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## Combined Method of Treatment of Ischemic Trophic Ulcers.

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### ABSTRACT

To restore damaged tissues, the effectiveness of growth factors contained in platelets has been proven, which can also be used to treat wounds with delayed regeneration. For this purpose, it is suggested to use plasma enriched with platelets. The prospect of treatment is the use of donor thromboconcentrate. In conditions of disturbed tissue trophism, collagen preparations are used as a stimulation factor for tissue regeneration. In this study, the effectiveness of using a combination of platelet-enriched donor plasma and collagen preparations for topical treatment of ischemic trophic ulcers was evaluated. During the treatment, the evaluation of efficacy was carried out on days 7, 14, 30 and 60 with the help of measuring the ulcerative defect, the state of the skin around the wounds, the cytological examination of wound impressions. The use of a combination of donor thrombocyte concentrate and collagen preparations statistically significantly speeds up the epithelization of the wound surface, more effectively stimulates granulation, significantly reduces the negative subjective sensations in the wound.

**Keywords:** ischemic trophic ulcers, regional treatment, donor platelet concentrate, collagen.

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## INTRODUCTION

Trophic ulcers on the background of chronic obliterating diseases of arteries of the lower extremities arise in 8-12% of cases. They are caused by decompensation of the arterial blood flow. More common trophic ulcers are found in older men [1-4]. Impossibility of conducting radical surgical intervention is associated with distal type of vascular lesion, high-level lesion, severe concomitant pathology [5]. The basis of treatment of this category of patients is conservative vascular therapy and local treatment aimed at stimulating regeneration [6,7].

To restore damaged tissues, the effectiveness of growth factors contained in platelets has been proven, which can also be used to treat wounds with delayed regeneration. For this purpose, it is suggested to use plasma enriched with platelets. To refer to a platelet-enriched plasma, a number of equivalent terms are used in the literature: "platelet gel," "concentrated platelet suspension," "platelet concentrate," Platelet Rich Plasma, PRP [8-11].

Platelet-enriched plasma is a plasma containing about 1,000,000 platelets per 1 microliter of plasma. This is 3-5 times more growth factors than in whole blood. The intracellular quantity and ratio of growth factors are determined genetically. They affect the receptors of cell membranes, causing proliferation of stem cells. Growth factors are oligopeptide structures that act on the receptors of type I and II cell membrane types, which leads to faster growth and differentiation of healthy progenitor cells. There are various ways to produce a platelet-rich plasma. At the same time, in any case, the trained medical personnel, the presence of a procedural room for blood sampling, a laboratory with the necessary equipment and time for carrying out the procedure for obtaining platelets enriched with platelets are required. Thus, in a number of medical institutions there are technical difficulties for widespread introduction of these methods into routine practice. A more perfect alternative can be the preparation of autologombokonsentrata with the use of hardware apheresis or platelets from the blood of the patient himself with specialized departments. In the treatment of patients with trophic ulcers of the lower extremities against the background of arterial pathology, the main burden falls on the outpatient and polyclinic link. In these conditions, the scope and scope of surgical care are limited, there is no possibility of round-the-clock monitoring of patients, a large flow of patients and a short reception time. These causes can become a limitation of auto-thromboconcentrate use in the majority of patients with long-term non-healing wounds. Based on these features, the prospect of treatment is the use of donor thrombocyte concentrate. [12-22]

In recent years, due to the expansion of state control in the organization of the blood service, the introduction of a quality system and technical re-equipment, the infectious and immunological safety of allogeneic blood components has significantly increased. The safety of the use of donor platelets in the local treatment of wounds is achieved by the following measures: screening of blood-borne infections; reduction of immunological risks; maintenance of an adequate stock of platelet concentrate; timely delivery of appropriate thrombocytes for all patients who need them; monitoring and prevention of side effects.

In conditions of disturbed trophism, the synthesis of tissue elements in wounds, especially collagen, is extremely slow. Collagen-containing drugs are used in various fields of medicine (surgery, cosmetology, dentistry, transplantology, traumatology, rheumatology) as an independent factor in tissue regeneration stimulation, and also as a component of complex combined drug systems [23-25].

The aim of the study was to evaluate the effectiveness of using a combination of donor thrombocyte concentrate and native unreconstructed collagen for local treatment of trophic ulcers in patients with chronic arterial insufficiency.

## MATERIALS AND METHODS OF RESEARCH

The study was conducted on the basis of the Voronezh Regional Clinical Hospital No. 1 and included 89 patients with the fourth stage of chronic arterial insufficiency of the lower limbs.

