



## CD25 and TNF- $\alpha$ in Patients with Critical! Ischemia of the Lower Extremities Under Conditions of Intravenous Laser Irradiation of Blood with Indirect Revascularization

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### Abstract

**Objective:** To immunocytochemically assess the CD25 (fixed cell receptor interleukin-2) positivity and TNF- $\alpha$  positivity of peripheral blood cells in patients with critical ischemia of the lower extremities (CILE) under intravenous laser irradiation of blood (ILIB) for indirect revascularization.

**Material and Methods:** The study was carried out in 66 patients with CILE who underwent indirect limb revascularization due to the impossibility of reconstructive operations and endovascular revascularization. In the perioperative period, 34 patients (the control group) underwent conventional treatment without ILIB. In 32 patients (the main group), ILIB was performed with the Mustang 2000 and Mustang 2000+ devices. CILE developed against the background of thromboangiitis obliterans and atherosclerosis obliterans.

**Results:** Prior to treatment, patients had a significantly increased number of CD25+ monocytes, granular leukocytes, and monocytes ( $p < 0.05$ ). In the control group, by the end of treatment, the number of CD25 + granular leukocytes ( $p < 0.05$ ) decreased, but the increased expression of CD25 by monocytes and lymphocytes did not normalize. The frequency of abnormal CD25-positive thrombo-leukocyte aggregates remains stable. In patients of the main group, the expression of CD25 remains elevated in lymphocytes and monocytes, but is completely normalized in granular leukocytes ( $p < 0.001$ ), and thrombo-leukocyte aggregates are reduced to a minimum. Increased number of TNF- $\alpha$  – positive formed elements (monocytes, granulocytes, lymphocytes) and TNF- $\alpha$  – positive thrombo-leukocyte aggregates ( $p < 0.05$ ). After treatment, the number of TNF- $\alpha$ -positive cells in the study group decreased significantly compared to the control group.

**Conclusion:** The use of ILIB in the perioperative period with indirect revascularization in patients with CILE completely normalizes CD25 expression in granular leukocytes, reduces the number of TNF- $\alpha$ -positive formed elements, the number of CD25 positive and TNF- $\alpha$ -positive thrombo-leukocyte aggregates in peripheral blood.

**Keywords:** Lower Extremity Critical Ischemia; Indirect Revascularization; Intravenous Laser Irradiation of Blood; CD25 Positivity of Peripheral Blood Cells; TNF- $\alpha$  Positivity of Peripheral Blood Cells

**Introduction**

Obliterating diseases of the arteries of the lower extremities occur in 3% of the adult population. In the coming years, according to the World Health Organization, the incidence of this pathology will increase by 5-7%. As a result of the progression of chronic occlusive arterial disease, 35% of this population develops critical ischemia [1,2]. The main methods of treatment of critical ischemia are reconstructive and hybrid operations [1,3]. In the absence of "outflow pathways", indirect methods of revascularization (lumbar sympathectomy, revascularizing osteotomies, gene therapy, *et al.*) are performed to save the limb [4-7].

In critical ischemia of the lower extremities, simultaneously with impaired blood flow in the main and collateral arteries, profound changes in homeostasis, morpho-functional and energetic disturbances are observed in the formed elements of peripheral blood and soft tissues of the extremity [8-10].

The effectiveness of the use of intravenous laser irradiation of blood (ILIB) in clinical practice, including in the treatment of patients with critical ischemia of the lower extremities (CILE), has been proven. There are reports of correction of hemostasis, lipid spectrum, humoral and cellular immunity, mediators of the inflammatory response, improvement of the morpho-functional state and metabolic status of leukocytes in patients with CILE [11-15]. However, the efficacy of ILIB on CD25 (fixed cell receptor interleukin-2) positivity and TNF- $\alpha$ -positivity of peripheral blood cells in patients with CILE remains poorly understood.

**Objective**

To immunohistochemically assess the CD25 (fixed cell receptor interleukin-2) positivity and TNF- $\alpha$  positivity of peripheral blood cells in patients with CILE under conditions of intravenous laser irradiation of blood (ILIB) with indirect revascularization.

**Material and Methods**

The study was carried out in 66 patients with CILE who underwent indirect limb revascularization due to the impossibility of reconstructive operations and endovascular revascularization. In 34 patients (control group), conventional treatment without ILIB was carried out in the perioperative period. In 32 patients (the main group), ILIB was performed with the apparatus "Mustang 2000" and "Mustang 2000+" (length -0.632; radiation power -5 mW, exposure -30 minutes, number of sessions - 10-12). The "reference" group consisted of 48 practically healthy individuals. CILE developed against the background of thromboangiitis obliterans and atherosclerosis obliterans.

CD25-positivity and TNF- $\alpha$ -positivity of formed elements were examined in peripheral blood samples from the ulnar vein of 66 patients and revealed by a streptavidin-biotin reaction modified to peripheral blood smears.

The numerical data are processed by statistical methods for nonparametric criteria.

