not determined distally. The right lower limb is normal in color and warm to the touch. Movement and sensation are preserved. Calf muscles with painless palpation. The pulsation of the main arteries is determined in Scarp's triangle.

Three-phase scintigraphy of soft tissues and bones with osteotropic radiopharmaceutical ^{99m}Tc-pyrfotech: in the tissue and bone phases, both lower extremities are visualized along the entire length in both phases. At the level of the hips, the blood flow is symmetrical. On the left, in the upper half of the leg in the projection of the posterior group of muscles, the zone of hyperfixation of radiopharmaceuticals in the tissue phase (RAC = 2.7) remains without its growth in the bone phase (RAC 1.7), which indicates the involution of the developing aseptic necrosis. Zones of absence of microcirculation are not determined (Fig. 4).

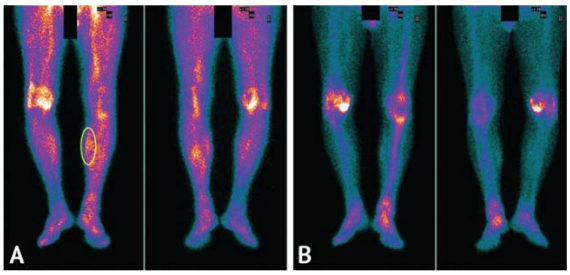

Fig. 4. Patient S. A – tissue phase; B – bone phase. Repeated study in 6 months

Clinical observation 2

Patient K., 53 years old, diagnosed with: "Atherosclerosis. Occlusion of the left superficial femoral artery. Occlusion of the left popliteal artery, arteries of the left leg. Critical ischemia of the left lower limb."

Complaints: coldness and pain in the left lower limb at rest, a feeling of numbness in the foot.

Medical history: suffers from intermittent claudication for a long time. Five years before admission, the patient underwent thrombectomy from the left superficial femoral artery with plastic repair with a synthetic patch. Present deterioration within 4 months before admission. He underwent courses of conservative therapy without significant effect. Concomitant diseases: CAD. Paroxysmal form of atrial fibrillation. Arterial hypertension. Bronchial asthma. Diabetes mellitus type 2, insulin-insensitive. Obesity.

Locally: the left foot is pale pink in color, cooler to the touch than the right. The saphenous veins of the foot are poorly filled. Movements in the foot are preserved. Sensitivity in the foot is reduced. The calf muscles are not tense and are slightly painful on palpation. The pulsation of the main arteries is detected in the femoral triangle, but is absent distally. There is a postoperative scar in the lower third of the left thigh. The right lower limb is normal in color and warm to the touch. Movements and sensitivity are preserved. The calf muscles are not tense and are painless on palpation. The pulsation of the main arteries is determined at all levels.

CT angiography: on the left there is occlusion of the SFA from the mouth, occlusion of the initial part of the DFA, the arteries of the leg are not contrasted, calcification of the arteries of the lower extremities (Fig. 5).

The patient was examined together with x-ray endovascular surgeons: given the extensive total damage to the arterial bed of the left lower limb, atheroscalcinosis due to diabetic angiopathy, there are no conditions for performing reconstructive vascular surgery and endovascular intervention.

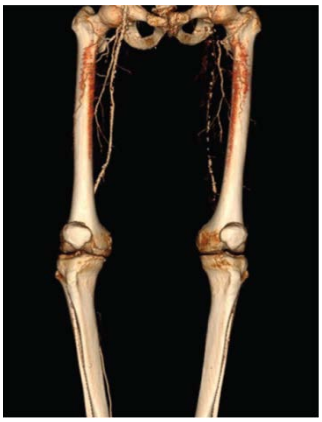
Fig. 5. CT-angio scan of patient 2

The patient was offered a therapeutic angiogenesis procedure. After obtaining voluntary informed consent, in preparation for receiving PFAPL, three-phase scintigraphy of the soft tissues and bones of the lower extremities was performed with osteotropic radiopharmaceutical ^{99m}Tc-pyrfotech.

Study protocol: radiopharmaceutical accumulation at the hip level is symmetrical. On the left, in the area of the proximal and middle thirds of the leg, there is a moderate decrease in the accumulation of radiopharmaceuticals (RAC 0.8–0.87), an area of impaired tissue blood flow; foci of necrosis and areas of lack of blood flow are not determined. At the level of the tarsus of the left foot, the accumulation of the indicator is moderately increased in both phases (RAC 1.17), which is more likely associated with infiltrative changes and degenerative-inflammatory changes in the joints of the foot (Fig. 6).