Criteria for including patients in the study:

- men and women between the ages of 20 and 80;

- chronic trophic ulcers of lower extremities against a background of small and medium-sized ischemia (up to 20 cm<sup>2</sup>);
- availability of written informed consent.

Exclusion criteria:

- a sign of acute ischemia;
- trophic ulcers of large and giant size;
- ulcers without spreading in depth (without exposing tendons and bones);
- gangrene;
- low compliance of patients;
- diabetes;
- decompensated heart failure;
- systemic connective tissue diseases;
- hormone therapy;
- lymphedema;
- pregnancy.

When collecting history and clinical examination, as a rule, the characteristic signs of arterial insufficiency were determined: intermittent claudication, numbness and coldness of the limb, muscle atrophy, impoverishment of the hair, dystrophic or fungal lesions of the skin of the feet and nail plates; sometimes signs of atherosclerotic lesion of other organs and systems, more often ischemic diseases of the heart and brain. Provoking moments of ulceration were domestic and industrial injuries, wearing narrow and uncomfortable shoes, hypothermia. In patients' complaints, pain syndrome predominated, provoked by walking and worsening when the elevated position was given to the affected limb. Ulcers are usually localized in the heel region, the terminal phalange of the thumb, on the back surface of the foot, less often on the sole and anterolateral surface of the lower third of the shin. The wound defects had a characteristic appearance: a semicircular shape, with sluggish granulations, dense uneven edges, rising against a background of pale yellow skin.

To study the clinical effectiveness of the combination of donor thrombocyte concentrate and native unreconstructed collagen for local treatment of ischemic trophic ulcers, a comparative study was conducted with control in parallel groups. Patients are randomly divided into 1 main and 3 control groups.

**The main group.** The main group included patients who used a combination of donor thromboconcentrate and native unreconstructed collagen for local treatment of ischemic trophic ulcers.

The main group included 26 patients with trophic ulcers of the lower extremities on the background of chronic arterial insufficiency in the II phase of the wound process: 23 men and 3 women, the mean age of which was  $76.2 \pm 8.4$  years. In patients in this group, trophic ulcers exist on average  $5.8 \pm 3.2$  months (from 1.5 to 9 months). The average area of the wound surface was  $6.1 \pm 4.8$  cm<sup>2</sup> (2.0 to 13.5 cm<sup>2</sup>). In 24 patients, the wounds were located on one lower extremity, in 2 on both legs. In the calcaneal region, trophic ulcers were located in 6 patients, in the terminal phalanges of the fingers - in 19 patients, on the sole - in 1 patient.

At the first dressing, 2-4 ml of donor thromboconcentrate, activated with 10% calcium chloride solution, was placed on the bottom of the wound, the top of the wound was covered with a membrane based on collagen. The drug Collost was used. This is a substance derived from collagen from the skin of cattle. Despite the fact that the method of production allows to preserve the three-helical structure of the fiber, the preparation passes a high degree of purification from ballast substances. As a result, a soluble, highly purified, maximally collagen-compatible preparation of collagen is obtained. The preparation of Collost is a matrix for directed tissue regeneration: fibroblasts, blood and lymph vessels, nerve fibers from the surrounding healthy tissue are introduced into the collagenic lattice and spread strictly along it. A transient matrix is formed that stimulates the body's immune system, accelerates the movement of growth factors, activates granulocytes, macrophages, fibroblasts, enhances the migration of the latter and the proliferation of epithelial cells. In the process of healing, the biomaterial is replaced with its own connective tissue, the disordered growth of the granulation tissue is prevented, as the body reacts to the prompt closure of the wound.

On top, the membrane was covered with a wound dressing or a sterile gauze cloth, treated with a 0.9% NaCl solution. The bandage was fixed on a healthy skin surrounding the damage, and moistened as it dries. Later the patient was given bandages every 3-5 days.

Before application of the preparation Collost<sup>®</sup>, an intradermal test sample was performed: 0.1 ml of the gel was injected intradermally into the region of the forearm. In all patients, the allergic test did not cause any abnormal reactions.

