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## ANALYSIS OF IMMUNE CELLS AND IMMUNOLOGICAL PROCESSES IN COVID-19

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**Bakridin Zaripov**

National University of Uzbekistan named after Mirzo Ulugbek, Tashkent, Uzbekistan

**Gulsara Akhmedova**

Khajand State University named after Academician Bobojon Gafurov, Tajikistan, Uzbekistan

**Bolta Kakhorov**

Khajand State University named after Academician Bobojon Gafurov, Tajikistan, Uzbekistan

**Behzod Shodiev**

Khajand State University named after Academician Bobojon Gafurov, Tajikistan, Uzbekistan

### ABSTRACT

The given scientific article analyses the dynamics of quantitative alterations in peripheral leukocytes and hematopoietic elements in blood during the period of COVID-19 and recovery. We studied the effect of SARS-CoV-2 infection on the hematopoietic system, lymphocytopenia and eosinopenia during the course of the disease, control of the recovery period from the disease by monitoring the dynamics of the number of formed elements.

### KEYWORDS

COVID-19, post-COVID-19, physiology, peripheral immune cells, blood, leukocytes, lymphocytes, monocytes, neutrophils, eosinophils.

### INTRODUCTION

COVID-19 has become a global health threat. Hematological alterations in patients with Covid-19 disease may be one of the indicators of functional recovery, along with the immune response during SARS-CoV-2 infection [1, 2, 16]. Therefore, quantitative alterations in peripheral leukocytes as well as hematopoietic elements are currently being studied to determine whether COVID-19 is associated with fatal outcomes as an early signal in patients [3, 4].

Analyzing the scientific literature, the most common haematological symptoms in COVID-19 are a decrease in the number of lymphocytes - lymphocytopenia [5, 6], an increase in the number of neutrophils - neutrophilia [7, 8], as well as a decrease in the number of eosinophils - eosinopenia [9] and mild thrombocytopenia ( 35%) [10]. In many ways, eosinophils have been considered antiviral cells[11]. Eosinophils contain and produce molecules with antiviral activity and, thus, are involved in adaptive immunity, the properties of which have been studied in vitro and in vivo against a number of respiratory viruses, including influenza [12]. Of note, lymphopenia and eosinopenia were observed in 73 (52.9%) of 138 hospitalized patients with COVID-19. There is a positive correlation between lymphopenia and thrombocytopenia in patients with severe disease ( $r = 0.486$ ,  $P < 0.001$ ) [13]. Therefore, in this article, a dynamic analysis of haematological indications for the

treatment of COVID-19 in the postoperative period has been performed.

## METHODS

General and biochemical analysis of blood was conducted mainly in the 16th family clinic of the Almazar district of Tashkent, in the multidisciplinary clinic of the Tashkent Medical Academy. Analyzes were performed on a biochemical analyzer BA-88A Mindray Co.Ltd (KNR). HUMAN (GmbH) reagents (Germany) were used. The study was conducted in a room with a moderate temperature (26°C) [16].

The participation of study participants was voluntary and not funded. The study was conducted in accordance with the rules of scientific ethics, while maintaining the anonymity of the participants [15]. Cytometric studies were carried out according to the standards set on a BM 1800 microscope. Leukocyte counts were performed on a BM 1800 biological microscope using a Goryaev camera and automatic counters. Blood was taken under sterile conditions, for counting leukocytes 0.4 ml of a 3-5% solution of acetic acid was added to the solution stained with methylene blue. 20 µl of fresh blood (diluted 20 times) was taken with a capillary pipette and leukocytes were counted. Platelets were also counted according to the standards set in the Goryaev chamber under a BM 1800 biological microscope. The results were processed using the Excel and OriginPro6.2017 programs (OriginLab

Corporation, USA). The results were processed using Fisher's test, Student's t-factor. The arithmetic mean (M), mean deviation ( $\pm m$ ), and statistical significance index (p) were determined. At  $p \leq 0.05$ , the results were considered 95% statistically significant.

The research participants were divided into 4 experimental and 2 control groups in the post-COVID-19 period. For the 1st group, on the basis of voluntary consent, individuals with a severe course of COVID-19, under the age of 40 years, without chronic diseases were selected ( $n = 25$ ). The 2nd group, on the basis of voluntary consent, were selected with moderate and mild forms of COVID-19, 2-3 months after recovery, not older than 40 years and without chronic diseases ( $n = 25$ ). The second control group consisted of healthy people aged 41-55 years who were not infected with COVID-19 ( $n=12$ ). The 3rd group, on the basis of voluntary consent, were selected with a severe course of COVID-19, 2-3 months after recovery, no older than 41-55 years, without chronic diseases ( $n=25$ ). The 4th group, on the basis of voluntary consent, were selected with moderate and mild forms of COVID-19, 2-3 months after recovery, not older than 41-55 years, without chronic diseases ( $n = 25$ ).

