

## Clinical and functional aspects of cord blood cell transplantation in patients with distal lesion of lower limb arteries

Dmitry Borisovich Dombrovskiy<sup>1</sup>, Volodimir Vasilyevich Savin<sup>2</sup>, Yuriy Vasilyevich Oliynyk<sup>2</sup>, Michael Ivanovich Sheremet<sup>1</sup>,\*, Yulia Romanivna Pshyborovska<sup>2</sup>

<sup>1</sup>Higher State Educational Institution of Ukraine «Bukovynian State Medical University» Department of Surgery No. 1, Ukraine

<sup>2</sup>Chernivtsi Regional Clinical Hospital, Department of Vascular Surgery, Chernivtsi, Ukraine

\*corresponding author e-mail address: [mihayl71@gmail.com](mailto:mihayl71@gmail.com) | Scopus ID [57193774935](https://orcid.org/0009-0001-9193-7493)

### ABSTRACT

To carry out a comparative analysis of the clinical condition of patients with distal lesions of the arterial bed using standard conservative therapy with cord blood cell transplantation and without it. The experimental group included patients with manifestations of chronic lower limb ischemia against the background of atherosclerosis, who underwent cord blood cell transplantation in combination with conservative therapy. The control group included patients with obliterating atherosclerosis and distal vascular lesions of the lower extremities, received a basic course of conservative therapy. To determine the quality of life before and after cord blood cell transplantation, we used a number of standardized questionnaires, which helped to determine the distance of intermittent claudication and make a subjective assessment of the personal well-being level of patients. To analyze the state of microcirculation in the ischemic limb, we used the laser Doppler flowmetry method. The following changes were observed after the cord blood cell transplantation: an improvement in the general state of patients, a decrease in the degree of ischemia according to Rutherford's classification, an increase in the distance and speed of painless walking during the first 3 months by 5-10%, and 1.3 times in 12 months compared with the control group. The improvement in the microcirculatory parameters of laser Doppler flowmetry was noted from the first to the 12th month after transplantation, which indicated an improvement in microhemodynamics. The use of cord blood cell transplantation in order to stimulate angiogenesis allows one to obtain a long-term positive clinical effect, which manifests itself in the form of an increase in the distance and speed of painless walking, improved working capacity, and improved personal well-being of patients in the physical, psychological and socio-economic field, correlated with laser Doppler flowmetry data and expands the possibilities of successful treatment of patients with chronic ischemia of the lower extremities on the background of obliterating atherosclerosis.

**Keywords:** *obliterating atherosclerosis; chronic lower extremity ischemia; transplantation; cord blood.*

### 1. INTRODUCTION

Chronic obliterating diseases of the arteries of the lower extremities occupy more than 20% of all lesions of the cardiovascular system in Ukraine [1, 2, 3]. Mortality among patients with the initial clinical manifestations of this pathology in the form of intermittent claudication is 3-5% per year, and with the presence of critical lower limb ischemia, the mortality rises to 20% per year [4, 5].

The frequency of amputations in vascular pathology both in Ukraine and abroad reaches 59%, and the mortality rate reaches 48%. Mortality remains the main issue in this group of patients: 30-40% of them live less than 5 years [3-4].

In the absence of a 'receiving' vascular bed or contraindications for reconstructive surgery, the only way is amputation or indirect revascularization, which enhances the compensatory capabilities of the microvasculature due to an increase in the density and total area of the capillary network [5-15].

Thus, the problem of treating patients with distal lesions of the lower extremity arteries is one of the most relevant in vascular surgery, and its solution will not only save the limb to inoperable patients but also significantly improve their quality of life.

There is enough randomized stem cell research in the world. Their diversity, degree of technical complexity and injury rate has been known for a long time and are widely discussed in the research literature.

