Journal of Surgery
kosayev JV,et al. J Surg 11: 11635
www.doi.org/10.29011/2575-9760.11635
www.gavinpublishers.com
Research Article

Morpho-Functional and Immunocytochemical Changes in the Formed Elements of Peripheral Blood in Patients with Critical Ischaemia of the Lower Extremities Under the Conditions of Complex Treatment in the Perioperative Period with Indirect Revascularization

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Citation: Kosayev JV, Hasanov IA, Abushov NS, Taghi-zade GT, Quliyev RA, et al. (2026) Morpho-Functional and Immunocytochemical Changes in the Formed Elements of Peripheral Blood in Patients with Critical Ischaemia of the Lower Extremities Under the Conditions of Complex Treatment in the Perioperative Period with Indirect Revascularization. J Surg 11: 11635 DOI: 10.29011/2575-9760.011635.

Received Date: 24 May 2026; **Accepted Date:** 28 May 2026; **Published Date:** 30 May 2026

Abstract

Objective: To study morpho-functional and immunocytochemical changes in peripheral blood cells in patients with critical ischaemia of the lower extremities lower (CILE) under the conditions of complex treatment in the perioperative period with indirect revascularization.

Material and methods: The study was conducted in 65 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 was carried out in the perioperative period. In 31 patients (the main group), ILIB with the apparatus "Mustang 2000" and "Mustang 2000+**",** cytokine therapy with the drug Roncoleukin, and intramedullary laser irradiation with the Mustang 2000 and Mustang 2000^{+}

devices: CILE developed against the background of thromboangiitis obliterans and atherosclerosis obliterans. **Results:** In patients of both clinical groups, a low content of G, RNP, ATP and low activity of ATP-ase in mature granulocytes and agranulocytes were revealed before treatment, an increase in the membrane adhesion of leukocytes, the frequency of thrombo-leukocyte aggregates was observed, and the number of CD25, TNF-α, and IL-6-positive monocytes, granular leukocytes, and monocytes was increased ($p < 0.05$). Complex treatment enhances the intracellular synthesis process (RNP), the production and utilization of energy (G, ATP, ATP-ase) in peripheral blood leukocytes in patients of the main group. After treatment in patients of the main group, the expression of CD25 remains increased in lymphocytes and monocytes, but is completely normalized in granular leukocytes ($p < 0.001$), and thrombo-leukocyte aggregates are reduced to a minimum. After treatment, the number of TNF-α-positive and IL-6-positive cells (monocytes, granulocytes, lymphocytes) in comparison with the control group significantly decreased in the study group. The number of TNF-α and IL-6 – positive thrombo-leukocyte aggregates – also decreased.

Conclusion: Complex treatment in the perioperative period for indirect revascularization in patients with CLI improves energy supply and intracellular expression in granular leukocytes, reduces the number of CD25, TNF-α and IL-6 positive formed elements, the number of CD25 positive, TNF-α positive and IL-6-positive thrombo-leukocyte aggregates in the peripheral blood.

Keywords: CD25 Positivity; Cytokine Therapy With Roncoleukin; Indirect Revascularization; Intramedullary Laser Irradiation; Energy Supply To Peripheral Blood Cells; Intravenous Laser Irradiation Of Blood; Lower Limb Critical Ischemia; TNF-A Positivity And IL-6 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, endovascular, and hybrid operations [1,3,4]. In the absence of "outflow pathways", indirect methods of revascularization (lumbar sympathectomy, revascularizing osteotrepanation, gene therapy et al.) are performed to save the limb [5-8]. In critical ischemia of the lower extremities, profound changes in homeostasis, morpho-functional and energetic disorders are observed in the formed elements of peripheral blood and soft tissues of the extremity [9-11].The effectiveness of the use of intravenous laser and ultraviolet irradiation of blood in clinical practice, including in the treatment of patients with critical ischaemia of the lower extremities(CILE). 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 [12-15]. Isolated reports have also been published on the use of cytokine therapy with Roncoleukin, intramusculear laser irradiation in the complex treatment of patients with CILE [16,17]. The effectiveness of the combined use of intravenous laser irradiation of blood (ILIB) remains poorly studied, cytokine therapy with Roncoleukine and intramuscullary irradiation in the perioperative period with indirect revascularization for metabolic status, membrane adhesion of leukocytes, frequency of thrombo-leukocyte aggregates, CD25-positivity, TNF-α-positivity and IL-6 positivity of peripheral blood cells in patients with CILE.