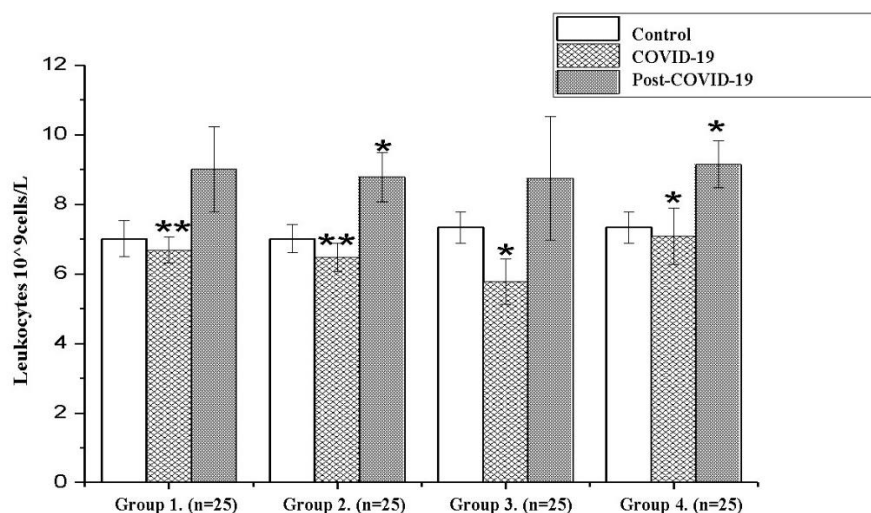
## RESULTS AND DISCUSSION

For the study, people without concomitant chronic diseases who recovered from COVID-19 were selected.

Functional parameters during the period of COVID-19 were obtained by retrospective analysis of case histories. Were obtained the results presented in fig. 1. for postoperative functional and haematological parameters.

In the group of 30-40-year-olds, whose average age is  $36.64 \pm 2.13$  years, a retrospective analysis showed that the number of leukocytes in the disease was  $6.68 \pm 0.37 \cdot 10^9$  cells/L in severe cases,  $6.47 \pm 0.41 \cdot 10^9$  cells/L in the lungs, which is somewhat lower than in the control norm, leukocytopenia was observed ( $P < 0.05$ ). On the 4th week after the disease it was  $9.01 \pm 1.22 \cdot 10^9$  cells/L in the first group,  $8.79 \pm 0.41 \cdot 10^9$  cells/L in the second group ( $P < 0.05$ ). It was found that leukocytes increased by 26% in both groups after recovery.

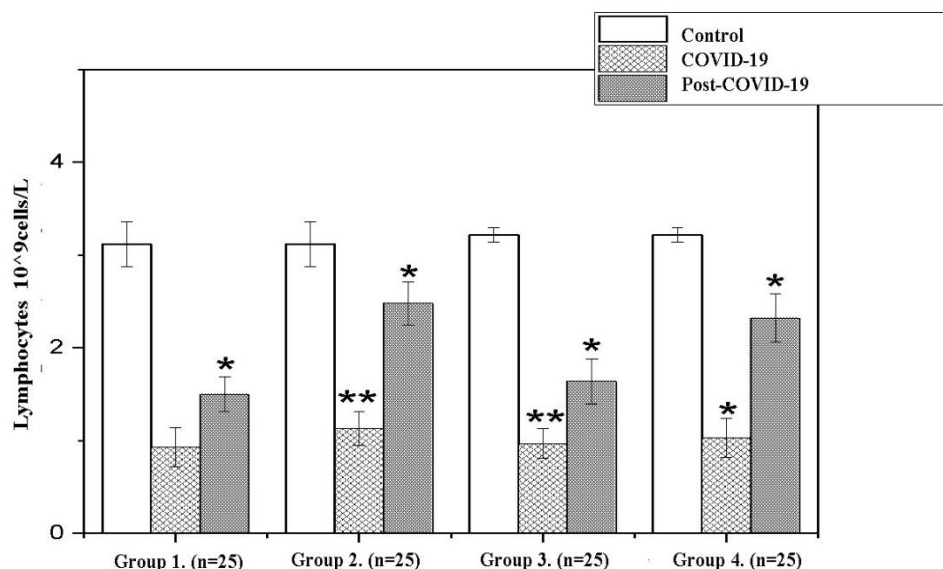
In the second large group, the average age of the subjects was  $46.1 \pm 1.87$  years; as a result of retrospective and laboratory analysis, it was found that the number of leukocytes in the disease was  $5.78 \pm 0.65$  ( $P < 0.05$ ) in severe cases, and in non-severe cases -  $7.08 \pm 0.81$  ( $P < 0.05$ ), mild leukocytopenia was observed somewhat less than the control norm ( $P < 0.05$ ), at the 4th week after the disease -  $8.75 \pm 1.78$  in the 3rd group,  $9.15 \pm 0$ , in the fourth group -  $68$  ( $P < 0.05$ ). Leukocytes increased by 19.2% in the first group and by 19.7% in the second group after recovery (Fig.1).