**First control group:** In the first control group, modern dressings were used in the complex treatment of patients.

The first control group included 20 patients with trophic ulcers of the lower extremities on the background of chronic obstructive diseases of arteries of the lower extremities in the II phase of the wound process. The group consisted of 1 woman and 19 men, whose average age was  $75.3 \pm 6.1$  years. The existence of long-term non-healing wounds varied from 2 months to 2 years, an average of  $7.1 \pm 4.2$  months. The area of wound surfaces in this group varied from 1.5 to 11.5 cm<sup>2</sup> and averaged  $4.1 \pm 1.8$  cm<sup>2</sup>. On one lower extremity trophic ulcers were located in 18 patients, in 2 - on both legs. In 18 patients, the wounds were single, and 2 - multiple. In the calcaneal region, trophic ulcers were in 1 patient, in the terminal phalanges of the fingers in 16 patients, on the sole - in 3 patients.

In accordance with the modern concept of the treatment of chronic wounds, the basis of modern local treatment is the means that provide healing in a humid environment. In the stages of proliferation and epithelialization, the tasks of treatment are also the creation of optimal conditions for regeneration, the mechanical protection of immature connective tissue, the acceleration of the formation and retraction of connective tissue scar. In this group, polyurethane spongy dressings, hydrogels, alginates, hydrocolumbic and lipid-colloidal dressings were used, the change of which occurred every 2-4 days.

**Second control group:** The second control group included patients who had only collagen-containing drugs in complex local therapy.

The second control group consisted of 3 women and 18 men with ischemic trophic ulcers of the lower limbs in the II phase of the wound process. The mean age of all patients was  $76.5 \pm 7.9$  years. Long-lasting non-healing wounds existed from 1.5 months to 2 years, an average of  $8.5 \pm 7.2$  years. The area of the wound surface is from 2.0 to 10.5 cm<sup>2</sup>, on the average,  $5.1 \pm 5.8$  cm<sup>2</sup>. In all patients, trophic skin defects were located on one lower limb and were single; In the calcaneal region, wounds were localized in 4 patients, in 15 patients - on the terminal phalanges of the fingers, on the rear of the foot - in 2 patients.

Collagen-containing drugs are used as a stimulation factor for tissue regeneration. We used a preparation made on the basis of soluble high-purified collagen of the skin of cattle, Collost. The preparation was applied to the wounds in the form of a membrane. Before use, the Collost<sup>®</sup> membrane was soaked in a 0.9% NaCl solution and cut into the shape of a wound defect. After performing the primary treatment, the gel "Collost" was injected into the wound edges intradermally along the entire circumference in the amount of 1.5-3.0 ml, depending on the size of the wound. Then the prepared membrane was covered on the wound surface, the latter was hemmed so that it did not move. An obligatory condition is the full adherence of the membrane "Collost" to the bottom of the defect. Above, the wound was covered with a material that provides healing in a moist environment (hydrocolumn and lipid-colloid bandages, a sterile gauze cloth treated with 0.9% NaCl solution). The dressing was fixed on a healthy skin surrounding the damage, changed or moistened as it dried. Later the patient was given bandages every 3 days.

Previously, all patients underwent an intradermal test-test - a negative reaction was detected.

**Third control group:** The third control group included patients who had a donor plasma enriched with platelets in complex local treatment. The group included 16 men and 6 women with ischemic trophic ulcers of the lower limbs, the average age of which is  $71.2 \pm 7.2$  years. The lifespan of trophic ulcers averaged  $6.5 \pm 8.2$  months (varied from 3 months to 1.5 years). The area of trophic ulcers averaged  $7.1 \pm 2.8$  cm<sup>2</sup> (1.0 to 14.5 cm<sup>2</sup>). In all patients, ulcers were located on one lower extremity, 17 patients had single ulcers, and 5 had multiple ulcers. Localization of trophic ulcers: in the heel area - 2 patients, terminal phalanges of the fingers - 13 patients, on

the sole - in 3 patients, on the rear of the foot - 4. The state of wounds also corresponded to the II phase of the wound process.

The results of the use of different methods were evaluated on days 7, 14, 30 and 60.

### METHODS OF CLINICAL RESEARCH

The clinical course of the wound process was analyzed, taking into account the number, location, depth, size and shape of ulcers. The smell, the consistency, the color of the wound detachable was assessed. To further assess the dynamics of wound regeneration, special attention was paid to measuring the magnitude of the ulcerative defect. To measure the area of wounds, a mobile application + WoundDesk was used, based on the use of a smartphone camera with the Android operating system. When taking a picture, the indicator scale "+ WD" (provided by the creators of the application) is used, with the help of which the contours of the examined wound are recognized.

