The combined approach to treatment of patients witn chronic ischemia and diabetic foot syndrome

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Motivation and Aim

The increase in the incidence of chronic diseases of the peripheral arteries and diabetes is associated with general aging of the population [1]. The most unfavorable signs of symptomatic atherosclerotic lesions of the arteries of the lower extremities are manifestations of critical limb ischemia, including pain at rest, ischemic ulcers and gangrene [2]. Traditional treatment includes drug therapy to reduce risk factors and vasodilation, as well as surgical interventions aimed at improving patency of the arteries and including open surgery, endovascular surgery, or their combination. Revascularization is usually advised to patients with foot ulcers with a decrease in the ankle-brachial index (ABI) of less than <0.8 or percutaneous oxygen tension (TcPO2) <40 mm Hg when confirming violations of patency of arteries using duplex scanning of arteries or computed tomography angiography, MRI angiography or contrast angiography [3]. However, infrapopliteal arterial lesion is one of the features of chronic lower limb ischemia in patients with diabetes, which has been shown by many studies [4]. Endovascular treatment using stents and drug-coated balloons shows good immediate results, especially with infrapopliteal lesions [5], but after endovascular revascularization in 35% of patients, repeated surgery is necessary within a year [6]. These data emphasize the need for close monitoring of patients after revascularization. In this regard, the research was aimed at the search for non-surgical methods of revascularization, including gene and cell therapy.

Methods and Algorithms

Mononuclear cells from peripheral blood after mobilization with granulocyte-colony stimulating factor (G-CSF) or from the bone marrow were taken patients of the main group took besides usual therapy. Every patient was assessed for the possibility of adequate peripheral venous access before making a decision of mononuclesar cells source. Central venous catheterization only for the purpose of lymphocytapheresis was considered inappropriate. The decision to obtain autologous mononuclear cells by iliac bone puncture was made in the absence of peripheral venous access. G-CSF (Neupogen (®) at a dose of 480 mg / day or its analogues) was subcutaneously administered in accordance with the instructions for use in order to mobilize human blood mononuclear lymphocytes for 5 days. The number of leukocytes in the peripheral blood was evaluated, the patient's condition was monitored, and adverse reactions were evaluated. The lymphocyte fraction was isolated from peripheral venous blood by lymphocytapheresis on a “Haemonetics MCS+” cell separator in accordance with the manufacturer's instructions on day 5. The iliac bone was punctured (posterior-superior spine, anterior-superior spine) to obtain autologous bone marrow mononuclear cells. Under local anesthesia using a special 15G puncture needle, 50 ml of red bone marrow was taken. The isolation of mononuclear cells from the bone marrow or cytapheresis product of human peripheral blood was performed according to the described procedure [6] using a ficoll gradient (density 1.077 g / ml) at a centrifugation speed of 3000 rpm for 20 min under sterile box conditions in the laboratory of cell technologies. A layer of mononuclear cells was collected at the interface over the entire cross-sectional area of the tube. Then it was washed twice with 0.9% sodium chloride solution, centrifuged at 1500 rpm for 10 minutes.

The suspension of autologous human mononuclear cells (50 million bone marrow cells or 1.5 billion cells of the peripheral blood cytapheresis product) was obtained after identification of the labeling and injected intramuscularly into the calf muscle of the affected limb from 20 points in equal doses of 0.5 ml per point. Before administration to the patient, the solution should be at room temperature. The procedure was carried out after standard skin treatment, in compliance with asepsis rules. During 24 hours after injecting the obtained suspension of autologous mononuclear cells, the patient was monitored for assessment of fractional thermometry and condition of the limb at the injection site.

The ABI was assessed by measuring systolic blood pressure (SBP) in both brachial arteries (BA), posterior tibial arteries (PTA), and anterior tibial arteries (ATA). The measurement was carried out in a supine position after keeping the patient in the same position for 10 minutes. Systolic blood pressure on the upper and lower extremities was measured using “Vivid Demension” ultrasound scanner (manufacturer GE) using a linear Doppler sensor with an operating frequency of 7-10 MHz. ABI was calculated according to the following formula: ABI = SBP on the ATA (PTA) / SBP on the BA. The highest of the two systolic blood pressure values obtained from measurements on the arteries of the lower extremity was taken as ankle SBP. It is necessary to compare systolic blood pressure in the posterior tibial artery and in the anterior tibial artery, and take into account the highest rate. The highest systolic blood pressure measured on the two upper extremities was taken for gad on the shoulder. We obtained as a result one value of systolic blood pressure of the upper extremities for further calculations of ABI. Pressure was recorded on both hands, due to possible damage to the subclavian or axillary arteries. The lowest value of the ABI recorded in hundredths was used as the worst result of the two ABI values obtained after the calculations. Unfortunately, ABI values are not always reliable in patients with diabetes because of media calcinosis of the lower leg arteries, as a result of which tibial arteries cannot properly compress and result in either a false increase in ABI or a value that is unattainable due to the inability to achieve vessel compression at a pressure of cuff significantly exceeding systolic pressure.