**Fig. 1.** Analysis of leukocytes during the recovery period ( $M \pm m$ ) (\*-  $P < 0.05$ ; \*\*-  $P < 0.01$ ; \*\*\*-  $P < 0.001$ ).

The number of lymphocytes was slightly below the norm and amounted to  $0.93 \pm 0.11 \cdot 10^9$  cells/L and  $1.03 \pm 0.18$  ( $P < 0.01$ ) during the course of the disease, and on the 4th week after the disease it was  $1.50 \pm 0.19 \cdot 10^9$  cells/L and,  $2.48 \pm 0.23$  ( $P < 0.01$ ), which was close to the norm in dynamics. The number of

lymphocytes in the 3rd and 4th groups of the disease was  $0.97 \pm 0.16$  ( $P < 0.05$ ) and  $1.03 \pm 0.18$  ( $P < 0.01$ ), in the 3rd group on composition  $1.64 \pm 0.24$  ( $P < 0.05$ ), in the 4th group  $2.32 \pm 0.26$  ( $P < 0.01$ ). In the 1st group, it was established that it was 3 times smaller, and the second group was close to the norm (Fig. 2.).

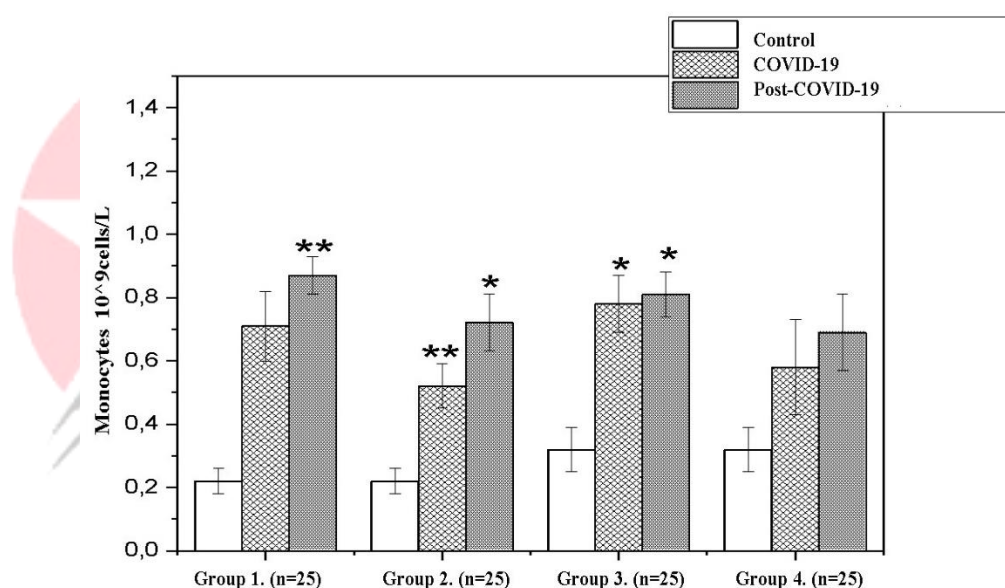


**Fig. 2. Dynamics lymphocytes in the recovery period**

(Note: Unit of measurement -  $10^9$ cells/L; \*-  $P<0,05$ ; \*\*- $P<0,01$ ; \*\*\*- $P<0,001$ )

In a number of monocytes in the first and second groups was  $0.71 \pm 0.17$  ( $P<0.05$ ) and  $0.52 \pm 0.27$  ( $P<0.05$ ) during the disease, and was within the upper limit of normal. On the 4th week after the disease, it was  $0.87 \pm 0.26$  ( $P<0.05$ ) in the first group and  $0.72 \pm 0.09$  ( $P<0.01$ ) in the second group. It is established, respectively, that it increased by 4.0 and 3.7 times. The number of monocytes in the third and

fourth groups, respectively, during the period of the disease was  $0.78 \pm 0.09$  ( $P<0.05$ ) and  $0.58 \pm 0.01$  ( $P<0.01$ ) and was within the upper limit of normal. After diseases in the 4th week -  $0.81 \pm 0.07$  ( $P<0.05$ ) in the 3rd group and  $0.69 \pm 0.12$  ( $P<0.01$ ) in the 2nd group, respectively, it increased by 2.5 and 2.1 times from the control. (Fig. 3.).