Objective

To study morpho-functional and immunocytochemical changes in peripheral blood cells in patients with lower limb critical ischaemia (CILE) under the conditions of complex treatment in the perioperative period with indirect revascularization.

Material and Methods

The study was conducted in 65 patients with CLI who underwent indirect limb revascularization due to the impossibility of reconstructive operations and endovascular revascularization. In 34 patients (control group), conventional treatment was carried out in the perioperative period. In 31 patients (the main group), ILIB was performed with the apparatus "Mustang 2000" and "Mustang 2000+" (wavelength -0.632; radiation power -5 mW, exposure -30 min., number of sessions – 10-12), cytokine therapy with Roncoleukin (two subcutaneous injections in the upper third of the upper arm of the drug after 3 days at a dose of 1000000 IU) and intramedullary laser irradiation (ILL) with the apparatus "Mustang 2000" and "Mustang 2000+" (length -0.632; radiation power - 2 mW, exposure -30 minutes, number of sessions - 6-7). The "reference" group consisted of 48 practically healthy individuals. CILE developed against the background of thromboangiitis obliterans and obliterating atherosclerosis. In both clinical groups, peripheral blood samples were taken from the ulnar vein before and after the completion of treatment. In smears stained with modified Brachet, Hodgkiss-McManus, Wachstein-Meisel and Hasanov methods with the setting of control samples, visually, semiquantitatively and cytophotometrically (by optical density) the content of glycogen (G), riborucleoproteins (RNP), macroergic nucleotide triphosphates (ATP), and adenosine triphosphatase (ATP-ase) activity. Leukocyte membrane adhesion (MAL) was studied by the cytochemical reaction with sour fuchsine + 0.1% metilenblau Thrombo-leukocyte aggregates (TLAs) were determined by the reaction with 0.1% Metile Blue + 0.05% Nile Blue Sulphate. CD25-positivity, TNF-α-positivity, and IL-6 positivity of formed elements were studied in peripheral blood samples from the ulnar vein of 65 patients and revealed by the streptavidin-biotin reaction. modified to peripheral blood smears. Digital data are processed by statistical methods for parametric and non-parametric criteria.

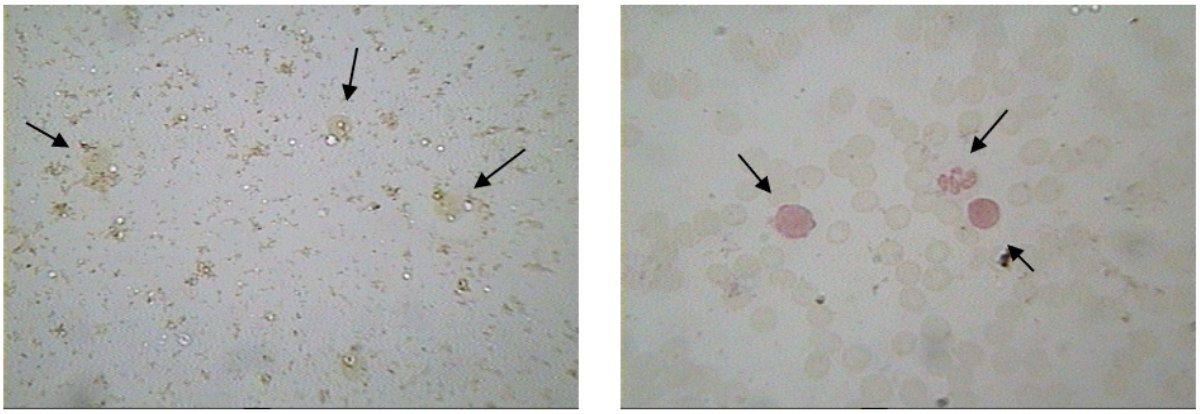
Results and Discussion

In patients of both clinical groups, a low content of G, RNP, ATP and low ATP-ase activity in mature granulocytes and agranulocytes were revealed before treatment. In the control group, at the end of treatment, the content of these substrates in segmented nuclei was almost unchanged, in monocytes and lymphocytes with an insignificant increase predominantly in lymphocytes ($p > 0.1$). ATPase remains inhibited and its potential only in monocytes exceeds 1.0 b. In the study group, after complex treatment in the perioperative period, an increase in polymorphism of lymphocytes and monocytes without deformation and formation of thrombo-leukocyte aggregates was noted in peripheral blood smears. The content of G is in comparison with the control group, without significant changes, with a tendency to decrease ($p>0.1$). RNP and ATP are elevated ($p<0.05$) in monocytes and lymphocytes. In parallel with a certain decrease in the G content and an increase in ATP, the potential of ATPase is enhanced. ($p <0.05$) in almost all types of leukocytes. Such a combination of quantitative indicators of the main energy raw material -G with the parameters of ATP and ATP-ase in the studied populations, as well as an increase in