In order to objectify the assessment of blood flow and microcirculation disorders, patients’ transcutaneous (percutaneous) oxygen tension on the lower extremities was determined using a portable transcutaneous Radiometer TCM 4 oximeter (Radiometer) with six modified Clark-type solid-state electrodes for long-term determination of TcPO2 with a special heating device. The electrodes were applied at standard points in accordance with the manufacturer’s instructions: control: in the right subclavian region, on the foot in the first interdigital space. The study was carried out in a horizontal position lying on your back; in the supine position with the limb elevation 30^0 relative to the horizontal position; in a sitting position with limbs down. For each measured value, the regional perfusion index (RTI) was calculated – the ratio of the transcutaneous oxygen tension in the lower extremity to the chest index multiplied by 100 [9], the indicator of which is considered more specific in patients with critical ischemia when tested with limb elevation on 30^0. The assessment of the dynamics of changes in values was carried out before treatment, 3 months, 6 months, 12 months.

The informed consent of the patient to participation in the trial was taken in accordance with the directives of the European Community (86/609 / EEC) and the Helsinki Declaration, in compliance with the «Ethical Principles for Scientific Medical Research with Human Participation» and in accordance with the «Rules of Clinical Practice in Russian Federation».

Results (1)

During the period of 2017-2019, as part of the development of a new medical technology, 16 completed cases had been analyzed in the main group, and 44 completed cases in the comparison group. Clinically significant atherosclerosis with multiple lesions of the coronary arteries and lower limb arteries was noted, as well as hypertension of a high functional class were observed in most patients (Tab. I). Almost one in five patients had a history of stroke or the presence of stenosis of the internal carotid artery (ICA). Patients of both groups were observed with a constant form of atrial fibrillation, threatened by the occurrence of arterial thromboembolism. The level of glycated hemoglobin (HbA1c) and leukocytosis at admission did not significantly differ in patients of both groups. Women in the main group were 31.25% (5/16), in the comparison group - 32.3% (11/34). Surgical revascularization was performed in 81.3% of patients in the main group and 68.2% of patients in the comparison group. (Tab.1)

The mobilized and isolated autologous peripheral blood mononuclear cells were injected into the gastrocnemius muscle of the affected limb in 8 patients. Autologous mononuclear cells isolated from bone marrow were injected into the gastrocnemius muscle of the affected limb in 6 patients. Adverse reactions associated with the administration of G-CSF or adverse reactions to the introduction of mobilized autologous mononuclear cells or mononuclear cells isolated from bone marrow were not observed.

Table 1. The clinical features of patients

	Main group n=16	*Comparison group n=44*
Age, years	67 [64; 70]	67 [62; 71]
Female, n (%)	5	11
Male, n (%)	11	33
Ulcers, n (%)	13 (81,3%)	31 (70,6%)
HbA1c, %	8.08 [7,32;8,59]	8,07 [7,12; 9,44]
ABI before treatment,	0,51 [0,10;0,59]	0,5 [0,4;0,65]
ABI before treatment	0,19 [0;0,45]	0,47 [0;0,54]
Arteriosclerosis, n	4	3
Arterial Hypertension	81,25%	79,4%
Heart failure	93,6%	61,7%
Stroke	18,8%	17,6%
Atrial fibrillation	18,8%	20,6%

Results (2)

The initial level of transcutaneous oxygen tension on the foot in the horizontal position was 1.8 times higher in the comparison group, but 6 months after the intervention, a multidirectional dynamic of this indicator in the groups was noted (Fig. 1).

During one year after the intervention, the main group showed a further trend towards an increase in the transcutaneous oxygen tension in the foot, as in the comparison group. Nevertheless, this indicator is at a level corresponding to tissue hypoxia and a low probability of wound healing.

Similar dynamics was observed in patients of both groups when performing a test with foot elevation 30^0 (Fig. 2), but the increase in this indicator in the main group is more significant than in the comparison group, the absolute value is more than 2 times higher.

The regional perfusion index measured horizontally differs before treatment and after 6 months, but does not show significant differences between the groups after a year of observation (Fig. 3). Changes in the local perfusion index obtained during the test with foot elevation 30^0 show that in the main group after 6 and 12 months of observation, the indicator was higher than in the comparison group (Fig. 4).