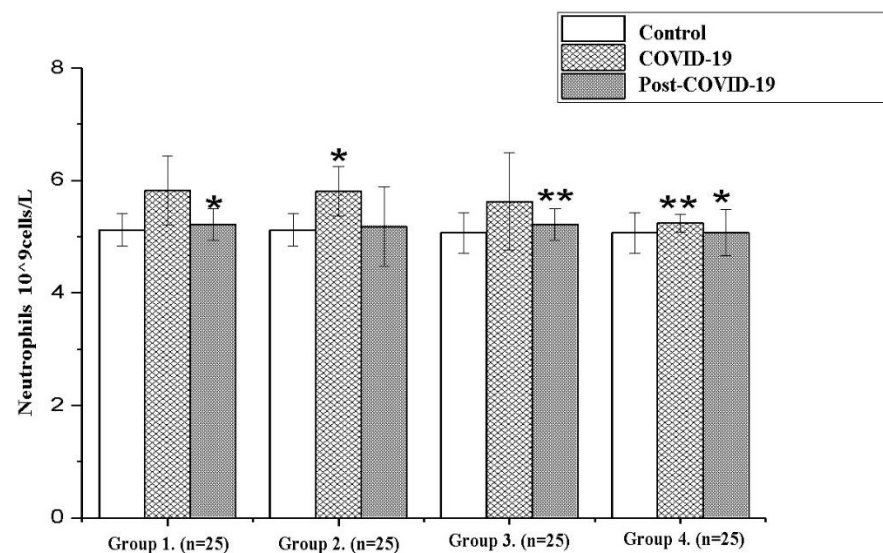


**Fig.3. Dynamics monocytes in the recovery period**

(Note: Unit of measurement -  $10^9$ cells/L; \*-  $P<0,05$ ; \*\*- $P<0,01$ ; \*\*\*- $P<0,001$ )

The number of neutrophils in the disease was  $5.82 \pm 0.61$  ( $P<0.05$ ) in the first group,  $5.16 \pm 0.44$  ( $P<0.01$ ) in the second group and was observed at the upper limit of normal. After the disease, the decrease in the number of neutrophils continued on the 4th week - in the first group it was  $5.18 \pm 0.30$  ( $P<0.05$ ), in the second group -  $5.81 \pm 0.62$  ( $P<0.05$ ) (3.15 - see figure). In the

third and fourth groups, it was  $5.63 \pm 0.87$  ( $P<0.05$ ),  $5.24 \pm 0.16$  ( $P<0.01$ ) and was observed at the upper limit of normal. In the following groups after the disease, the decrease in the number of neutrophils continued and on the 4th week it was  $5.22 \pm 0.28$  ( $P<0.05$ ) in the third group, and  $5.07 \pm 0.51$  in the fourth group ( $P<0,05$ ) (Fig. 4).



**Fig.4.** Dynamics neutrophils in the recovery period

(Note: Unit of measurement -  $10^9$  cells/L; \*-  $P<0,05$ ; \*\*- $P<0,01$ ; \*\*\*- $P<0,001$ )

In a number of eosinophils, weak eosinopenia was observed, the number of which was  $0.017 \pm 0.01$  ( $P<0.01$ ) and  $0.021 \pm 0.03$  ( $P<0.01$ ) in the first and second groups, respectively. In the dynamics after the disease, an increase in the number of eosinophils was observed and on the 4th week it was  $0.09 \pm 0.04$  ( $P<0.05$ ),  $0.20 \pm 0.11$  ( $P<0.05$ ). Mild eosinopenia was observed in patients with severe disease.

During infection with COVID-19, high levels of chemokines are synthesized to recruit effector inflammatory cells due to the immune response. This inadequate immune response leads to lung infiltration and hyperactivation of monocytes and macrophages as a result of chemokine secretion. A large number of inflammatory cytokines, chemokines and monocytes in the lung tissue attract neutrophils, which causes alveolar edema and a decrease in gas exchange [14].

This process explains the pathogenesis of changes in the number of peripheral immune cells.

### CONCLUSIONS

Thus, COVID-19 is a systemic disease that significantly affects the haematopoietic system and haemostasis. Since lymphocytopenia and eosinopenia are observed during the course of the disease, dynamic control of the number of hematopoietic elements is one of the most important factors in recovery. As a result, monitoring of haematological parameters, the dynamics of the number of lymphocytes helps to control the period of recovery from the disease and take timely preventive measures.

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