cellular polymorphism, as well as an increase in intracellular synthesis (RNP), energy production and utilization (G, ATP, ATP-ase) in peripheral blood leukocytes in patients of the main group (Table 1; Figures 1-3). In individuals of the "reference group", the membrane adhesion of leukocytes was 22.0 ±1.2 optical-cytochemical units. Membrane adhesion of peripheral blood leukocytes in both groups before treatment was abnormally high (36.9 ±1.9 and 36.9 ±2.0, respectively), especially in monocytes and segmented leukocytes. After treatment in patients of the control group, MAL decreased by 18.7%. In the patients of the study group, after complex treatment with the use of ILIB, cytokine therapy, and IMLI in the perioperative period, MAL decreased by 25.2% ($t=3.80$, $p< 0.01$). (Table 2). In physiologically healthy individuals, TLAs are not found in the peripheral blood (with the exception of isolated cases). Before treatment, TLA was found in 73.5% of patients in the control group, and in 83.9% of patients in the study group.

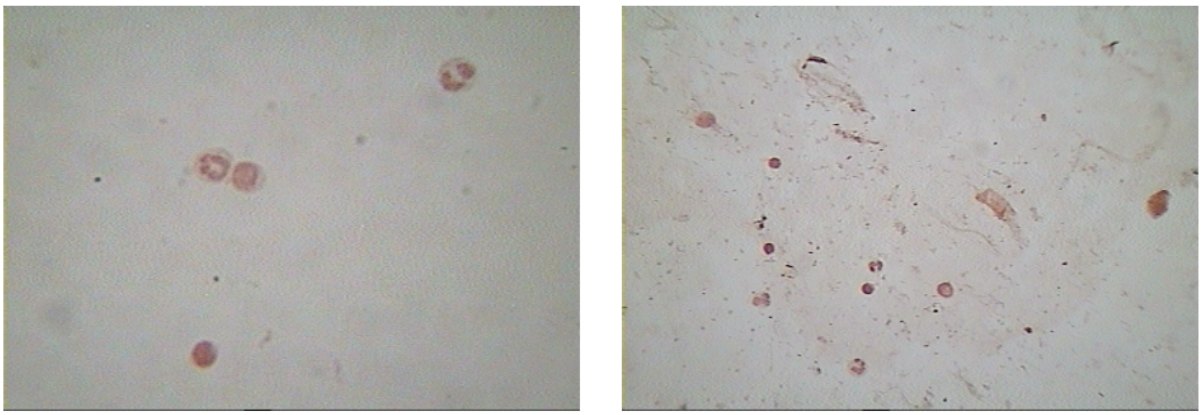
Group / Patients / Indicators	Reference Group (n=34)	Control group		Core Group	
		Before treatment (n=34)	After treatment (n=34)	Before treatment (n=32)	After treatment (n =32)
Glycogen (conventional unit)	2.9 ± .22	2.1 ± 0.16	2.3 ± 0.18	2.1 ± 0.15	2.6 ± 0.26
Ribonucleoproteins (conventional unit)	3.0 ± 0.27	2.1 ± 0.15	2.3 ± 0.19	2.0 ± 0.17	2.6 ± 0.25
Adenosine triphosphate (conventional unit)	3.0 ± 0.27	2.0 ± 0.15	2.1 ± 0.15	2.0 ± 0.17	2.8 ± 0.25
Adenosine triphosphate ase (conventional unit)	3.5 ± 0.31	2.2 ± 0.19	2.5 ± 0.21	2.1 ± 0.18	2.9 ± 0.26

Table 1: Cytochemical characteristics of metabolic status and energy supply in the formed elements of peripheral blood in patients with critical ischaemia of the lower extremitie.



