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Research Article

## Analysis Study of Visual and Physiological Functions, Expression of Various CD Markers of the Immune System, Clinical Course and Treatment of Complications in Patients with COVID-19 When Using Drug Mercureid

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### Abstract

The coming era of COVID-19 introduces critical challenges for researchers - what will happen to the patients who have suffered from long COVID-19? What diseases threaten them? What happens to the patients' immunity after the action of antigen SARS-CoV-2? To what extent do the changes caused by it contribute to or, on the contrary, prevent from the development of long-term protective immunity? And how effective will COVID-19 vaccine be in these patients?

We tried to answer some of these questions in our research. The study was conducted in 49 patients with ophthalmic pathologies who had previously undergone COVID-19. On the one hand, ocular pathologies are important because they allow in a non-traumatic way to obtain lifetime visualization of the state of blood vessels and capillaries, as well as to assess the effect of the virus on the central nervous system. On the other hand, immunological studies made it possible to draw a conclusion about the state of antiviral immunity, immune status and its

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correlation with the severity of inflammatory processes in the eye structure, the central nervous system including the vascular endothelium.

The novelty of the study is that we have established a causal relationship between SARS-CoV-2 infecting, formed dysfunction of immune parameters that caused the manifestation of chronic inflammatory diseases. As a result, light adaptation was impaired by 2.3 times, due to the damage to blood circulation owing to the neurotoxic effect of SARS-CoV-2 and hypoxemia. The corrective effect of drug Mercureid was fixed in 73.4 % of patients.

The most dramatic cases were observed in the group of patients with damage to the retinal vascular system: the phenomenon of re-thrombosis of both the central retinal vein and its branches, as well as circulatory disorders in the optic nerve trunk - ischemic optic neuropathy with a sharp deterioration in vision. In these patients, the combination of vascular drugs and drug Mercureid allowed stabilizing the patients' state, achieve remission and in some cases reach high visual functions in 50.0% of cases.

Mercureid made venotonic and angioprotective effect. It reduced vein elasticity and capillary permeability. Also it improved venous outflow and microcirculation that allowed in some cases to restore lymphatic drainage.

According to the results of the immune study, the targeted effect of the new drug Mercureid, aimed at modulating the activity of several critical target proteins, such as CD3, CD4, CD8, CD25, CD38, CD54, CD95 was revealed. The therapeutic efficacy of Mercureid was 75.1%.

The second research finding is that in patients with manifestation of chronic inflammatory diseases who have previously been infected with SARS-CoV-2, the production of specific protective antibodies is likely to be impaired (as these patients often have pathologically low levels of CD4, CD8, CD25 and overexpression of CD38, ICAM-1, CD95 that causes apoptosis of immune cells, lymphopenia and also forms the phenotype of exhausted T-cells with activation of the expression of inhibitory receptors) and vaccination may be ineffective for them, due to the presence of a compromised immune system. Accordingly, the provision of corrective multitarget immunotherapy aimed at several target proteins, which are critical for the formation of long-term effective post-viral immunity to SARS-CoV-2, is an extremely important therapeutic need. This immunotherapy can be carried out both before and after vaccination in order to achieve the maximum protective effect from the vaccine. But the definite answer to this research question will require another type of study design, which we plan to conduct in the future.

## Keywords

Long COVID-19; SARS-Cov-2; Antiviral Immunity; Mercureid; Role of T-Lymphocytes; Immune Homeostasis; Viral Immune-Inflammatory Lesions after COVID-19

## Introduction

One of the most pressing problems of medicine in the modern world and issues of the mankind survival is the high morbidity and mortality from viral infections, which are widespread in the human population, capable of affecting almost all organs and systems of the host's body. Over the past decades, humanity has experienced serious trials. There have never been such rapid pandemics in the world history, which during a short period of time took away many lives not only of people suffering from chronic diseases, but also of those people who, before the disease, considered themselves practically healthy, regardless of age, gender, social and material status in the society. Viral infection is a cofactor for the progression of HIV infection and AIDS (Isakov VA, et al., 2006; Samgin MA, et al., 2002). Unfortunately, the lessons from the past pandemics have taught little to humanity.

## SARS-CoV-2

Since December 2019, COVID-19 has evolved into a global pandemic of severe acute respiratory syndrome caused by a new highly transmissible RNA virus identified as a member of the family of Coronaviridae. SARS-CoV-2 is a beta coronavirus. It enters the cells of the human body using two proteins: the first one is ACE2 cell receptor (Angiotensin-Converting Enzyme 2), connected with the angiotensin converting enzyme and the other is TMPRSS2 enzyme.

The ophthalmologist was the first to report the virus in Wuhan. He himself contracted and died from the disease while treating a patient for glaucoma. Ophthalmic manifestations vary in form, severity and timing. Wu, et. al., suggested that ophthalmic manifestations are more common in patients with severe systemic diseases with abnormal blood parameters and inflammatory processes [1]. Obviously, when infected with SARS-Cov-2 virus, which causes COVID-19, the respiratory tract, intestines, kidneys and eyes are endangered first of all, as both proteins necessary for the virus are present in these organs. Even in the study of atypical pneumonia SARS and Middle East Respiratory Syndrome (MERS), it was found that coronaviruses are able to penetrate through nerve cells into the brain.

MRI studies have shown an increase in the signal from the white matter of the posterior rectus gyurus in the cerebral hemispheres and from the olfactory bulbs. Taking into account the study

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of the patients ill with COVID-19, we have come to the conclusion that the virus causes changes in white matter only during the first days of infection and in some patients, respiratory failure may appear due to the effect of the virus on the brain structures that are part of the limbic system. However, the organ of vision is also affected, because ACE2 receptor is known to be widely expressed in many tissues, including the retina, which is an extension of the central nervous system. It should be noted that ACE2 receptor is involved in the pathogenesis of diabetic and hypertensive retinopathy. Expression of ACE2 has also been found in the cornea, human aqueous humor and retinal tissue, pigment epithelial cells, photoreceptors and Müller cells. In addition, coronaviruses are known to cause inflammation in animals directly in the organ of vision, including retinitis and optic neuritis.

According to meta-analysis 2020, among patients with COVID 19, the overall prevalence of ophthalmic pathology is estimated at 7% (95% CI: 0.03-0.10). It is known that conjunctivitis can be the entrance gate of infection [2]. The authors have also concluded that the spread of the virus in the conjunctiva may persist even after a nasopharyngeal swab becomes negative for SARS-Cov-2 virus [3].

Conjunctivitis is the most common ophthalmic manifestation which is found in patients with COVID-19. In a great number of cases of mild COVID-19 infection, Sindhuja, et al., reported that 8.66% of patients had conjunctivitis. All symptomatic patients had a history of redness in one or both eyes. Respiratory tract symptoms have been connected with conjunctival congestion.

## Conjunctivitis in Children

A 30 times higher increase in morbidity of Kawasaki disease in children has been reported in some parts of Italy that are strongly connected with COVID-19. This atypical manifestation is known as Multisystem Inflammatory Syndrome in Children (MIS-C) [4]. Kawasaki disease, a form of self-limiting vasculitis, is connected with iridocyclitis, punctate keratitis, vitreous opacity, papilloedema edema and subconjunctival hemorrhage [5]. In the available literature on MIS-C, ophthalmic manifestations have been predominantly in the form of conjunctivitis.

## Pathogenesis of COVID-19

According to the world research literature, three main COVID-19 pathogenetic mechanisms are described: inflammatory syndrome as a result of viral invasion; pro-inflammatory state with hypercoagulability and "cytokine storm"; hypoxia and hypertension. Virus damage to endothelial cells via ACE-2 receptor has been shown in the lungs, heart, kidneys, intestines and brain. Histopathological studies have demonstrated direct viral invasion of endothelial cells,

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endotheliitis and vasculitis in both arterial and venous beds. Inflammation of endothelial cells causes edema, blockage and thrombosis of small vessels that ultimately leads to organ ischemia [6,7]. One theory of hypercoagulation associated with COVID-19 suggests that SARS-CoV-2 promotes the recruitment of inflammatory cells into the blood vessels, resulting in the release of inflammatory markers and cytokines, which subsequently activate the coagulation cascade [8].

Clinical results show significantly increased prothrombin time, high D-dimer levels and increased concentrations of proinflammatory cytokines and inflammatory biomarkers in patients with more severe disease, indicating the likelihood of disseminated intravascular coagulation or thrombotic microangiopathy [9].

Patients with COVID-19 suffer from moderate to severe hypoxemia, which can contribute to dysfunction of many organs, especially the central nervous system. Hypoxemia causes functional changes in ion channels sensitive to oxygen, the Na<sup>+</sup>/K<sup>+</sup> pump, disrupts the processes of excitation and inhibition of neuronal and glial cells, activates excitotoxicity [10].

In COVID-19 patients, ACE-2 receptor function is exhausted immediately after binding to SARS-CoV-2. There is an increase in Ang II - type II angiotensin and a decrease in the levels of Ang 1-7 (angiotensin 1-7) in tissues that leads to an aggravation of hypertension [11].

Currently, it has been suggested that antihypertensive drugs, the effect of which is based on inhibiting ACE or blocking ACE-2 receptor, can either aggravate or, conversely, improve the clinical course of COVID-19 [12].

COVID-19 has a wide range of clinical manifestations, from asymptomatic to symptomatic disease progression. Infection with the virus causes latent, acute, chronic and slow forms of infection. The immune response of the human body to a viral infection is divided into two phases, the phase of localization of the virus in a limited anatomical area and the phase of late specific influence, during which the localized infection is removed.