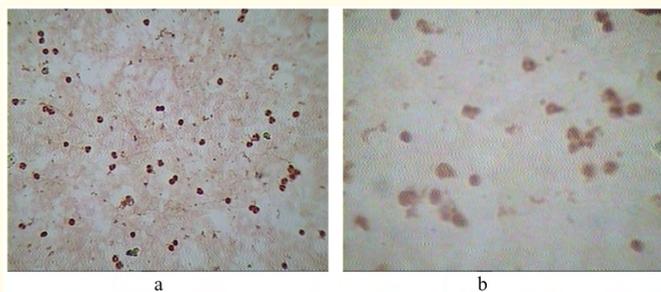
**Results**

In patients of the "reference group" CD25-positive lymphocytes, granulocytes and monocytes were found in 17.0% of formed elements (Table 1).

Groups patients Indicators	Reference Group (n = 48)	Control group		Main Group	
		Before treatment (n = 34)	After treatment (n = 34)	Before treatment (n = 32)	After treatment (n = 32)
CD25-positive count Formed elements (%)	17.0 ± 1.4	39.0 ± 2.8	31.5 ± 2.7	42.5 ± 3.8	30.3 ± 2.6
Number of TNF- $\alpha$ -positive form elements (%)	3.4 ± 0.3	23.0 ± 21.5	21.5 ± 1.8	24.5 ± 2.1	21.9 ± 1.9

**Table 1:** CD25 positivity and TNF- $\alpha$  positivity in peripheral blood elements in patients with critical ischaemia lower extremities.

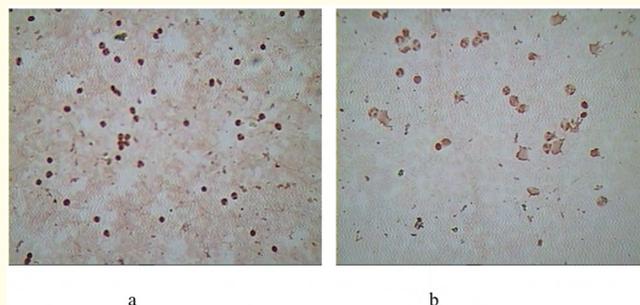
In patients before treatment, the number of CD25+ monocytes, granular leukocytes, and monocytes was significantly increased ( $p < 0.05$ ). There are also thrombo-leukocyte aggregates with sharp CD25-positivity. In the control group, by the end of treatment, the number of CD25 + granular leukocytes decreased ( $p < 0.05$ ), but the frequent CD25 compression by monocytes and lymphocytes did not normalize. The frequency of abnormal CD25-positive thrombo-leukocyte aggregates remains stable. In patients of the main group, the expression of CD25 remains elevated in lymphocytes and monocytes, but is completely normalized in granular leukocytes ( $p < 0.001$ ), and thrombo-leukocyte aggregates are reduced to a minimum (Figure 1).



**Figure 1:** CD25 positivity in peripheral blood. a – before treatment (male patient aged 64 years); b – standard treatment in the perioperative period (the same patient) Staining: Immunocytochemical reaction with CD25 monoclonal antibodies Magnification: a – x200; b – x480.

In patients of the “reference group” TNF- $\alpha$ , positive lymphocytes, granuloocytes and monocytes were found in 3.4% of the formed elements. Platelets in these individuals are TNF- $\alpha$  negative (Table

1). In patients of the control group TNF- $\alpha$ , positive lymphocytes, granuloocytes and monocytes were found in 23.0% of the formed elements. The identified TLAs have always been TNF- $\alpha$  positive. After treatment with TNF- $\alpha$ , positive formed elements decreased to 21.5%. In the study group, as a result of TNF- $\alpha$  treatment, positive formed elements decreased from 24.5% to 21.9%. The number of TNF- $\alpha$ -positive thrombo-leukocyte aggregates significantly decreased, but these complexes did not completely dissolve (Figure 2).



**Figure 2:** TNF- $\alpha$  – positivity in peripheral blood. a – before treatment (male patient at the age of 55); b – standard treatment in the perioperative period (same patient) Stain: Immunocytochemical reaction with TNF- $\alpha$  monoclonal antibodies Magnification: a – x200; b – x240.

The dependence of interleukin-positivity of formed elements of peripheral blood on treatment tactics in the perioperative period after the operation of indirect revascularization in patients with critical ischaemia of the lower extremities was also studied IL1B, which is an indicator of correction of inflammatory activity. A decrease in inflammatory activity and a decrease in the number of thrombo-leukocyte aggregates are prerequisites for improving microcirculation in ischemic soft tissues of the limb (Table 2).

Indicator		Interleukin-positivity of formed elements of peripheral blood	$\chi^2$
Group of patients			P
			r
Control group b (n = 34)	Improvement	11	$\chi^2 = 4.833$ $p < 0,05$ $r = 0,4$
	no change	23	
Main Group (n = 32)	Improvement	19	
	no change	13	

**Table 2:** Dependence of interleukin-positivity of formed elements of peripheral blood on treatment tactics in the perioperative period after indirect revascularization in patients with critical ischaemia of the lower extremities.

## Conclusion

The use of ILIB in the perioperative period with indirect revascularization in patients with CLI completely normalizes CD25 expression in granular leukocytes and reduces the number of CD25-positive thrombo-leukocyte aggregates in peripheral blood. ILIB reduces the number of TNF- $\alpha$ -positive formed elements of peripheral blood and TNF- $\alpha$ -positive thrombo-leukocyte aggregates.

## Conflict of Interests

The authors state that this work, its theme, subject and content do not affect competing interests.

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