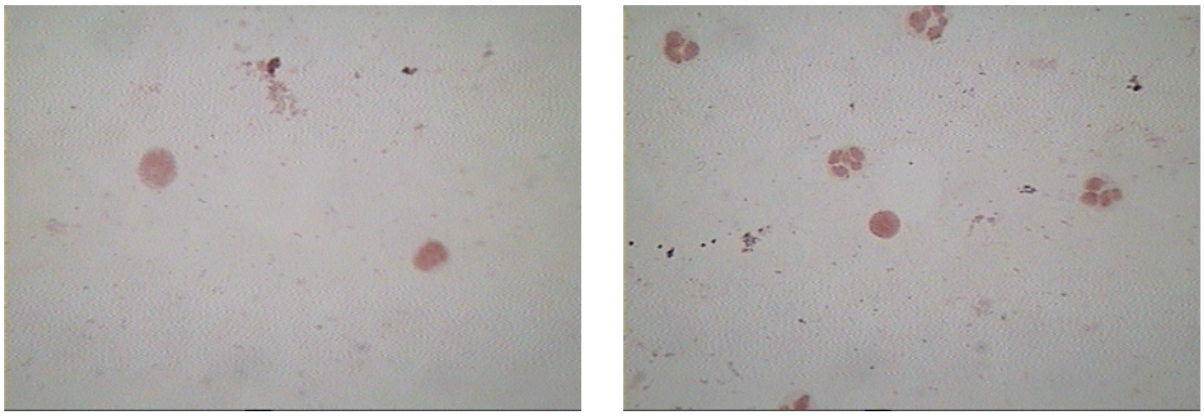
a b

Figure 1: Cytochemical study of ribonucleoproteins (RNPs) in various leukocytes in peripheral blood clotting: a – before treatment; b – after treatment (a 57-year-old male patient); traditional treatment + intravenous laser radiation + cytokinotherapy + intramedullary laser radiation). Significant increase in RNP in monocytes and segmented leukocytes after treatment. Dye: Brush reaction (methyl-aged - pyronine G); Magnification: x400.



a b

Figure 2: Cytochemical study of adenosine triphosphate (ATF) in various leukocytes in peripheral blood clotting: a – before treatment; b – after treatment (a 57-year-old male patient; traditional treatment in the perioperative period + venous laser radiation + cytokinotherapy + intramedullary laser radiation). In the case of monocytes, segmental leukocytes and an increase in the cytochemical amount of ATF in lymphocytes. Dye: J.L. Griffin, G. Pezeshkpour (1988) method for the determination of adenosine triphosphate (ATF); Magnification: a – x400; b – x200.



a b

Figure 3: Cytochemical study of adenosine triphospate ase (ATF-azane) in various leukocytes in peripheral blood clotting: a – before treatment; b – after treatment (a 61-year-old male patient; traditional treatment + intravenous laser radiation + cytokinotherapy + intramedullary laser irradiation during perioperative period). After treatment, an increase in the number of ATF-positive cells in monocytes, segmental leukocytes, and lymphocytes.

Dye: Adenosine triphosphate (ATP-monophosphate; 3.6.1.3.; J.L. Griffin, G. Pezeshkpour (1988) for the determination of ATF-aza; Magnification: a – x400; b – x200.

After treatment, the incidence of patients with TLA decreased to 55.9% in the control group. In patients of the study group, after complex treatment with the use of ILIB, cytokine therapy, and IMLI in the perioperative period, TLA occurred in 22.6% of patients (Table 2, Figure 4).

Groups / patients / Indicators	Reference Group (n=34)	Control group		Core Group	
		Before treatment (n=34)	After treatment (n=34)	Before treatment (n=31)	After treatment (n=31)
Membrane adhesion of leukocytes (optical cytochemical unit)	22.0 ± 1.2	36.9 ± 1.9	30.0 ± 1.8	36.5 ± 2.0	27.3 ± 1.5
Frequency of thrombo-leukocyte aggregates (%)	0.0	25 (73.5%)	19(55.9%)	26 (83.9%)	7 (22.6%)

Table 2: Cytochemical characteristics of membrane adhesion of leukocytes and the frequency of thrombo-leukocyte aggregates in the formed elements of peripheral blood in patients with critical ischaemia of the lower extremities.