In patients with COVID-19, other infections (viral, bacterial, fungal) occurring parallel to COVID disease were often found [13].

Thus, a positive test result for another pathogen does not exclude infection with COVID-19 and vice versa. The prevalence of acute acquired concomitant or secondary infections occurring in the context of COVID-19 is not precisely determined, but, apparently, it is low and it depends on local factors, including the presence of endemic and new infections in the region [14-18].

Overuse of antibiotics is one of the contributing factors to the disease. It increases the risk of the emergence and spread of multidrug-resistant bacteria. Infections caused by these bacteria are more difficult to treat and they increase morbidity and mortality.

## COVID-19 and Ophthalmology

According to the recent data from the research literature, it is known that:

1. The full range of clinical manifestations of ophthalmic pathologies connected with COVID-19 is not completely cleared out; new clinical symptoms are still being described.
2. Structural and functional changes in the organ of vision both as a result of the direct viral effect of COVID-19 and as a result of a violation of homeostasis are still being studied.
3. Some researchers suggest that post-COVID syndrome and disorders in the visual analyzer may be considered as an underestimated large-scale problem [19].

## Post-COVID Syndrome in Ophthalmology

All of the above-mentioned factors in the pathogenesis of COVID-19 and post-COVID syndrome are also relevant for ophthalmology. The global coronavirus pandemic has far-reaching and long-term consequences. The full spectrum of the disease is still under examination. The disease consequences for various organs, multiple views, theories of pathogenesis and associations with SARS-CoV-2 severe acute respiratory syndrome are being examined at a fast pace today.

An ordinary search for term "COVID-19" in search engines offers about a hundred thousand articles. If we specify this term by adding the word "ophthalmology", the results are less overwhelming, but still about 1000 publications can appear. To reduce the number of publications to more realistic values, the words "ophthalmic manifestations" were introduced. These words were found a little more than 100 times.

The literature search was carried out in PubMed for COVID-19, SARS-CoV-2, "ophthalmology", "ophthalmic manifestations", "anterior segment", "conjunctiva", "eye surface", "retina", "vascular membrane of the eye", "uveitis", "neuro-ophthalmology". The articles in English published from January 1, 2020 to January 31, 2020 have been included to summarize the current understanding of the ophthalmic manifestations of SAR-CoV-2 virus. Although the search is not exhaustive, we have tried to examine the more important and unique articles. We reviewed 46 case reports, 11 crossover / cohort observational studies, 5 prospective interventional studies, 3 animal models / autopsies and 6 reviews / meta-analyses.

## Ophthalmotoxicity of Drugs Used To Treat COVID-19

Medicines used to treat COVID-19 are toxic to the eyes. Long-term use of chloroquine and hydroxychloroquine may result in retinal toxicity. Medicines Lopinavir / ritonavir may cause

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reactivation of autoimmune diseases. Ribavirin is seldom used to treat COVID-19, but it is known to cause retinopathy, retinal vein occlusion, serous retinal detachment, non-arterial ischemic optic neuropathy and Vogt-Koyanagi-Harada disease (VKH). Interferon was associated with retinopathy, conjunctivitis, uveitis, optic neuropathy, corneal ulcers, epithelial defects and Sjogren's syndrome. It has been reported that tocilizumab causes retinal hemorrhages. Systemic corticosteroids are known to cause cataracts, glaucoma and central serous chorioretinopathy. These points should be taken into account by an ophthalmologist during the history, examination and treatment of patients [20].

Therefore, it is important for ophthalmologists to know not only about the ophthalmological manifestations of a new viral infection in order to diagnose correctly, but also treat with drugs that have a high profile of toxicological safety, therapeutic efficacy, but their pharmacological properties were unknown to physicians.

## Innovations in the Treatment of Post-COVID Syndrome

The search for new opportunities in the treatment and prevention of complications caused by COVID-19 is undoubtedly an urgent challenge of the present time. Our attention was attracted by a new original drug that has no world analogues Mercureid, a targeted (targeted action) drug aimed at reducing the overexpression of the proinflammatory cytokine  $TNF\alpha$  and normalizing the production of the most important, if not the main immunological factor of mucosal immunity (mucosalimmunity)-SIgA [21].

Swiss molecular biologist V. A. Loroeh (Ph.D in Biology, Experimental research strategies and research methods in molecular genetics and molecular biology, Switzerland), said that Mercury's interaction with the immunoglobulin superfamily changes the paradigm of our thinking about the mode of action of a drug, as it leads to multiple mechanical biological responses (available at the link <https://vimeo.com/435729749>)

1. Stimulation of secretory immunoglobulin A. Secretory IgA on the surface of the mucous membrane of the respiratory tract prevents the adhesion of viruses to the surface of the epithelium. Secretory IgA has a non-inflammatory protective function: antibodies of this type can bind to the virus without activating complement and without releasing inflammatory mediators
2. Stimulation of neutralizing immunoglobulin G. Neutralizing IgG prevents the systemic spread of the virus
3. Activation of CD4 +, CD8 + and CD16 + T-cells, which kill virus-infected cells
4. Activation of macrophages. Macrophage-driven phagocytic elimination of apoptotic cells is an immunologically silent response; there is no inflammation. In fact, it is anti-

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inflammatory because macrophages consume apoptotic neutrophils and other cells that produce and secrete pro-inflammatory cytokines as a result of viral infection [22-24]

Thus, we can state that after suffering COVID-19, a negative PCR test after an illness is only the beginning of a difficult path to complete recovery. And in 61% of patients, the signs of post-COVID syndrome are kept for a long time [25].

## Aims of the Study

- To investigate the functional state of the Visual Analyzer (VA), regional hemodynamics and immune status in patients after suffering COVID-19 and patients with post-COVID syndrome complicated by ischemic optic neuropathy, as well as the possibility of correction effect in the course of therapy with Mercureid on the identified disorders;
- To study the level of expression of apoptosis markers (CD 95), intercellular adhesion ICAM-1 (CD54), as well as CD3, CD4, CD8, CD25, CD38 in healthy individuals and in the patients who have undergone COVID-19.

In respect that while searching the information in English on the topic: "the level of expression of markers of apoptosis (CD95), intercellular adhesion ICAM-1 (CD54), CD3, CD4, CD8, CD25, CD38 in patients undergoing COVID-19" we haven't found any information available to the public, this immunological study provides a new and extremely important material for practitioners and scientists, allowing for better understanding of the immunopathogenesis of COVID-19.

## Materials and Methods

This study is open, according to the Declaration of Helsinki Ethical Principles for Medical Research, the Council of Europe Convention on Human Rights and Biomedicine, as well as the relevant laws of Ukraine.

The studies were carried out with the informed consent of the patients who were examined and treated in the Department of Inflammatory Eye Pathology of the State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine".

All patients had a history of COVID-19 about 2-3 months before the examination. At the time of treatment and examination, all SARS-CoV-2 IgM antibodies were negative.

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## Study Population

Under our control there were 49 patients who had undergone COVID-19. They were divided into three groups.

Group 1 included 17 patients who had undergone COVID-19 and did not have eye diseases (a group of volunteers) who received an outpatient examination and drug correction.

Group 2 included 18 patients with high-risk chronic eye diseases: with posterior uveitis - focal chorioretinitis (7 patients) and disseminated chorioretinitis (11 patients) of various etiology.

Group 3 included 14 patients with ophthalmopathy of vascular genesis: 9 patients with thrombosis of the central retinal vein and its branches and a group of 5 patients who received eye complications after COVID-19 disease, but hadn't had previous eye pathology. These 5 people were admitted with a diagnosis of acute vascular optic neuropathy in both eyes.

The average age of the patients was  $30 \pm 19.5$  (from 30 to 50 years old). The average gap between the symptoms of COVID-19 manifestation and ophthalmic pathology manifestation was 5.6 (average  $4.2 \pm 8.2$ ) days.

The immunological study was carried out in the laboratory of immunology of the State Institution "The Filatov Institute of Eye Diseases and Tissue Therapy" and it was aimed at the determination of the immune status, including the determination of antigens of the eye structure and the expression of biomarkers such as: markers of T-lymphocytes CD3, T-helper CD4, cytotoxic T-cell antigen CD8, Interleukin-2 receptor alpha chain (also called CD25), activator of B-cells and T-cells - CD38; ICAM-1 (Intercellular Adhesion Molecule1) also known as CD54, Fas/CD95 death receptor. They were determined by the immunohistochemical method in 49 patients (the average age of patients is  $30 \pm 19.5$  (from 30 to 50 years old)) within 1-2 months after recovery from COVID-19 and in 53 practically healthy people of the control group (age  $31 \pm 20$ , 4 years old).

The patients of the first group were taking Mercureid as monotherapy for 1 month. The patients of the second and third groups were undergoing a course of traditional anti-inflammatory therapy with Mercureid taking for a 14-day period and subsequently they had a course of Mercureid as monotherapy which lasted 1 month.

## Statistical Analysis

Statistical analysis was carried out by means of STATISTICA 8.0 application (StatSoftInc). When analyzing the data, the Mean values (M) and the error of the mean (m), Standard Deviation (SD) were taken into account. To determine the paired differences, Student T-test and Wilcoxon test were used.