Also, the state of wounds was assessed by bacteriological and cytological studies. In the bacteriological study of the wound detachable to determine the stage of the wound process and prevent complications, the species belonging to microorganisms was most often assessed using differential diagnostic and chromogenic media. For adequate antibacterial therapy, the sensitivity of microorganisms to antibacterial drugs was determined by the disco-diffusion method.

To monitor the dynamics of the regeneration process and the state of the wound, a cytological examination of the smears-prints of the wound surface was performed. Smears - prints were applied to pre-skimmed glass (or took the material by lightly scraping the surface layer of the wound with a blunt edge of the blade or the handle of a surgical scalpel, a special spatula), and the resulting material was evenly distributed on the glass with a thin layer. In the cytological laboratory, fingerprints were dried, fixed, stained by the method of Romanovsky-Giemsa and Gram (for detection of microorganisms).

To assess the state of the vascular bed of the lower extremities, patients were selectively conducted with arteriography, ultrasound angioscanning, ultrasound dopplerography on VOLUSON E devices, Angiodin. According to the indications, ultrasound scanners were performed with the help of ultrasound scanners LOGIC 7, VIVID I, VIVID E, PRO FOCUS, computer and magnetic resonance imaging on a multislice CT scanner Aquilion 64 manufactured by Toshiba and a high-field magnetic resonance imager Signa HDx 1.5T from General Electric, Holter monitoring of blood pressure, transcutaneous determination of oxygen tension in tissues.

Statistical processing of data was carried out in accordance with the requirements for research in the field of medicine, using licensed electronic analysis packages "STATISTICA 10.0" and "Microsoft Excel". Calculations were carried out using parametric statistics methods. The arithmetic mean and the standard deviation were calculated. To determine the reliability of the differences, the t-test of the Student was used (confidence level  $p < 0.05$ ).

### RESULTS AND ITS DISCUSSION

**Results of the study on the 7th day:** In the main group, the subjective feelings of the patients are as follows: 7 (27%) patients noted positive changes in the form of pain in the wound, 16 (61.5%) patients did not notice changes, 2 (7.7%) - still had a burning sensation and drawing pains in the wound. After removal of bandages: in 22 (84.6%) patients the skin around the wound without signs of inflammation, the collagen membrane swollen, loose, tightly fixed to the wound surface, in 4 (15.4%) - the membrane was partially lysed, turning into a gel-like mass, fragments of the membrane were left on the wound, in patients who noted painful pains, after macular removal, maceration of the skin with excoriation sites was noted, signs of pronounced inflammation were not present, the membrane was lying on the wound. The tactics of wound management remained the same - the wounds were closed with sterile gauze napkins moistened with sterile 0.9% NaCl solution.

In the first control group, patients did not notice a change in the sensations from the wounds. With an objective assessment of the state of trophic ulcers, there was no perifocal edema and hyperemia, the appearance of the wounds corresponded to the second phase of the wound process, and granulation, marginal epithelization appeared in 4 (20%) patients.

Patients of the second control group noted the following subjective sensations: absence of negative sensations in the field of trophic ulcers - 15 (71.4%) patients, 5 (23.6%) patients did not notice changes in sensations, 3 (14.2%) complained of occasionally a slight burning sensation in the wound. During dressing after removal of the bandage, 20 (95.2%) patients had skin around the wound without signs of inflammation, the collagen membrane swollen, loose, densely on the surface of the wound, partially lysed in 1 (4.8%) of the diseased membrane, turning into a gel-like membrane mass, on the wound were fragments of the membrane. The tactics of wound management remained the same.

In the third control group, subjectively, all patients noted a reduction in pain from the wounds. Also in all patients of the group during the dressing the appearance of bright juicy granulations was noted, fragments formed the cushion of marginal epithelization. 5 patients had a condition without dynamics.

**Results of the study on the 14th day:** Patients of the main group did not notice cardinal changes in the sensations from the wound. On the dressing after removal of the bandage: in 22 (84.6%) patients the membrane was tightly attached to the wound surface, the skin around was not changed, in 2 (7.7%) patients the membrane remained fragmentarily on the wound, the latter granulated, epithelialized, in 2 (7.7%), the membrane was completely lysed, the wound area decreased by 1.5 cm<sup>2</sup> and 2 cm<sup>2</sup>, bright juicy granulations were found on the bottom of the wounds, the marginal epithelization beads were expressed.