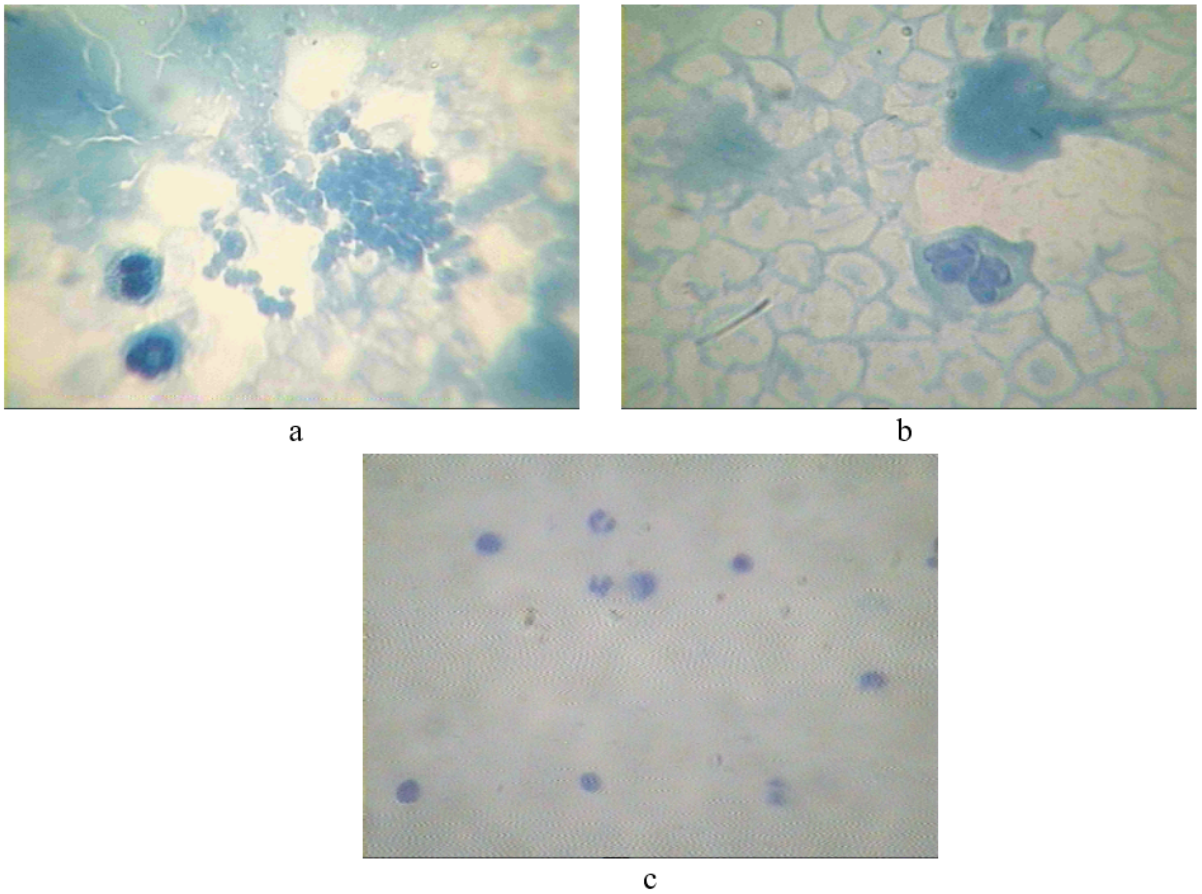


Figure 4: Thrombo-leukocytic aggregates in peripheral blood: a – before treatment (a 62-year-old male sick); b – remaining aggregates; End of traditional treatment during perioperative period; c – actual absence of aggregates; Traditional treatment in the perioperative period + intravenous laser radiation + cytokinotherapy + intramedullary laser radiation (a 60-year-old male patient). Dyeq: 0.1% methylene + 0.05% Nile sulfate (nilblausulfat); Magnification: a, b – x1000; C – x400.

In persons of the "reference group" CD25-positive lymphocytes, granulocytes and monocytes were found in 17.0% of the formed elements. 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 patients of the control group, by the end of treatment, the number of CD25 + granular leukocytes decreases ($p < 0.05$), but the frequent CD25 compression by monocytes and

lymphocytes does 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 (Table 3, Figure 5).

Groups / patients / Indicators	Reference Group (n=48)	Control group		Core Group	
		Before treatment (n=34)	After treatment (n=34)	Before treatment (n=31)	After treatment (n = 31)
Number of CD25-positive formed elements (%)	17.0 ±1.4	39.0 ±2.8	31.5 ±2.7	43.3 ±3.8	25.7 ±2.0
Number of TNF-α-positive form elements (%)	3.4 ±0.3	23.0±21.5	21.5 ±1.8	25.8 ± 2.3	20.5±1.7
Number of IL-6 positive form elements (%)	3.3 ±0.3	12.8 ±1.1	11.3 ±0.8	14.0 ±1.2	11.6 ±1.0

Table 3: CD2 5 positivity, TNF-α positivity and IL-6 positivity in peripheral blood elements in patients with lower limb critical ischaemia.