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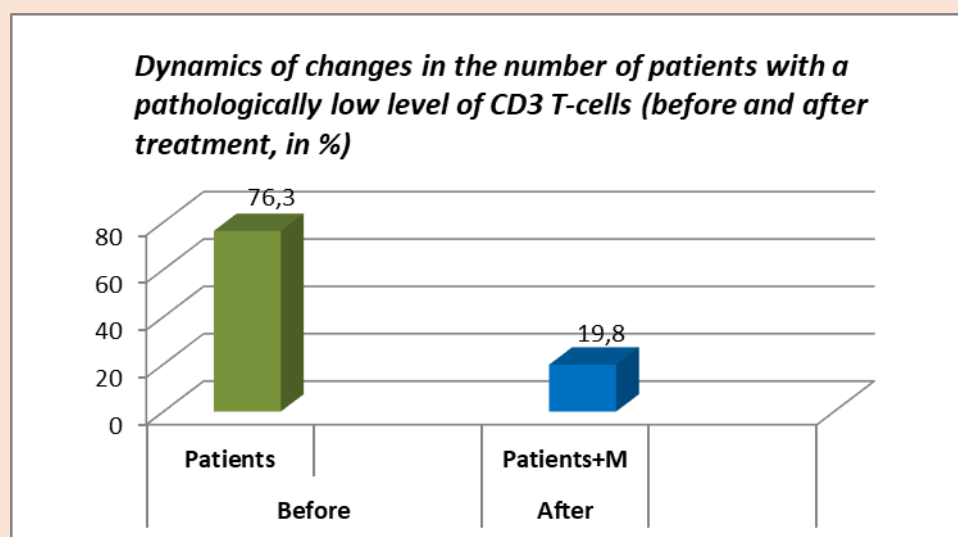
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## Immunological Test Results

### Studying the expression of CD3, CD4, CD8, CD4/CD8

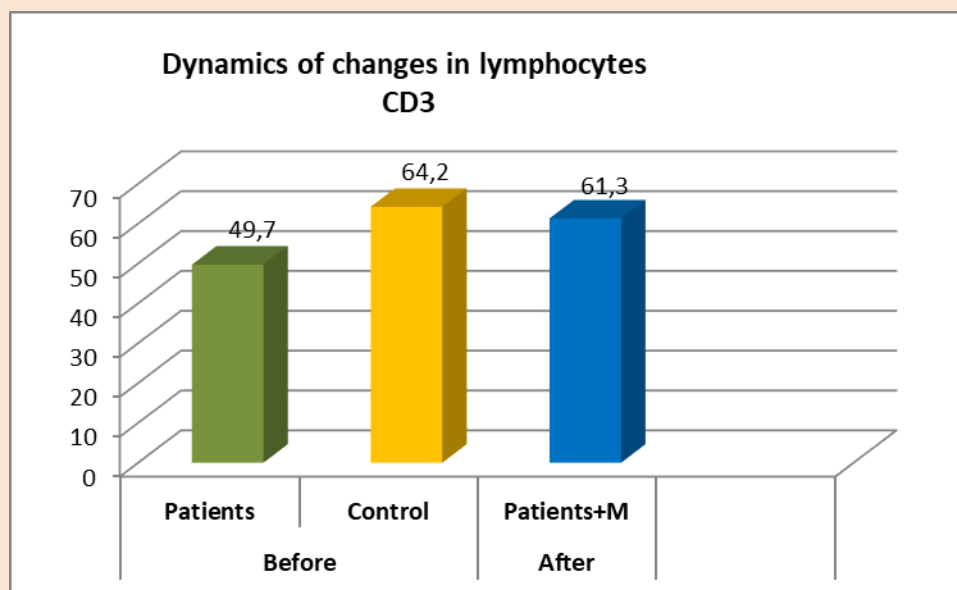
The response to viral infections is accompanied by activation of the innate and acquired immune systems. The most effective response against various viral infections is the activation of the cellular immune response, especially the activation of T-cells. CD8 Cytotoxic T-Cells (CTLs), by secreting a range of molecules including perforin, granzyme and Interferons (IFNs), remove viruses from the host. CD4+ T-cells (Th) also help to remove viral infection by assisting cytotoxic T-cells and B-cells.

In our study, the absolute value of CD3 T-cells was below norm in 76.31% of patients ( $p < 0.05$  according to Mann-Whitney test) (Fig. 1).



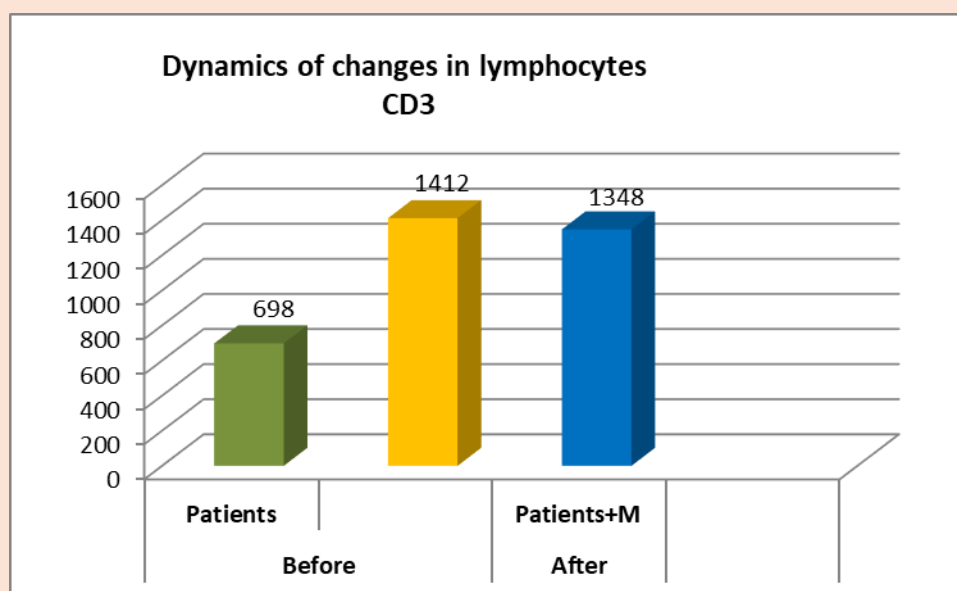
**Figure 1:** Dynamics of changes in the number of patients with a pathologically low level of CD3 T-cells (before and after treatment, in %).

In patients who underwent COVID-19: CD3 was  $49.7 \pm 7.1\%$  in percentage value and  $447.42 \pm 61.71 / \mu\text{L}$ , ( $n = 49$ ) in absolute value. In healthy individuals, these indicators were higher and equal to  $64.2 \pm 3.9\%$  and  $1412.31 \pm 69.6 \text{ cells} / \mu\text{L}$  ( $n = 53$ ) (Fig. 2).



**Figure 2:** Dynamics of changes in the number of CD3 T-cells (before and after treatment, in %).

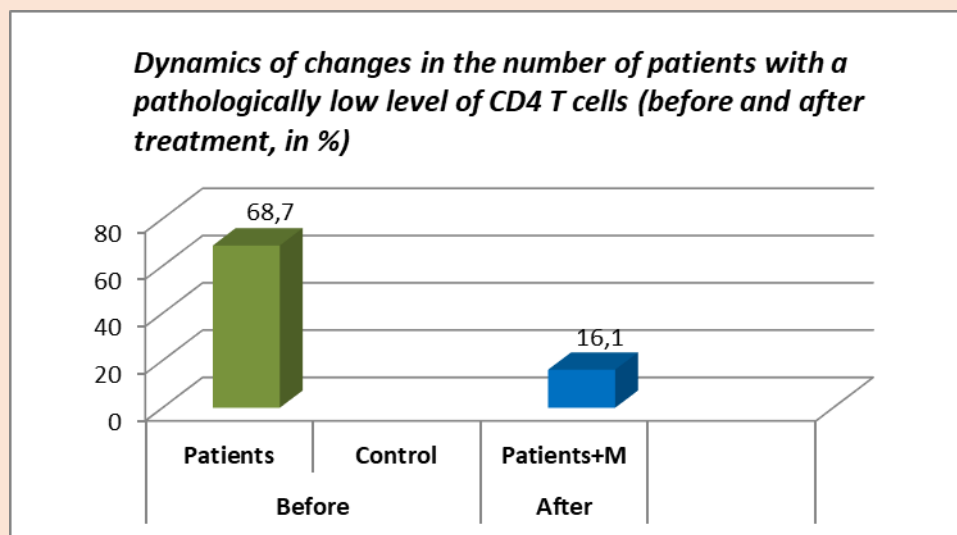
After taking Mercureid, CD3 T-cell value was below norm in only 19.77% of patients ( $p < 0.05$  according to Mann-Whitney test). The number of CD3 T-cells increased and amounted to  $-61.3 \pm 4.2\%$  in percentage value and  $1348.42 \pm 74.8$  cells /  $\mu\text{l}$  in absolute value (Fig. 3).