Due to the diffuse nature of changes in tissue blood flow, the volume of the area of ischemic changes was calculated using the formula for the volume of a

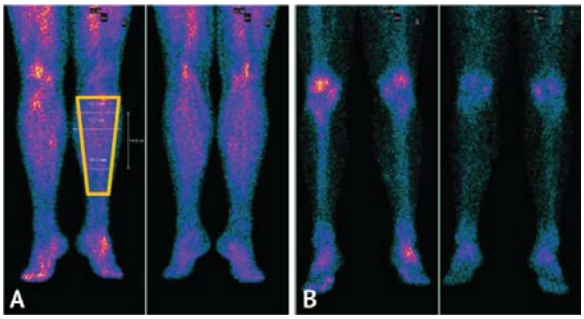

Fig. 6. Patient K. Three-phase scintigraphy with 99 m Tc-pyrfotech (A – tissue phase, B – bone phase), diffuse decreased accumulation of a radiopharmaceutical in the soft tissues of the proximal and middle thirds of the leg in the phase of tissue blood flow; in the bone phase, no foci of hyperfixation suspicious for areas of necrosis were identified

803×0.05=40 ml. The amount of patient venous blood required to formulate the drug according to the truncated cone. By calculation, the volume of the ischemic zone was 803 cm3 . The dose of PFAPL was empirical form was 400 ml. However, due to the fact that the concentration of platelets in the patient's blood was 390 cells × 10^9 /l with a proportion of complete platelets of 49%, it was sufficient to take 200 ml of blood to achieve the required number of functionally complete platelets. The patient had 200 ml of blood taken from the cubital vein into tubes with EDTA anticoagulant while maintaining sterility.

Next, the PFAPL preparation was obtained according to the standard method in a volume of 20 ml.

The procedure for administering the drug: the patient was lying on his stomach, the skin of the left leg in the projection of the posterior muscle group was extensively processed in accordance with the rules of asepsis and antisepsis. The points for drug administration were previously marked with a skin marker. The drug was administered with a syringe through a needle to a depth of 5 cm. After all injections, an aseptic dressing was applied. No local complications were noted.

The patient was discharged in satisfactory condition after 5 days. Readmission after 6 months.

Complaints: pain in the left lower limb when walking 50–70 m.

Locally: The left lower limb is of normal color and warm to the touch. Movement and sensation are preserved. The calf muscles are painless on palpation. The pulsation of the main arteries is determined at the level of the inguinal fold, where it is weakened, and is not determined more distally. The right lower limb is normal in color and warm to the touch. Movement and sensation are preserved. The calf muscles are painless on palpation. The pulsation of the main arteries is determined in Scarp's triangle.

Three-phase scintigraphy of the lower extremities with osteotropic radiopharmaceutical 99 m Tc-pyrfotech: in the TV and CF phases on the left and right, the accumulation of radiopharmaceuticals is determined at all levels up to the feet inclusive. The distribution of radiopharmaceuticals is quite symmetrical; normalization of RAC values is noted in both phases of the study (RAC 0.97–1.0). Zones of lack of microcirculation and foci of radiopharmaceutical hyperfixation (foci of necrosis) are not determined (Fig. 7).

The table presents summary data of semi-quantitative indicators for both patients and their changes over time.

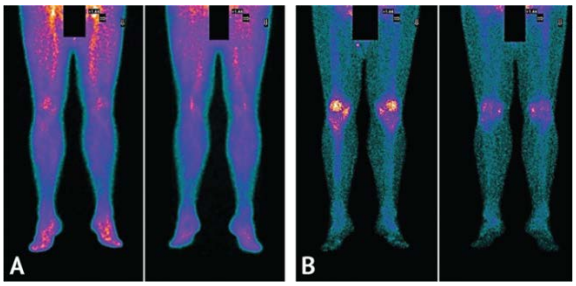

Fig. 7. Patient K., repeated study in 6 months. Three-phase scintigraphy with 99m Tc-pyrfotech (A – tissue phase, B – bone phase). In the tissue and bone phases on the left and right, the accumulation of radiopharmaceuticals is determined at all levels including the feet

Table

Semi-quantitative data

	Patient 1	Patient 2
RAC TP1	1.9	0.8
RAC TP2	**2.7**	1.0
RAC BP1	**5.7**	0.9
RAC BP2	**1.7**	0.9

Notes: RAC – relative accumulation coefficient; BP – bone phase; TP – tissue phase. Index 1 – before treatment. Index 2 – repeated study in 6 months

CONCLUSION

These clinical observations demonstrate an improvement in blood circulation and thus a decrease in the degree of ischemia in patients with critical limb ischemia when treated with plasma-free autologous platelet lysate. In both patients, after a follow-up period of 6 months, a positive clinical effect was achieved, which was confirmed by scintigraphy data.