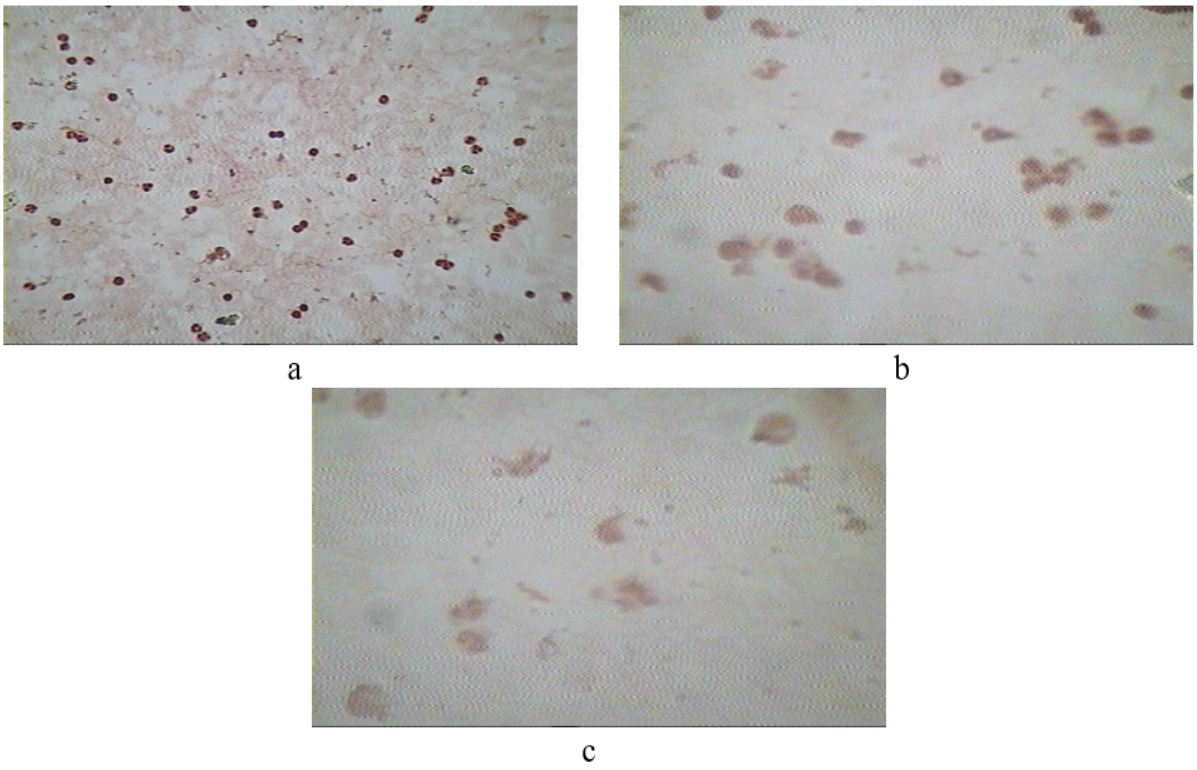


Figure 5: CD25-positivity in peripheral blood. a – before treatment (a 64-year-old male patient); b – standard treatment during perioperative period (same patient); c – traditional treatment + intravenous laser radiation + cytokinotherapy + intramedullary laser radiation (a 60-year-old male patient). Dye: Immunocytochemical reaction with CD25 monoclonal antibodies; Magnification: a – x200; b,c – x480.

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 increased in lymphocytes and monocytes,but is completely normalized in granular leukocytes (p

<0.001), and thromboleukocyte aggregates are reduced to a minimum (Figure 4). In persons of the "reference group" TNF-α, positive lymphocytes, granulocytes and monocytes were found in 3.4% of the formed elements. Platelets in these individuals are TNF-α negative (Table 3). In patients of the control group TNF-α, positive lymphocytes, granulocytes and monocytes were found in 23.0% of the formed elements. The identified TLAs have always been TNF-α positive. After treatment with TNF-α, positive formed elements decreased to 21.5%. In the main group, as a result of TNF-α treatment, positive formed elements decreased from 24.5% to 21.9%. The number of TNF-α-positive thrombo-leukocyte aggregates decreased significantly, but these complexes were still present (Figure 6).