**Figure 3:** Dynamics of changes in the number of CD3 T-cells (before and after treatment, in cells/ $\mu\text{l}$ ).

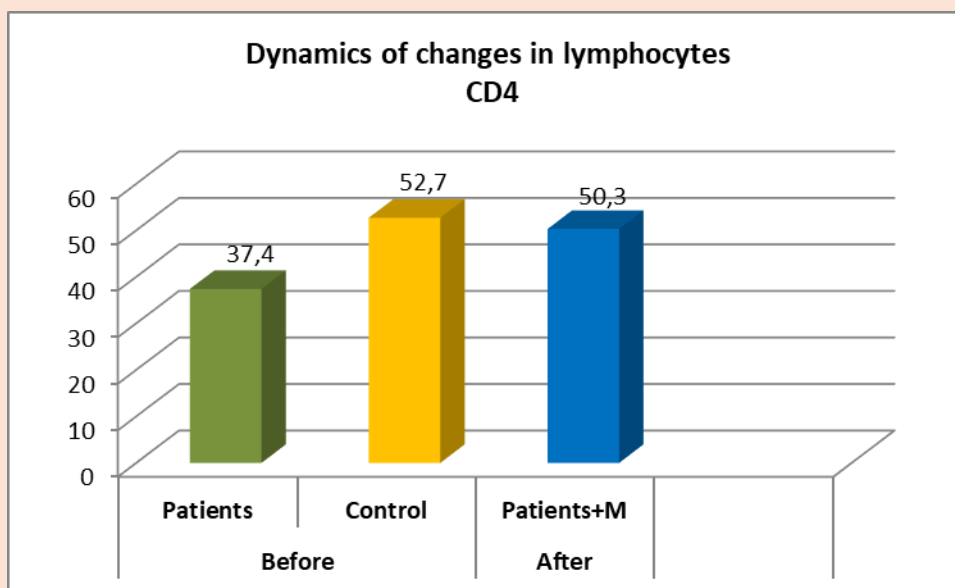
### Target CD3, Therapeutic Efficacy-74,1%.

The absolute value of CD4 T-cells was below norm in 68.72% of patients. The number of CD4 T-cells, was  $-37.4 \pm 5.2\%$  in percentage value and  $-366.73 \pm 52.8$  cells/ $\mu\text{L}$ , ( $n = 49$ ) in absolute value. In healthy individuals, this indicator was stable and amounted to  $52.7 \pm 4.1\%$  and  $1159.75 \pm 32.8$  cells/ $\mu\text{L}$ , ( $n = 53$ ) (Fig. 4).

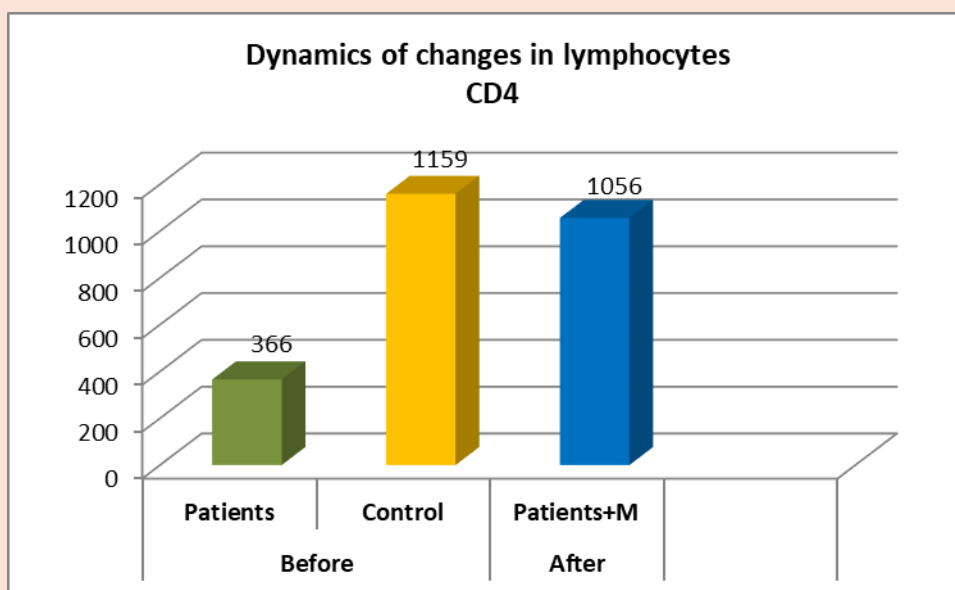


**Figure 4:** Dynamics of changes in the number of patients with a pathologically low level of CD4 T-cells (before and after treatment, in %).

After taking Mercureid, the CD4 T-cell value was below norm only in 16.14% of patients ( $p < 0.05$  according to Mann-Whitney test). The number of CD4 T-cells increased, which was  $-50.3 \pm 3.9\%$  in percentage value and  $-1056.62 \pm 69.4$  cells/ $\mu\text{L}$ , in absolute value (Fig. 5 and 6).



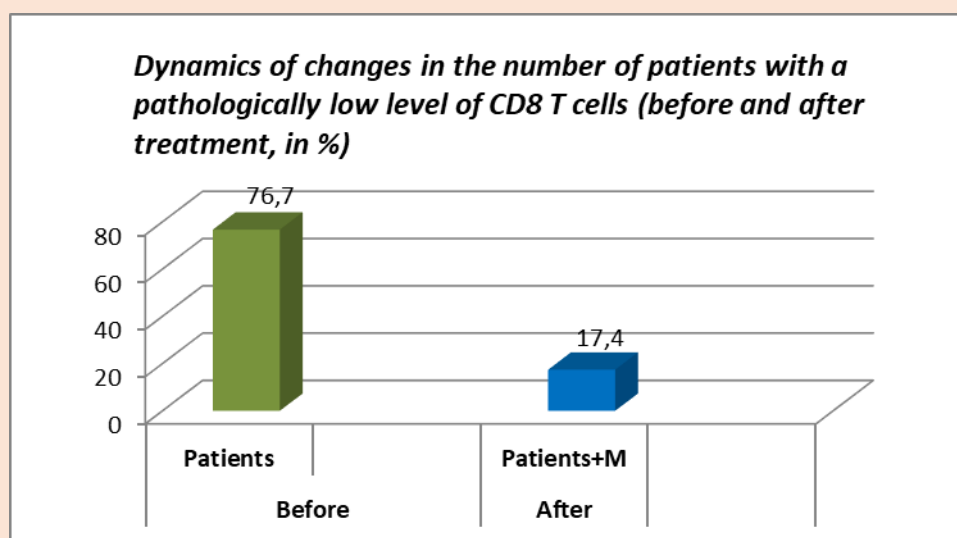
**Figure 5:** Dynamics of changes in the number of CD4 T-cells (before and after treatment, in %).



**Figure 6:** Dynamics of changes in the number of CD4 T-cells (before and after treatment, in cells/μl).

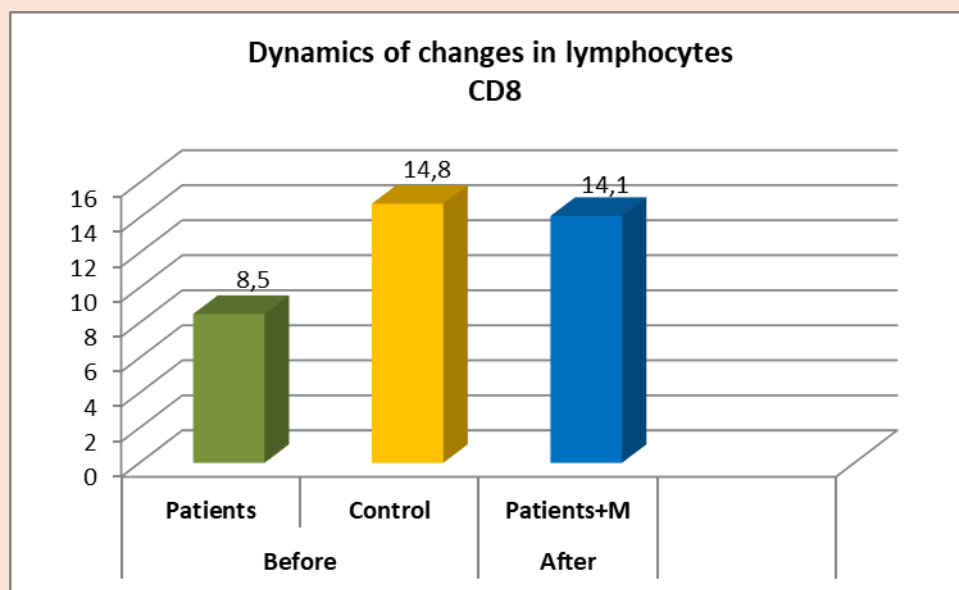
## Target CD4, Therapeutic Efficacy-76,6%

The absolute value of CD8 T-cells was below norm in 76.73% of patients. Similar to CD4 T-cells, their decrease was registered. In percentage value it was  $-8.5 \pm 2.9\%$ , in absolute value  $-103.36 \pm 36.4$  cells/ $\mu\text{l}$ , ( $n = 49$ ). In healthy individuals, these indicators were  $14.8 \pm 3.2\%$ , in absolute value they were  $314.35 \pm 48.3$  cells/ $\mu\text{l}$ , ( $n = 53$ ) (Fig. 7).