Joshua M. Hare *et al.* [16] published in 2017 the results of a randomized POSEIDON-DCM Trial study comparing the safety and efficacy of autologous and allogeneic mesenchymal bone marrow stem cells in the treatment of chronic non-ischemic dilated cardiomyopathy, conducted at the University of Miami Hospital, USA, from 2011 to 2015.

In 2018, scientists from Duke University and the Houston Methodist Neurological Institute (USA) published the results of phase 1 of multiple site open prospective clinical trials that examined the safety of cord blood cell transplantation in patients after ischemic stroke. The study proved that this technique is safe, well-tolerated and possible. A larger, randomized, placebo-controlled, double-blind phase 2 trial has now been initiated to evaluate the ability of cord blood cells to improve functional outcomes in patients with acute ischemic stroke [17].

### 2. MATERIALS AND METHODS

We selected 16 patients to the experimental group in the Vascular Surgery Department of the OCI Chernivtsi Oblast Clinical

Hospital – 12 men (75%) and 4 women (25%) with manifestations of chronic lower limb ischemia against the background of

atherosclerosis who underwent treatment in the form of cord blood cell transplantation in combination with conservative therapy. The control group included 11 patients: 9 men (82%) and 2 women (12%) with obliterating atherosclerosis and distal vascular lesions of the lower extremities, who received a basic course of conservative therapy in average doses.

The presence of chronic ischemia in patients was confirmed using instrumental research methods and the appropriate clinical symptoms: pain in the lower limb, which occurred during physical exertion or at rest, the presence of trophic disorders of the foot soft tissues or without them, the long-term course of the disease. The nature of the manifestation of clinical symptoms was different depending on the degree of limb ischemia (Table 1).

For the purpose of an additional degree of severity of lower limb ischemia, we used the classification according to R.B. Rutherford [15], because here variants of alternating claudication, different in degree of expression, are singled out as separate independent points. It is based on the functional data of the arteries of the lower extremities and fully reflects the clinical condition of the lower extremities, including the features of regional hemodynamics.

The criteria for selecting patients for the study were: distal damage to the arteries of the lower extremities, the impossibility of performing "direct" reconstructive surgery, belonging of patients to Rutherford grade 4 and 5 (grade III and IV of Fontaine ischemia with minor trophic disorders), negative tests for tumor markers and the absence of cancer pathology in the history. Exclusion criteria: the possibility of reconstructive surgery on the main arteries, belonging to the 6th class according to Rutherford (degree IV ischemia according to Fontaine with severe trophic disorders), positive tests for tumor markers or burdened cancer history, diabetes mellitus. The presence of other concomitant pathology in

patients allowed them to participate in the experimental group formation.

In order to improve revascularization, the patients of the study group underwent cord blood cell transplantation against the background of the prescribed conservative therapy. The cell transplant was received from the cord blood bank of the Institute of Cell Therapy, LLC, under a contract. The suspension was injected into the ischemic limb using a long thin cannula subfascial on the medial and lateral surface of the lateral lower leg surface according to the technique developed during the study (Patent of Ukraine No. 13117 of January 10, 2019). The total amount of injected suspension containing cord blood stem cells in one limb was  $50 \pm 5$  ml (the content of the host cell nucleus was from  $47 \times 10^6$  to  $356 \times 10^6$ , the cell viability was not less than  $87 \pm 5\%$  of the initial one).

To evaluate the effectiveness of cord blood cell transplantation, we used the unified Walking Impairment Questionnaire (WIQ), which was recommended as a specific questionnaire for patients with intermittent claudication with the determination of the distance of painless walking and the possible walking speed in patients with distal lesions of the lower limb arteries.

An additional survey was conducted to determine the Quality of Life Index (Spitzer *et al.*), which criteria were the patient's assessment of the level of personal well-being in physical, mental and socio-economic terms. The maximum number of points that the patient could score was 10 points, the minimum – 0 points

According to the standardization recommendations (European Contact Dermatitis Society, since 1994), we analyzed laser Doppler flowmetry in patients with chronic limb ischemia where we determined the hemodynamic type of microcirculation and gave several indicators that, in our opinion, quite clearly characterized the state of microhemodynamics.