In the first control group, the subjective feelings of the patients were as follows: 8 (40%) patients noted painless dressings, reduced pain and burning sensation in the wound region at rest. When dressing: the area of trophic ulcers in 15 (30%) patients decreased by an average of 1.43 cm<sup>2</sup> ( $p < 0.05$ ) due to marginal epithelization, in 17 (85%) cases - the area and appearance of wounds did not change ( $p > 0.05$ ).

In the second control group, the subjective sensations from the wound did not change. Condition of the wound on the dressing: in 17 (81%) cases, the membrane tightly adhered to the wound surface, the skin around is not changed, in 4 (19%) patients the membrane remained fragmentarily on the wound, the latter granulates and epithelialized.

In the third control group, in assessing the state of wounds, it was revealed that the area of trophic ulcers in 16 (72.7%) patients decreased by 1.1 cm<sup>2</sup> ( $p < 0.05$ ) due to marginal epithelization, the wound bottom was made with juicy red granulations, in 1 (4.5%), there was an increase in exudation from the wound and steps were taken to protect the skin around ulcers, absorbent wound coverings were used, in 5 (22.7%) patients the wounds were still clean, with single sluggish granulations, the depth and area of the wound not changed.

**Results 1 month after the start of treatment:** All patients of the main group after the use of a set of methods of stimulation of reparative processes noted positive dynamics. Objective assessments of the state of trophic ulcers: in 14 (53.8%) patients of this group, it was possible to reduce the wound area by 50%, in 7 (27%) cases, the area of trophic ulcers decreased by an average of 20% due to marginal epithelization, in 5 (19%) patients, the wound area did not statistically significantly change ( $p > 0.05$ ), but there are juicy granulations and signs of marginal epithelialization.

In 4 (20%) patients of the first control group, the area of ulcers decreased on average by 2.3 cm<sup>2</sup> ( $p < 0.05$ ). In the remaining patients of this group, wounds without signs of active granulation and epithelialization.

In the second control group, 14 patients (66.7%) reported positive dynamics (reduction of pain in the wound at rest, lengthening the walking distance without pain in the wound) and an 18% decrease in the wound surface area ( $0.9 \pm 0.7$  cm<sup>2</sup>). In 5 (23.8%) patients of this group the condition of the wound surface did not change, in 2 patients the area of trophic ulcers with a tendency to increase, the pain intensified.

In the third control group, the area of ulcers in 16 (72.7%) patients decreased on average by 1.7 cm<sup>2</sup> ( $p < 0.05$ ) due to the sites of marginal epithelialization, in 6 patients the depth and area of wounds did not change.

**Results of the study 2 months after the start of treatment:** In 14 (53.8%) patients of the main group, 60% reduction in wound area was achieved, in 7 (27%) cases the area of trophic ulcer decreased on average by 27%

due to marginal epithelization, in 3 (11.5%) patients the area of the wound has not statistically significantly changed ( $p > 0.05\%$ ), granulations and signs of marginal epithelialization are present, in 2 (7.7%) cases granulation is weak, epithelization is slow.

In the first control group, the area of trophic ulcers decreased by an additional 0.5 cm<sup>2</sup> on the average, signs of granulation and marginal epithelization were noted in 4 (20%) patients.

In the second control group, by the end of the second month of treatment, the area of the wound defect in 14 (66.7%) patients was reduced by 19.3% compared to the baseline level, in 5 patients the condition was stable, the dynamics of the area and the state of the wounds were not noted, granulation sluggish, epithelization is slow. In 2 patients it was possible to stabilize the course of the wound process.

In the third control group, in 16 cases, the area of trophic ulcers decreased by 2.5 cm<sup>2</sup> ( $p < 0.05$ ), in 2 patients the wounds were completely buried. In 5 patients, the wound surface is clean with sluggish granulations, the marginal epithelialization is not very pronounced. In one case, signs of infection appeared in the wound: hyperemia of perifocal tissues, exudation increased, necrosis areas appeared.

### CONCLUSION

When comparing different methods aimed at stimulating reparative processes in the wound, the use of a combination of donor thromboconcentrate and collagen preparations most effectively stimulates regeneration processes in the wound, even under conditions of chronic ischemia. So, after a month of using the combination of donor thromboconcentrate and collagen preparations, the wound area was reduced by half in 53% of patients, by the end of 2 months, the wound area in these patients was reduced by 60%. Thus, the use of a combination of donor thrombocyte concentrate and collagen preparations, statistically significantly accelerates the wound surface epithelization, more effectively stimulates granulation, significantly reduces the negative subjective sensations in the wound.

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