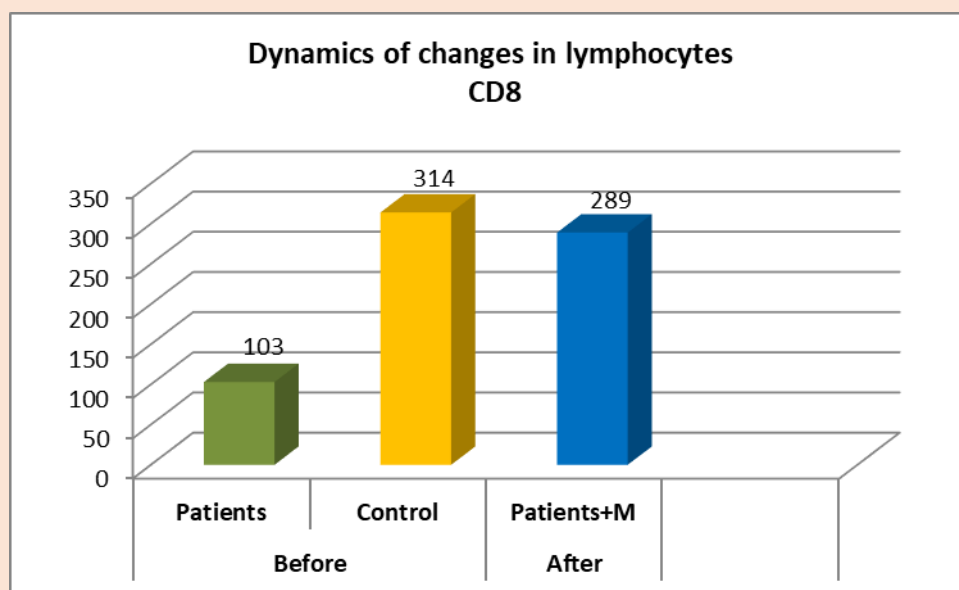


**Figure 7:** Dynamics of changes in the number of patients with a pathologically low level of CD8 T-cells (before and after treatment, in %).

After taking Mercureid, CD8 T-cell value was below norm in 17.45% of patients ( $p < 0.05$  according to Mann-Whitney test). The number of CD8 T-cells increased and amounted to  $-14.1 \pm 2.8\%$  in percentage value and  $-289.19 \pm 26.7$  cells/ $\mu\text{l}$  in absolute value (Fig. 8 and 9).



**Figure 8:** Dynamics of changes in the number of CD8 T-cells (before and after treatment, in %).

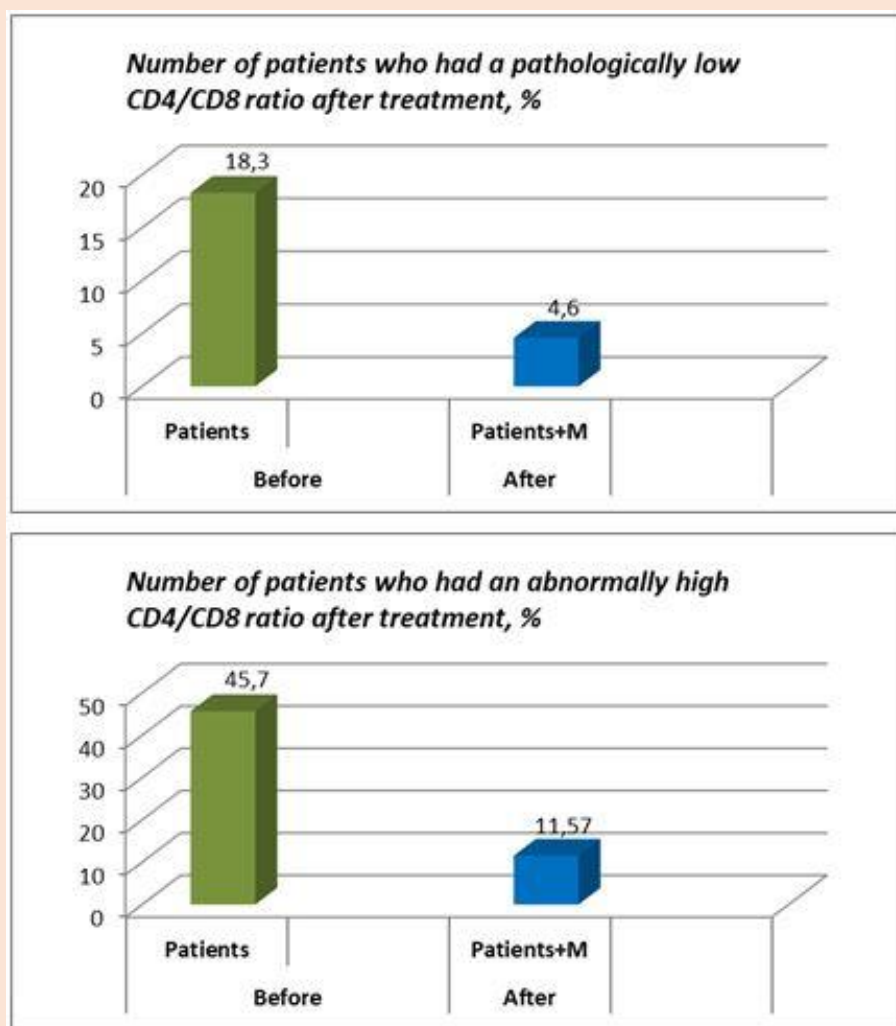


**Figure 9:** Dynamics of changes in the number of CD8 T-cells (before and after treatment, in cells/μl).

### Target CD8, Therapeutic Efficacy-77,1%

Meanwhile, CD4 / CD8 ratio was multidirectional and was below norm in 18.31% of patients and above norm in 45.67% of patients. Wang et, al., emphasized that in the context of lymphopenia, low CD8 cell amount and increased CD4 / CD8 ratios were connected with the inflammatory status of COVID-19 patients. Accordingly, dysregulation of CD4 / CD8 can be considered as a predictor of the manifestation of inflammatory diseases.

After taking Mercureid, CD4 / CD8 ratio remained below norm in 4.62% of patients with initially low ratio and remained increased in 11.57% of patients with initially increased ratio ( $p < 0.05$  by Mann-Whitney test) (Fig. 10).

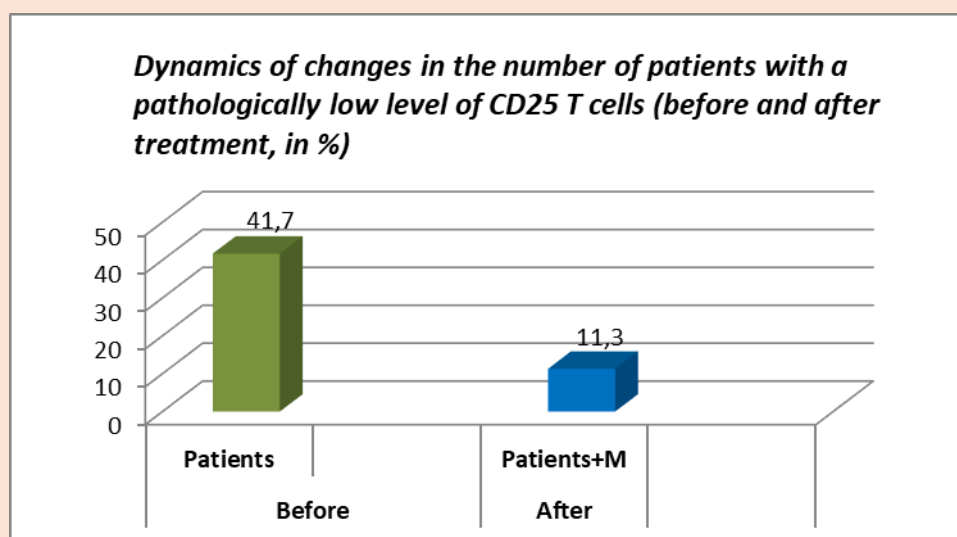


**Figure 10:** Dynamics of changes in CD4/CD8 ratio (before and after treatment).

## Studying the Expression of CD25

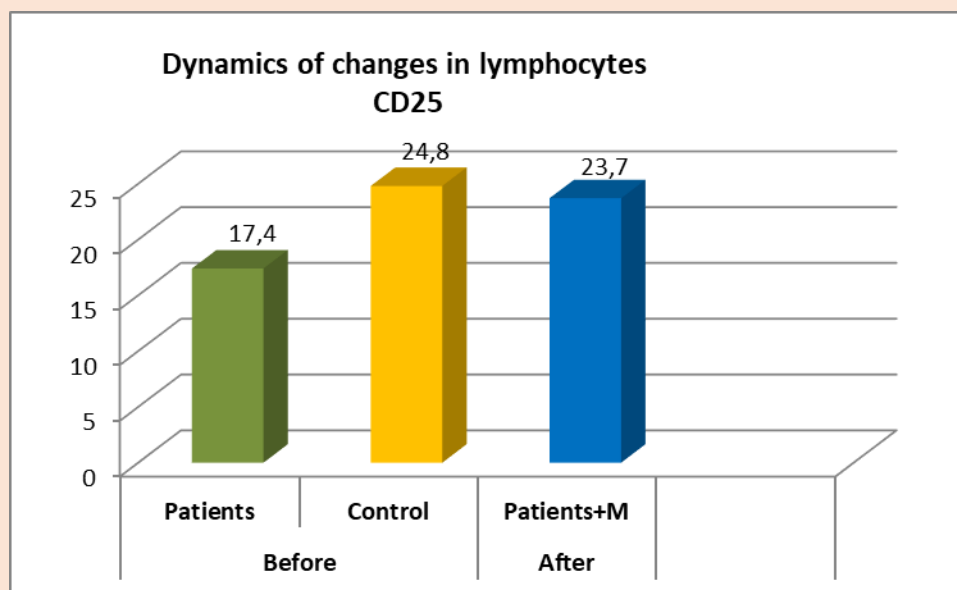
CD25 ( $\alpha$ -chain of the high-affinity IL-2 receptor). T regulatory cells constitutively express CD25 and respond to IL-2 generated by T-cells during the immune response. CD25 deficiency is known to lead to severe enteropathy, diabetes mellitus, autoimmune hemolytic anemia, eczema and impaired lymphoproliferation. It is important to note that patients with CD25 deficiency often have chronic herpes viral infections and an increased susceptibility to infections. CD25 expression occurs primarily in CD4 T-cells and it is likely that CD4 deficiency may also be associated with low CD25 expression. In addition, low CD25 levels may contribute to lymphopenia by inhibiting IL-2 signalling.