### 3. RESULTS

Positive clinical dynamics were observed in all patients of the experimental group after the transplantation of cord blood cells. During the first month after cell transplantation, patients noted a gradual improvement in well-being, pain at rest decreased, and then vanished, trophic disorders decreased and marginal necrosis of the fingers healed, the distance and speed of painless walking gradually increased. In addition to improving the local status, an improvement in general condition was noted, and working capacity and overall activity increased in 3 months after cell transplantation. The state of patients in 6 months after cell transplantation: all patients were able-bodied and led an active social and home life.

After 12 months post-transplant patients there is a shift in the group with a low degree of ischemia that seen in the table (Table. 2). The distance and speed of patient painless walking increased by 5-10% during the first 3 months, and after 12 months – 1.3 times compared with the control group (Fig. 1).

A progressive increase in the quality of life index was observed 6 months after transplantation, and after 1 year this indicator increased by 18.2% compared to the same indicator of the control group (Fig. 2).

When conducting laser Doppler flowmetry, we investigated the type of hemodynamics, the background index microcirculation (BIM), the reserve of capillary blood flow of the occlusal test

(RCBFo) and the microcirculation index of the first toe (MI). Analyzing the indicators before treatment, it was noted that the value of BIM from the left forearm was significantly higher ( $7.02 \pm 1.04$ ), which indicates changes in the vascular bed, compared with the norm (Fig. 3).

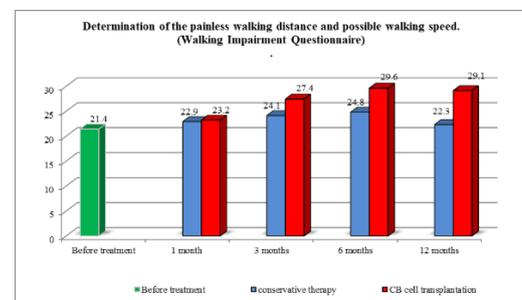


Figure 1. Determination of the painless walking distance and possible walking speed in dynamics.

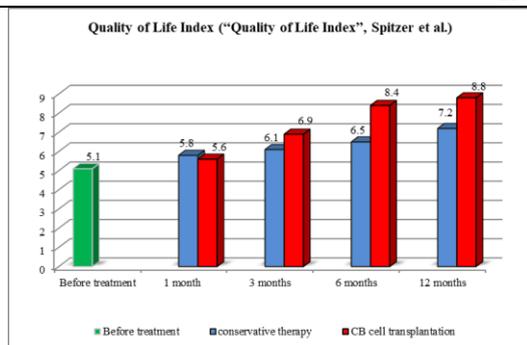


Figure 2. Determining the quality of life index in dynamics.

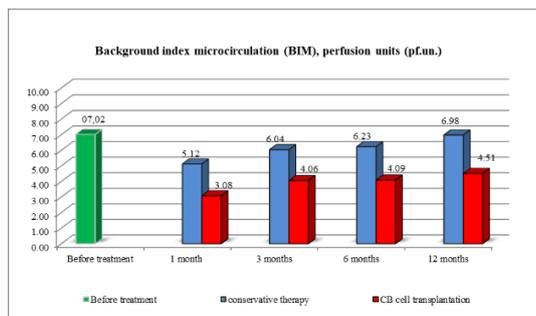


Figure 3. Dynamics of BIM indicators in patient groups.

One month after transplantation, a significant decrease in the background microcirculation index was determined in the experimental group, and in 3 months and further its normalization could be noted.

In the control group, 1 month after conservative therapy, this indicator decreased to a normal level, but after 3 months and further, its value increased to the initial level.