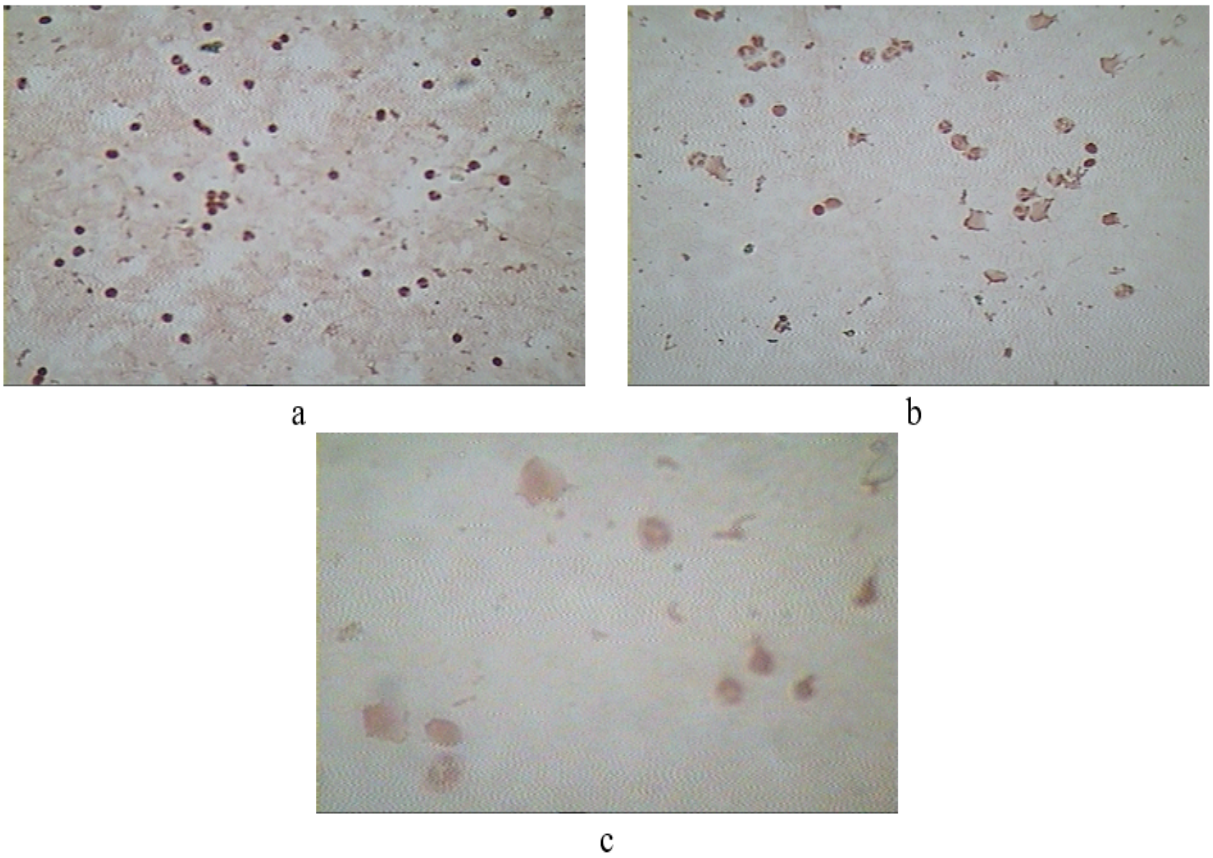


Figure 6: TNF-α-positivity in peripheral blood: a – before treatment (a 55-year-old man is sick); b - Traditional treatment during perioperative period (same patient); c – traditional treatment + intravenous laser radiation + cytokinotherapy + intramedullary laser radiation (a 67-year-old male patient). Dye: Immunocytochemical reaction with monoclonal antibodies α SNF; Magnification: a - x200, b - x240; c - x480.

In apparently healthy individuals, IL-6-positive cells are found in 3.3±0.3% of peripheral blood cells, and positivity is often observed in lymphocytes, monocytes, and segmented leukocytes. In conventional perioperative treatment, IL-6-positive cells were found in 12.8±1.1% of the formed elements in the perioperative period, and after treatment, the incidence of IL-6-positive cells decreased by 11.7%. In perioperative complex treatment with VLOK, cytokine therapy, and intramedullary laser irradiation together with conventional treatment, the number of IL-6-positive cells decreased by 17.2% (from 14.2±1.2% to 11.6±1.0%) (Table 3, Figure 7).