The study revealed that the number of T-lymphocytes carrying CD25 on their surface was reduced in 41.67% of patients ( $p < 0.05$  according to Mann-Whitney test) (Fig. 11).



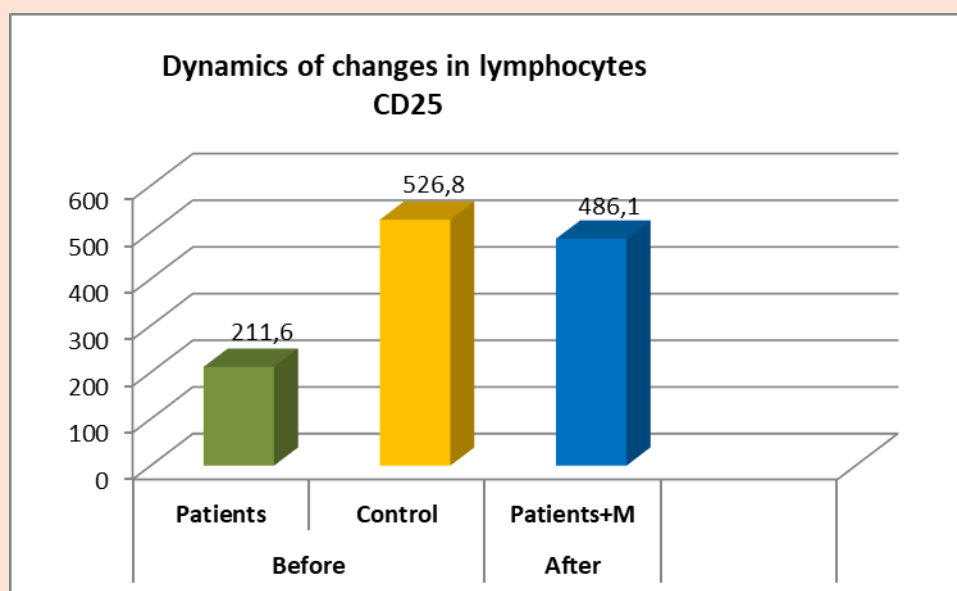
**Figure 11:** Dynamics of changes in the number of patients with a pathologically low level of CD25 T-cells (before and after treatment, in %).

The number of CD25 T-cells amounted to  $-17.4 \pm 3.4\%$  in percentage value and to  $-211.58 \pm 32.7$  cells/ $\mu\text{l}$ , ( $n = 49$ ) in absolute value. In healthy individuals, this indicator was stable and amounted to  $24.8 \pm 3.7\%$  and  $603.12 \pm 68.4$  cells/ $\mu\text{l}$  ( $n = 53$ ) (Fig. 12).



**Figure 12:** Dynamics of changes in the number of CD25 T-cells (before and after treatment, in %).

After taking Mercureid, CD25 T-cell value was below norm in 11.26% of patients ( $p < 0.05$  according to Mann-Whitney test). The number of CD25 T-cells increased, which was  $-23.7 \pm 4.1\%$  in percentage value and  $-486.09 \pm 46.7$  cells/ $\mu\text{l}$  in absolute value (Fig. 13).



**Figure 13:** Dynamics of changes in the number of CD25 T-cells (before and after treatment, in cells/ $\mu\text{l}$ ).

It should be taken into account that CD25 is also expressed on a subset of T-cells endowed with regulatory / suppressive abilities that are critical for maintaining immune tolerance (Belghith et al., 2003; Sakaguchi, 2004). Thus, restoration of CD25 expression is an important therapeutic task, both for the induction of tolerance and restoration of the patient's antiviral immunity.

## **Target CD25, Therapeutic Efficacy-73,4%**

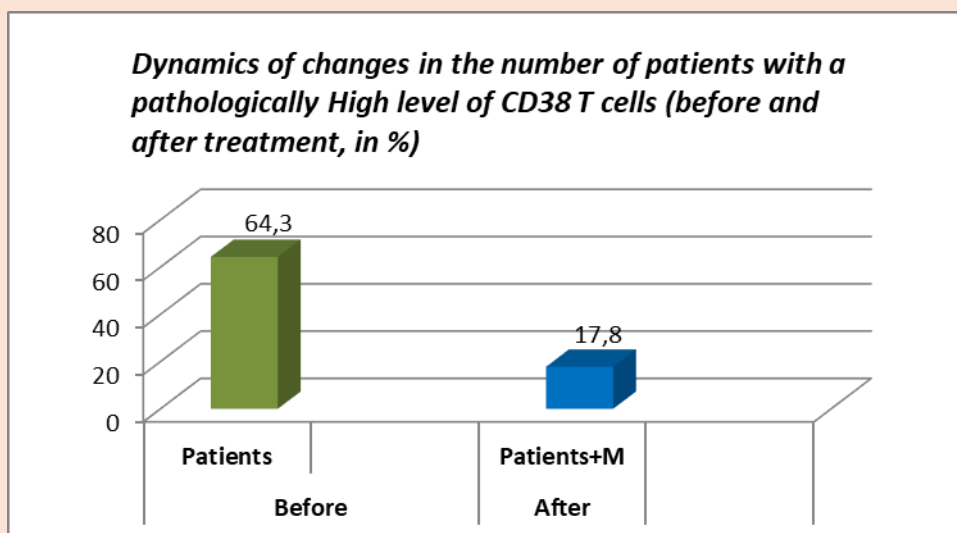
### **Studying the Expression of CD38**

CD38 is a multifunctional cell protein endowed with signaling receptor and enzymatic features and was initially identified as a lymphocyte antigen. CD38 protein, as a transmembrane receptor, affects both innate and adaptive immune response by regulating the movement of cells (e.g., macrophages, dendritic cells, lymphocytes and neutrophils) to the sites of inflammation. CD38 is not only a marker of differentiation and activation of surface cells, but can also induce the release of various cytokines. These findings correlate with the role of CD38 as an inflammatory marker of human macrophages / monocytes in inflammatory processes.

COVID-19 viral infection previously experienced by patients may have caused excessive antigenic stimulation, resulting in an abrupt breakdown of circulating immune cells with progressive T-cell anergy or exhaustion. The mechanisms leading to lymphopenia may result from:

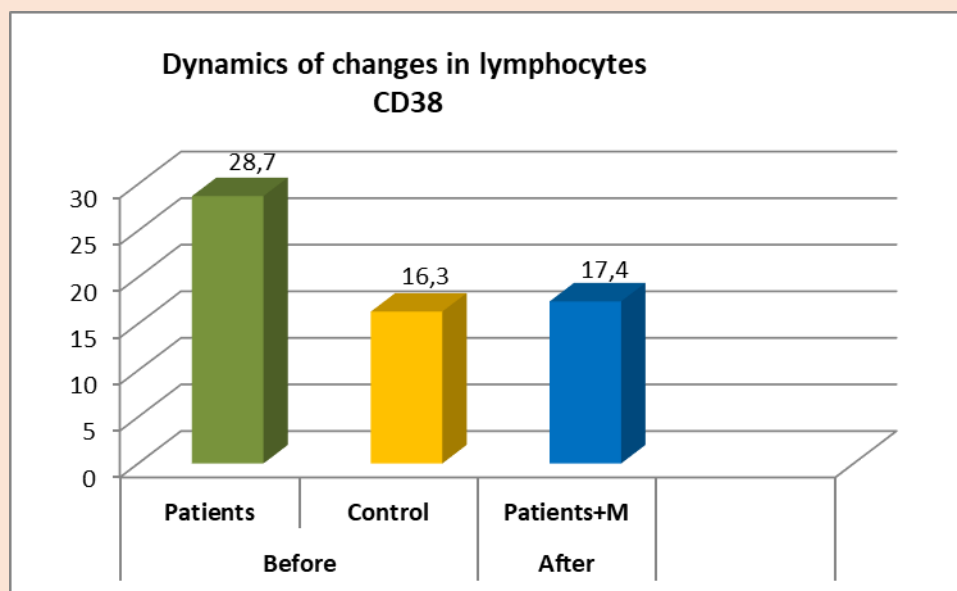
1. Direct affect on lymphocytes or indirect action that destroys lymphatic organs
2. Impaired inflammatory cytokine reaction leading to apoptosis of lymphocytes. And CD38 can be directly involved in these actions
3. Overexpression of CD38 causes cell death, mainly through exhaustion of NAD + (Badawy, et al., 2020)

The study revealed that the number of CD38 T-lymphocytes was increased in 64.31% of patients ( $p < 0.05$  according to Mann-Whitney test) (Fig. 14).