Therapeutic angiogenesis through the injection of plasma-free autologous platelet lysate into the calf muscles can be considered as an alternative revascularization of the ischemic limb. The developed method for assessing tissue ischemia and the volume of foci of necrosis makes it possible to calculate the volume of the required amount of the drug and the areas of its preferential administration. This method can significantly increase the effectiveness of treatment of critical ischemia of the lower extremities even in inoperable patients.

It should be noted that the use of plasma-free lysate of autologous platelets is economically more profitable compared to other methods of therapeutic angiogenesis (autotransplantation of stem cells, use of genetically engineered drugs).

Thus, the results obtained demonstrate the need for additional clinical research in this direction.

REFERENCES

1. Chervyakov YV, Vlasenko ON, Kha KN. Five-Year Conservative Therapy Results in Patients with Lower Extremity Arterial Atherosclerosis at Critical Ischemia Stage. *Perm Medical Journal*. 2017;34(5):20–27. (in Russ.) https://doi.org/10.17816/pmj34520-27
2. Kozlovsky BV, Mikhailov IP, Isayev GA, Kudryashova NE, Leshchinskaya OV. Evaluation of the Efficacy of Surgical Treatment of Patients with Chronic Critical Ischemia of Lower Extremities at the Stage of Trophic Complications. *Russian Sklifosovsky Journal Emergency Medical Care*. 2020;9(4):545–550. https://doi.org/10.23934/2223-9022-2020-9-4-545-550
3. Zubova ES, Vavilov VN, Artyushin BS, Movchan KN, Krutikov AN, Romanenkov NS, et al. Possibilities of the Application of Hemopoetic Cells of Monocytary Series in the Treatment of Patients with Critical Limb Ischemia. *Modern Problems of Science and Education*. 2019;(3). (In Russ.) Avalable at: https://science-education.ru/ru/article/view?id=28990 [Accessed Nov 2, 2022]
4. Reinecke H, Unrath M, Freisinger E, Bunzemeier H, Meyborg M, Lüders F, et al. Peripheral arterial desiase and critical limb ischemia: still poor outcomes and lack of guideline adherence. *Eur Heart* J. 2015;36(15):932–938. PMID: 25650396 https://doi.org/10.1093/eurheartj/ehv006
5. Kret MR, Perrone KH, Azarbal AF, Mitchell EL, Liem TK, Landry GJ, et al. Medical comorbidities but not interventions adversely affect survival in patients with intermittent Claudication. *J Vasc Surg*. 2013;58(6):1540–1546. PMID: 23972525 https://doi.org/10.1016/j.jvs.2013.07.012
6. Conte MS, Bradbury AW, Kolh P, White JV, Dick F, Fitridge R, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia. *Eur J Vasc Endovasc Surg* . 2019;58(1S):S1–S109. PMID: 31182334 https://doi.org/10.1016/j.ejvs.2019.05.006
7. Sukovatykh BS, Orlova AYu. Stimulation of Angiogenesis by Bone Marrow Cells in Experimental Ischaemia of Lower Limb. *Angiology and Vascular Surgery* . 2017;23(1):43–50. (In Russ.)
8. Martínez CE, Smith PC, Alvarado VAP. The influence of platelet-derived products on angiogenesis and tissue repair: a concise update. *Front Physiol* . 2015;6:290. PMID: 26539125 https://doi.org/10.3389/fphys.2015.00290
9. Sukovatyh BS, Orlova AYu, Artyushkova EB. Influence of plasma enriched with platelets and of the preparation "Myelopid" on the course of acute and chronic ischemia of the lower limbs. *News Khirurgii* . 2012;20(2):41–48. (In Russ.)
10. Dragunov AG, Aleksandrov YuV, Khripunov SA. Primenenie vnutritkanevogo vvedeniya autoplazmy, obogashchennoy trombotsitami (AOT), pri lechenii ishemii nizhnikh konechnostey. *Angiology and Vascular Surgery*. 2008;14(4):17–21. (In Russ.)
11. Goshchynsky V, Migenko B, Lugoviy O, Migenko L. Perspectives on Using Platelet-Rich Plasma and Platelet-Rich Fibrin for Managing Patients with Critical Lower Limb Ischemia After Partial Foot Amputation. *J Med Life*. 2020;13(1):45–49. PMID: 32341700 https://doi.org/10.25122/jml-2020-0028

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