We can also observe that after transplantation of cord blood cells, there is an improvement in microcirculatory indicators, which are reflected by the level of RCBFo, starting from the end of the first and up to the 12th month (Fig. 4). These changes indicate an improvement in the state of microhemodynamics due to an

improvement in venous outflow, as well as an improvement in endothelium-dependent vasodilation in the experimental group. In the control group patients, RCBFo values tended to increase after one month of treatment, however, in a 1 year they were approaching the initial values, which indicated a short and insufficient effect of standard conservative therapy.

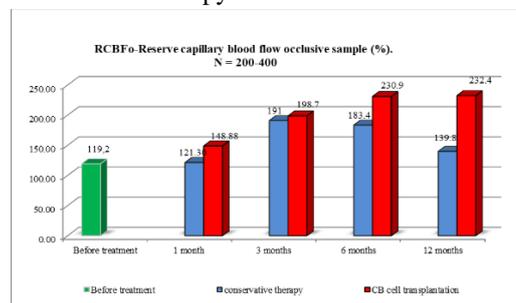


Figure 4. Dynamics of RCBFo indicators in patient groups.

In addition, after transplantation of cord blood cells, there was a steady tendency toward an increase in the microcirculation index of the first toe, and from the 3rd month onward, the studied parameter did not differ from the norm (Fig. 5).

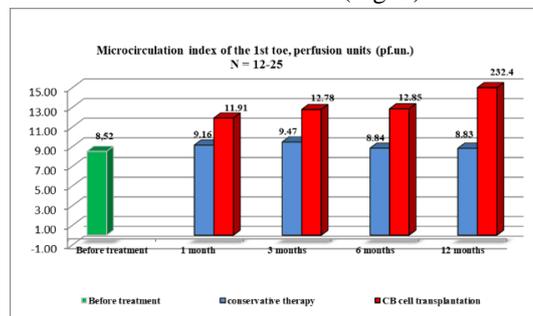


Figure 5. Dynamics of the microcirculation index in groups of patients.

Patients in the control group had a slight improvement in blood circulation at the beginning of the study. However, at the end of the study, they were approaching the original data.

Table 1. Distribution of patients according to the degree of lower limb ischemia.

According to Fontaine	Symptoms		According to Rutherford		Experimental group (n=9)	Control group (n=11)
			Grade	Class		
I	asymptomatic course		0	0	-	-
IIa	claudication > 200 m	light claudication	I	1	-	-
IIb	claudication < 200 m	moderate claudication	I	2	-	-
		hard claudication	I	3	-	2
III	pain at rest		II	4	11	5
IV	necrosis and ischemic ulcers	slight tissue damage	III	5	5	4
		significant tissue damage	IV	6	-	-

Table 2. Dynamics of limb ischemia degree after transplantation

The chronic ischemia degree according to Rutherford	Class	Before cell transplantation	12 months after cell transplantation
0	0	-	-
I	1	-	2
I	2	-	6
I	3	-	5
II	4	11	3
III	5	5	-
IV	6	-	-

#### 4. CONCLUSIONS

Positive clinical dynamics were observed in all patients of the experimental group after transplantation of cord blood cells. During the first month after cell transplantation, patients noted a gradual improvement in well-being, pain at rest decreased, and then vanished, trophic disorders decreased and marginal necrosis of the fingers healed, the distance and speed of painless walking gradually increased. In addition to improving the local status, an improvement in general condition was noted, and working capacity and overall activity increased in 3 months after cell transplantation. The state of patients in 6 months after cell transplantation: all patients were able-bodied and led an active social and home life. At 12 months after

transplantation clearly visible transition patients into the low degree of ischemia. Cord blood stem cell transplantation leads to activation of compensatory-restorative reaction in ischemic muscle tissue and stimulation of angiogenesis processes.

Due to the use of the indirect revascularization method through cord blood cell transplantation, a long-term positive clinical effect can be obtained, which manifests in the distance and speed of painless walking increase, improved performance, improved personal well-being of patients in the physical, psychological and social and economic sphere, correlates with laser Doppler data flowmetry.

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