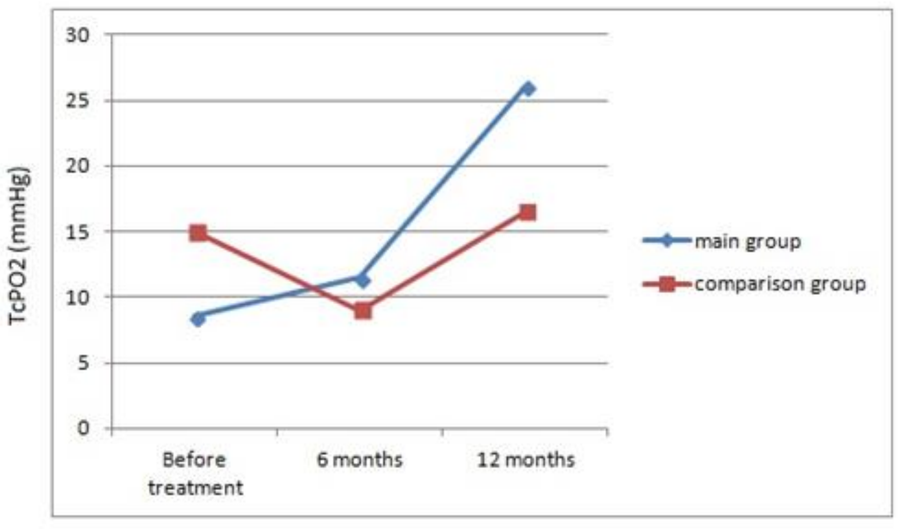

Fig.1 Transcutaneous oxygen tension in patients before treatment and at the follow-up visits

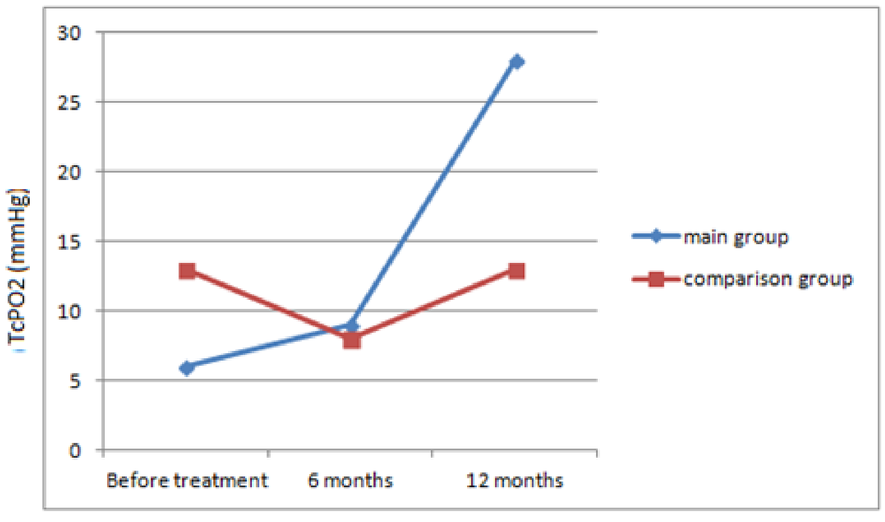

Fig.2. Transcutaneous oxygen tension with foot elevation 30^0 in patients before treatment and at the follow-up visits

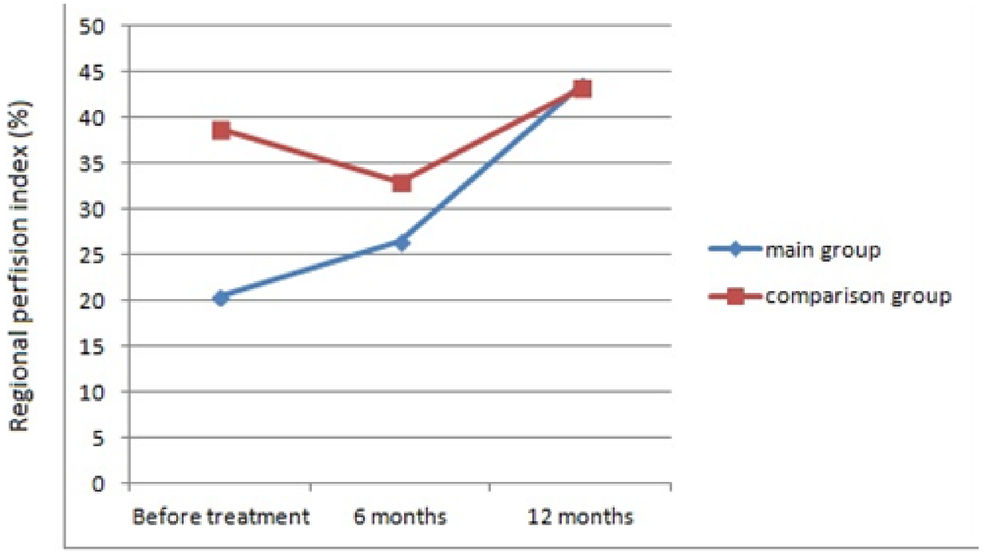

Fig.3. Regional perfusion index in patients before treatment and at the follow-up visits