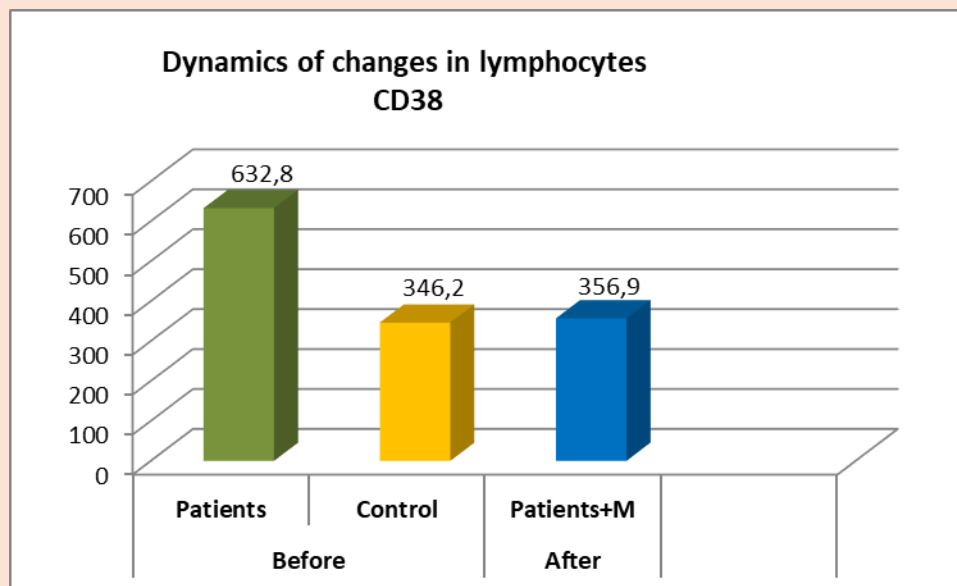
**Figure 14:** Dynamics of changes in the number of patients with a pathologically low level of CD38 T-cells (before and after treatment, in %).

The number of CD38 T-cells amounted to  $-28.7 \pm 2.6\%$  in percentage value and to  $-632.77 \pm 81.3$  cells/ $\mu\text{l}$ , ( $n = 49$ ) in absolute value. In healthy individuals, this indicator was stable and amounted to  $16.3 \pm 2.9\%$  and  $346.21 \pm 53.1$  cells/ $\mu\text{l}$  ( $n = 53$ ) (Fig.15).



**Figure 15:** Dynamics of changes in the number of CD38 T-cells (before and after treatment, in %).

After taking Mercureid, CD38 T-cell value remained increased in 17.81% of patients ( $p < 0.05$  according to Mann-Whitney test). The number of CD38 T-cells decreased and amounted to  $-17.4 \pm 3.5\%$  in percentage value and to  $-356.87 \pm 41.8$  cells/ $\mu\text{l}$  in absolute value (Fig. 16).



**Figure 16:** Dynamics of changes in the number of CD38 T-cells (before and after treatment, in cells/ $\mu\text{l}$ ).

## Target CD38, Therapeutic Efficacy-72,3%

Inhibition of CD38 reduces the polarization of proinflammatory macrophages, thereby reducing inflammation and improving the course of pathologies connected with it.

## Studying the Expression of CD54, CD95

The level of expression of the molecular marker of apoptosis CD95 on blood lymphocytes in patients who underwent COVID-19 is 1.7 - 2.5 times higher than the norm and the molecular marker of intercellular adhesion CD54 is 2.9 - 4.4 times higher that indicates a persistent high level of the immune response in the short term after recovery. The severity of the expression of the intercellular adhesion molecule (ICAM-1, CD54) shows the involvement of the endothelium of the vascular wall as one of the mechanisms of the pathogenesis of long COVID syndrome in the inflammatory process.

Fas (CD95), a cell surface receptor of the tumor necrosis factor super family, has been long viewed as a death receptor that mediates apoptosis to maintain immune homeostasis [29].

CD95 is widely expressed in memory and effector T-cells upon contact with antigen, while naive T-cells are typically negative [30].

Programmed cell death 1 (PD-1, CD279), an antigen of effector T-cells, is considered an exhaustion marker, also expressed during antigen-mediated T-cell activation [31].

Upregulation of PD-1 is observed during acute infections and after infection with persistent virus, including HIV, HBV and HCV. In particular, PD-1 expression in HIV-specific CD4 + and CD8 + lymphocytes is associated with T-cell exhaustion and disease progression [32].

Both antigens are known to be activated upon activation of T-cells and can signal a propensity for apoptosis (CD95) or T-cell exhaustion (PD-1). We observed an increased CD95 expression in T-cells with older age, according to previously described higher susceptibility to CD95-induced apoptosis in the elderly [32].

Our data on an increase in CD95 expression, which can cause PD-1 activation, also correlate with the data of Zheng, et al., showing the phenotype of exhausted T-cells in patients with severe COVID-19 infection [34].

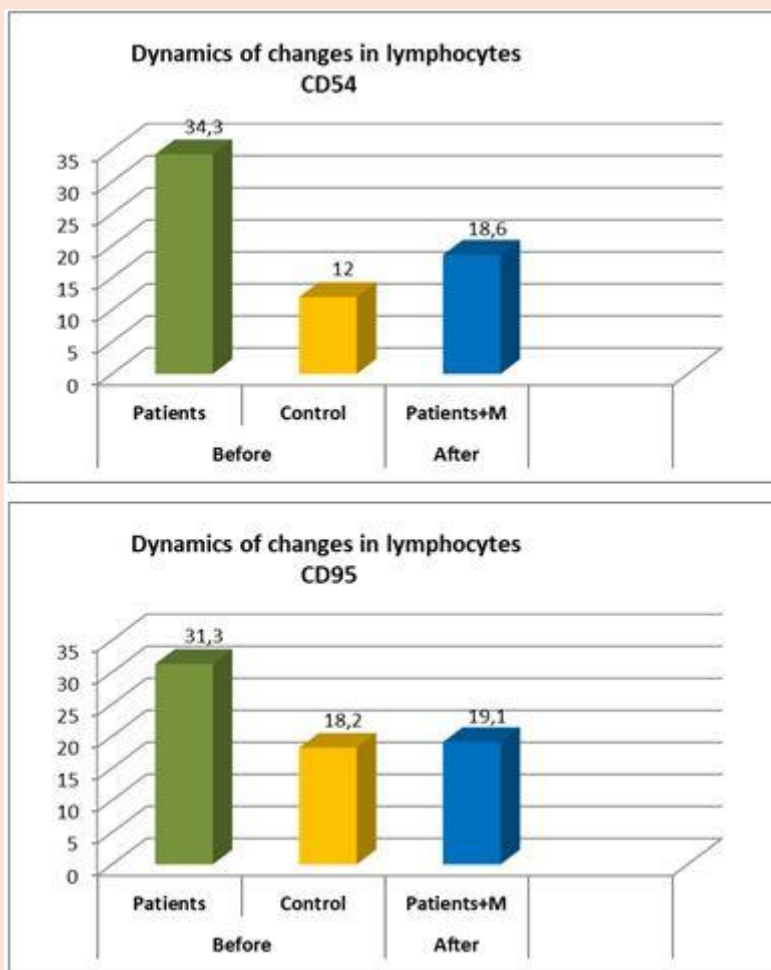
Apoptosis via CD95 may be a possible mechanism for lymphopenia caused by COVID-19 and our data provide new insights into the functional competence of T-lymphocytes when COVID-19 infection.

A high level of expression of the molecular marker of intercellular adhesion CD 54 on lymphocytes in patients who underwent COVID-19 was revealed: it amounted to  $34.3 \pm 5.6\%$  in percentage value and  $756.13 \pm 58.4$  cells /  $\mu\text{L}$  (n = 49) in absolute value. In healthy individuals, these indicators were 2.9-4.4 times lower (p <0.05 according to Mann-Whitney test), respectively, that was equal to  $12.0 \pm 4.0\%$  and  $173.5 \pm 22.1$  cells /  $\mu\text{L}$ , (n = 53).

After taking Mercureid, the number of CD54 T-cells decreased and amounted to  $19.4 \pm 3.8\%$  in percentage value and  $521.64 \pm 57.3$  cells /  $\mu\text{L}$  in absolute value.

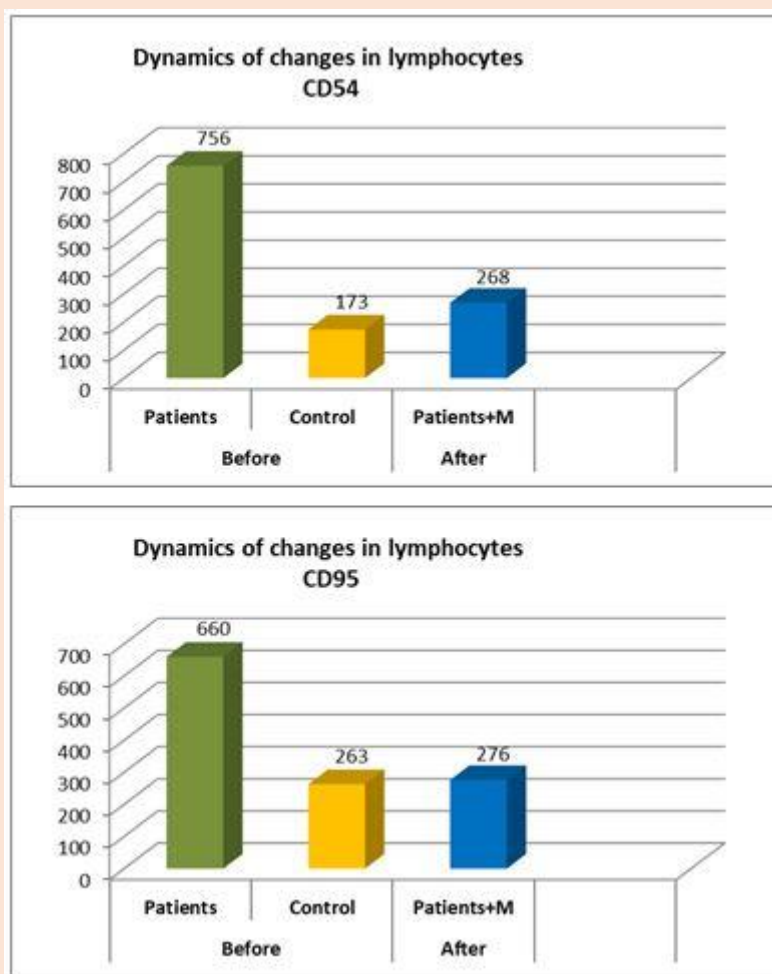
The expression level of the molecular marker of apoptosis CD95 on blood lymphocytes in patients who underwent COVID-19 also exceeded the relative and absolute standard values by 1.7-2.5 times (p <0.05 according to Mann-Whitney test). In percentage value it was  $31.3 \pm 6.8\%$ , in absolute value  $660.11 \pm 68.4$  cells /  $\mu\text{L}$ . So, in healthy individuals, these indicators were  $18.2 \pm 4.1\%$  and  $263.5 \pm 32.1$  cells /  $\mu\text{L}$ , respectively.