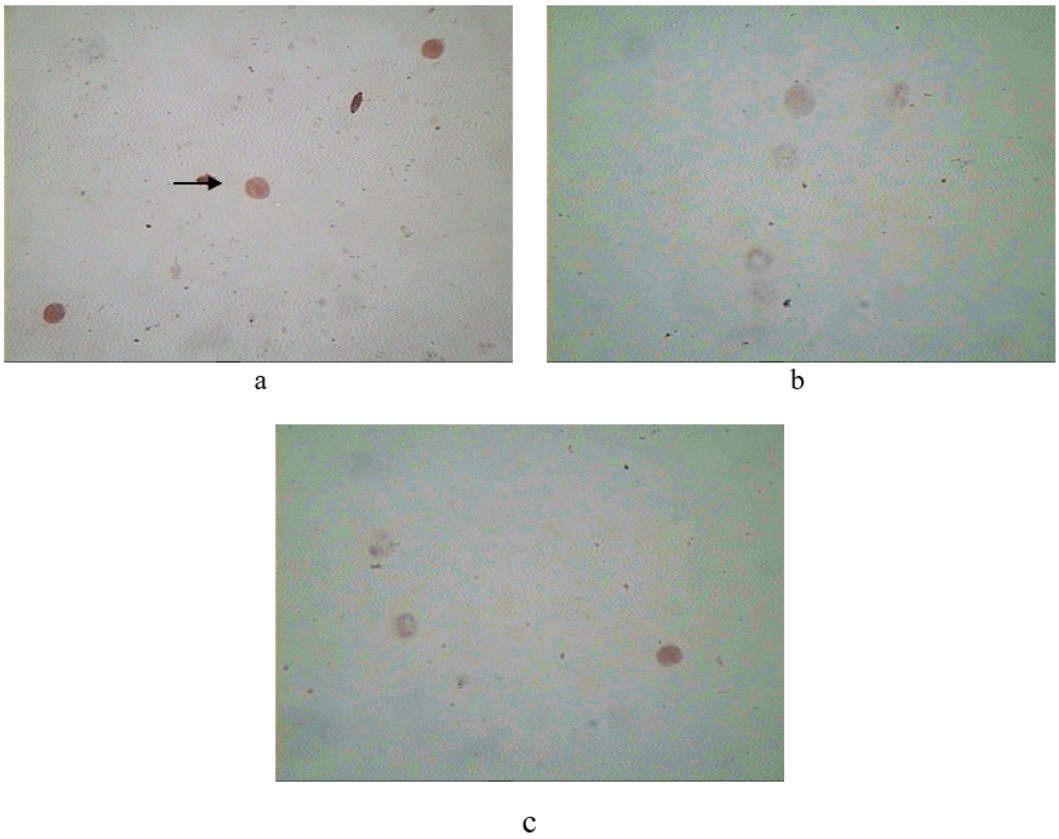


Figure 7: Interleukin-6 in peripheral blood – microscopic view of positivity. a – specific stained platelets along with positive lymphocytes (↑); before treatment (a 60-year-old male patient); b – traditional treatment during perioperative period (same patient); c – traditional treatment during perioperative period + intravenous Laser radiation + cytokinotherapy + intramedullary laser radiation (a 67-year-old male patient). Dye: IL-6 - immunocytochemical reaction with monoclonal anti-bodies; agnification: a, c – x400, b – x440.

An analysis of the literature indicates profound changes in hemostasis and hemorheology, lipid spectrum, cellular and humoral immunity in patients with critical lower limb ischaemia [13,15].

Morpho-functional, immunocytochemical changes in the formed elements of peripheral blood have been revealed [9,10,11,14]. These changes lead to progressive reduction of microcirculation in the soft tissues of the affected limb. For the prevention and treatment of these changes in homeostasis in patients with lower limb critical ischemia, various therapeutic measures have been proposed in the perioperative period [4,8,10,12-17]. A comparative analysis of the data obtained by us and the literature data allows us to state the effectiveness of the combined use of ILIB + cytokine therapy with Ronkoleukin + intramedullary laser irradiation in the perioperative period with indirect revascularization in patients with critical ischaemia of the lower extremities. Since the proposed method of managing the perioperative period with indirect revascularization improves the metabolic status, reduces the membrane adhesion of leukocytes, thrombo-leukocyte aggregates, it neutralizes immunocytochemical changes in the formed elements of peripheral blood. These factors lead to improved microcculation and metabolism in the ischemic soft tissues of the limb.

Conclusion

- The use of ILIB, cytokinetherapy and IMLI in the perioperative period for indirect revascularization in patients with CILE improves energy supply and enhances intracellular synthesis in granulocytes and agranulocytes of peripheral blood.
- The proposed method of perioperative management in patients with CILE reduces MAL in monocytes and segmented leukocytes of peripheral blood, decreases TLA circulating in peripheral blood

- Complex treatment in the perioperative period for indirect revascularization in patients with CLI completely normalizes CD25 expression in granular leukocytes, reduces the number of CD25-positive thrombo-leukocyte aggregates in peripheral blood, reduces the number of TNF-α- and IL-6-positive formed elements of peripheral blood and TNF-α- and IL-6-positive thrombo-leukocyte aggregates.

Conflict of interests: The authors state that this work, its theme, subject and content do don't affect competing interests

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