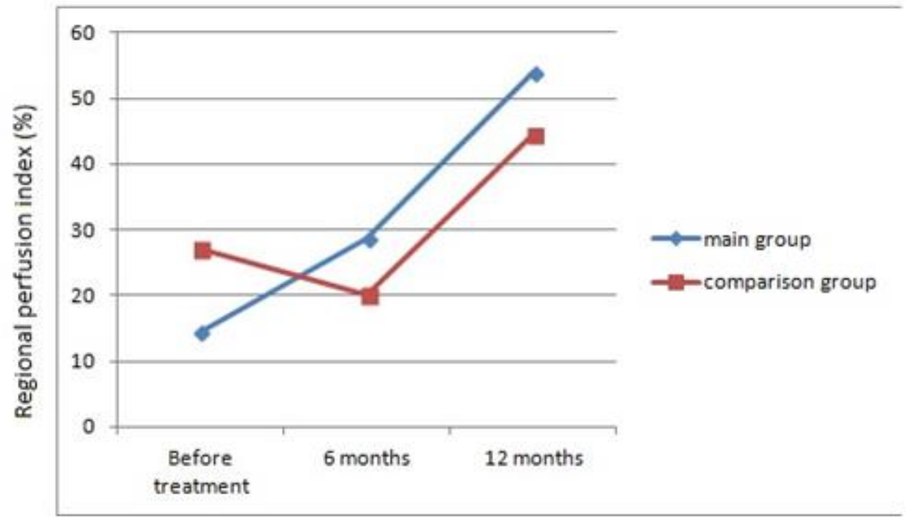

Fig.4. Regional perfusion index with foot elevation 30^0 in patients before treatment and at the follow-up visits

***Results* (3)**

During the observation period, two patients were undergoing necrosectomy, four patients - small amputation and two patients - skin grafting in the main group. Amputation above knee was performed in 4 patients (9.1%), amputation below knee in 1 patient (2.2%), necrosectomy in 4 patients (9.1%), small amputations in 8 patients (32%) in the comparison group with subsequent follow-up. Ulcer healing in 6 patients (16.6%) or significant decrease in ulcer defects in size in 5 patients (13.8%) was observed on 12 months follow-up visits. The absence of dynamics in the state of ulcers was noted in 6 (16.6%) patients. When selecting a subgroup of patients with trophic ulcers who underwent endovascular revascularization (17 patients), the healing rate of trophic ulcers was 35.2%, and the frequency of reduction of existing trophic ulcers was 29.4%. There was no progression of the disease and the appearance of trophic ulcers in the subgroup of patients with critical lower limb ischemia without trophic ulcers. Two patients underwent reoperation percutaneous balloon angioplasty due to reocclusion of the lower leg arteries and the absence of positive dynamics of the wound process on the part of the lesion.
Complete epithelialization of ulcerative defects was observed in 5 patients (41.6%) of the main group. On follow-up visits after 12 months, 8 (66.6%) patients had no trophic ulcers, 2 patients (18.8%) showed a significant decrease in the size of trophic ulcers. Moreover, optimal results were obtained in patients with a combination of restoration of arterial blood flow by endovascular revascularization and intramuscular administration of autologous mononuclear cells. Two patients underwent reoperation percutaneous balloon angioplasty like in the comparison group.

Conclusion

Thus, in this patient sample group the combination of cell therapy with surgical revascularization has shown its effectiveness in healing or reducing the size of trophic ulcers, in reducing the number and volume of surgical interventions on the foot, and maintaining the supporting function of the limb. The positive effect of the combined approach develops gradually, which is confirmed by an increase in the index of local perfusion by 6 months after the start of treatment. The regional perfusion index, when measured horizontally after a year of observation, does not show significant differences between the groups, and its values by this time correspond to the ones described by Hauser C.J. in patients with intermittent claudication [7]. The procedures for stimulating leukopoiesis, lymphocytapheresis, or taking mononuclear cells of the bone marrow and introducing mononuclear cells into the calf muscle are well tolerated by patients with chronic lower limb ischemia and diabetes mellitus, and in this sample of patients there were no undesirable adverse reactions and complications. The developed combined technology, including surgical treatment of chronic lower limb ischemia in diabetic foot syndrome can be recommended for implementation as part of additional randomized clinical trials in patients with a threat of limb loss. According to the authors of the latest international clinical recommendations, the amount of data that reliably confirm the effectiveness of cell and gene therapy methods in treating patients with ischemia threatening limb loss are still insufficient and these types of treatment should be considered only for research purposes [8].

References

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