After taking Mercureid, the number of CD95 T-cells decreased and amounted to  $19.1 \pm 3.8\%$  in a percentage value and to  $276.2 \pm 36.4$  cells /  $\mu\text{L}$  in an absolute value (Fig. 17).



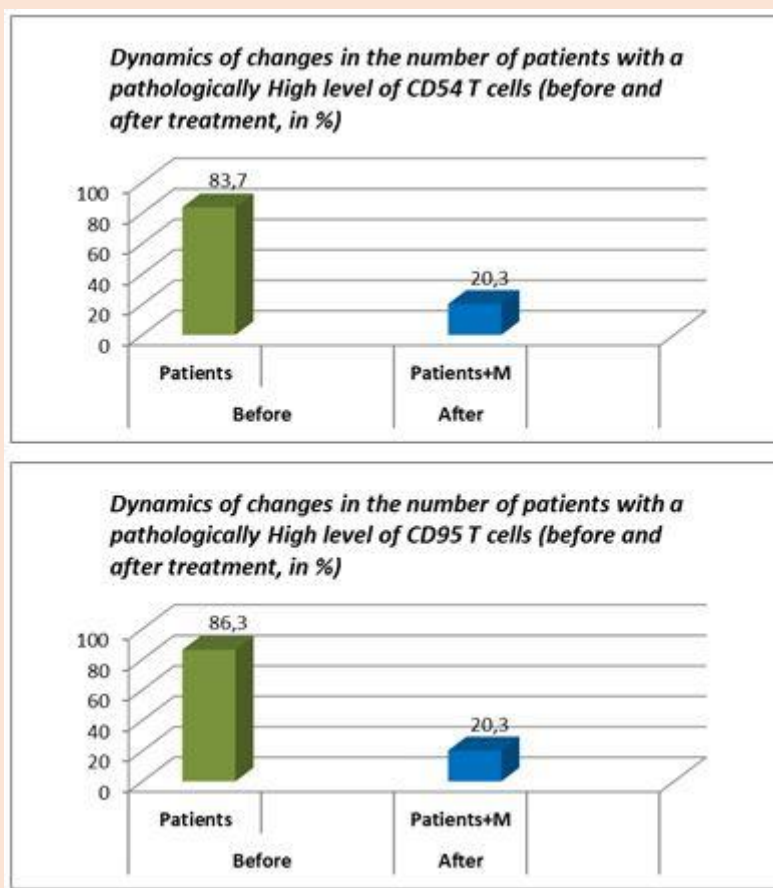
**Figure 17:** Dynamics of changes in the number of CD54, CD95 T-cells (before and after treatment, in %).

The number of T-lymphocytes expressing CD54 was higher than norm in 83.68% of patients, CD95 was higher than norm in 86.32% of patients ( $p < 0.05$  according to Mann-Whitney test) (Fig. 18).



**Figure 18:** Dynamics of changes in the number of CD54, CD95 T-cells (before and after treatment, in cells/ $\mu$ l).

After taking Mercureid, CD54 T-cell value remained exceeded in 20.31% of patients; CD95 - in 20.36% ( $p < 0.05$  according to Mann-Whitney test) (Fig. 19).



**Figure 19:** Dynamics of changes in the number of patients with pathologically high levels of T-cells CD54 and CD95 (before and after treatment, in %).

Target CD54, therapeutic efficacy-75,7%

Target CD95, therapeutic efficacy-76,4%

## Discussion

The patients who underwent Covid-19 are faced with long-term problems of immunity recovery. Besides, the state of the immune system is still below the norm for several months after recovery.

Aberrant T-cell differentiation and lymphopenia are signs of severe COVID-19 disease. As T-cells must race to discard infected cells, they rapidly differentiate and achieve cytotoxic function. With such reactivity, accelerated apoptosis occurs due to the coupled mechanism of death and differentiation of both CD4 and CD8 lymphocytes via CD95 (Fas) and serine threonine kinase (Akt). Lymphopenia of T-cells in severe cases can mean cell death by apoptosis. This provides insight into SARS-Cov-2 as a lymphatic manipulative pathogen; it

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distorts T-lymphocytes function, their number and death and creates a dysfunctional immune response.

T-cells play a fundamental role in viral infections: CD4 T-cells help B-cells make antibodies and organize the response of other immune cells, while CD8 T-cells kill infected cells to reduce viral load. However, dysregulation of T-cell response can lead to immunopathology.

Taking into account the convincing evidence for the role of T-lymphocytes in maintaining immune homeostasis and, in particular, in controlling the severity of viral immune-inflammatory lesions after COVID-19, this study is of great importance. Changes in the activity of surface antigens (CD) in the patients who underwent COVID-19 indicate the exhaustion of peripheral blood T-cells that predetermines poor functional effector, stable expression of inhibitory receptors. In its turn, exhaustion of T-cells prevents optimal control of both infection and tumor. This is a discovery that could explain lymphopenia in COVID-19 which may be associated with direct destruction of lymphocytes by the virus, abnormal apoptosis and the effect of pro-inflammatory cytokines aimed at inhibiting the functions of T-lymphocytes.

Impaired immune homeostasis in cases of COVID-19 can disrupt the delicate balance between the regulatory and effector links of the immune system, which leads to the massive proliferation and activation of neutrophils, macrophages, dendritic cells and masT-cells. Uncontrolled innate inflammatory reactions and imbalance can lead to harmful tissue damage, both locally and systemically. Thus, dysfunction of T-lymphocytes leads to the increase of tissue damage and growth of morbidity and mortality, Trandem, et al.

The use of Mercury made it possible to have a multi-target effect on the immunoglobulin superfamily as one of the largest families of proteins in the body that enables cellular communication in immune responses. Its 765 members include antigen receptors, co-receptors and co-stimulatory molecules, proteins involved in antigen presentation to lymphocytes, cell adhesion proteins and many cytokine receptors.

Target corrective effect on the following proteins has been noted:

- CD3 - increase to physiological norm in 74.1%
- CD4 - increase to physiological norm in 76.6%
- CD8 - increase to physiological norm in 77.1%
- CD25 - increase to physiological norm in 73.4%
- CD38 - decrease to physiological norm in 72.3%
- CD54 - decrease to physiological norm in 75.7%
- CD95 - decrease to physiological norm in 76.4%

Assessment of phenotypic characteristics will allow tracking the development of the disease and response to treatment and makes an important contribution to understanding the

immunopathogenesis of COVID-19, especially during the rehabilitation phase and reducing the risk of developing post-COVID-19 syndrome.

The second, equally important conclusion is that the formation of immunity against SARS-CoV-2 coronavirus is of primary importance in controlling COVID-19 pandemic, protecting vulnerable people from serious illnesses and restricting the spread of the virus. Our immune system protects against SARS-CoV-2 either through reaction to infection or in response to vaccination. The three main pillars of the antiviral response are immune cells, called cytotoxic T-cells, which can kill infected cells and neutralizing antibodies, which prevent the virus from infecting the cells, namely, secreted by immune cells called plasma cells. The third pillar of an effective immune response is the production of helper T-cells (CD4), which coordinate the immune response. It is also extremely important that CD4s are required for the creation of immunological memory in particular, for the emergence of long-lived plasma cells, which continue to secrete antiviral antibodies, even when SARS-CoV-2 virus has been eliminated from the body [35].

## Conclusion

With a high degree of probability, we can assume that in patients with manifestation of chronic inflammatory diseases who have had previous infection with SARS-CoV-2, the production of specific protective antibodies will be impaired (often these patients have pathologically low levels of CD4, CD8, CD25 and overexpression of CD38, ICAM-1, CD95 that causes apoptosis of immune cells, lymphopenia and also forms the phenotype of exhausted T-cells with activation of the expression of inhibitory receptors). And even vaccination may be ineffective for them due to the compromised immune system. Accordingly, the implementation of corrective multitarget immunotherapy aimed at several target proteins, which are greatly important for the formation of long-term effective post-viral immunity to SARS-CoV-2, is an extremely important therapeutic need which can be carried out both before and after vaccination in order to achieve the maximum protective effect from the vaccine. But the answer to this research question will require another type of study design, which we plan to conduct in the future.

## Conflicts of Interests

The authors declare that have no competing interest and not any conflict of interest.

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