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IMMUNOGLOBULIN ISOTYPES AND BLOOD MONOCYTE SUBPOPULATIONS IN COVID-19 FEMALE PATIENTS WITH DIFFERENT DISEASE SEVERITY

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COVID-19, an acute respiratory infection caused by the SARS-CoV-2 virus, manifests itself in various severity forms - mild, moderate and severe, caused by the reactions of the patient's immune response.

Aim. To evaluate the serum levels of immunoglobulins G, M, and A and the number of circulating monocytes of different phenotypes in female patients with the abovementioned forms of COVID-19 severity.

Methods. Blood samples of 53 women with SARS-CoV-2 infection were studied. Flow cytofluorimetry was used to estimate monocyte subpopulations by the expression of CD14 and CD16. Concentrations of IgM, IgG, and IgA in the serum were determined in radial immunodiffusion test according to Mancini.

Results. The relative number of non-classical monocytes with CD14+-CD16++ phenotype was significantly decreased in the blood of COVID-19 patients from all 3 clinical severity groups, while changes in the number of classical and intermediate monocytes were insignificant. The levels of IgA in COVID-19 patients significantly decreased after recovery as compared to the acute phase of the infection.

Conclusion. The results emphasize the importance of monocyte subpopulation analysis in COVID-19 diagnosis and indicate dynamic changes in IgA levels depending on disease severity. The research data may help in the development of new diagnosis methods and therapy for SARS-CoV-2 infection.

Key words: COVID-19, circulating monocytes, immunoglobulin isotypes M, G and A.

SARS-CoV-2 infection involves various forms of human diseases, namely mild, moderate and severe ones depending on the patient's immune response [1]. The humoral immune response to SARS-CoV-2 is mediated by antibodies directed against the surface glycoproteins of the virus, mainly the S-glycoprotein and the nucleocapsid protein [2]. Monocytes are the main effector cells of the cytokine syndrome: they produce inflammatory cytokines and regulate the recruitment and activation of new tissue-damaging cells from the blood [3]. However, the connection between the disease's clinical forms and the functioning level of humoral and cellular immunity is still poorly explored. Coronavirus infection diagnosis is often based on the assessment of blood immunoglobulin content [4]. The evaluation of innate cellular immunity might enable the development of new approaches both for diagnosis and therapy [5].

This research was targeted to assess humoral and cellular immune response in patients suffering from COVID-19 of different disease severity, namely, to determine the number of different sub-populations of monocytes and the levels of IgG, IgM and IgA

in the COVID-19 female patients' blood before and after treatment.

Methods

Flow cytofluorimetry of heparinized blood samples was applied to determine monocyte populations by CD14 and CD16 expression. The method of radial immunodiffusion in agarose gel according to Mancini was used for the quantitative estimation of immunoglobulins G, A, and M in the patient's blood serum.

Participants and settings. 53 women took part in the experiment. The average age was 35 years (the youngest was 26 and the oldest was 44). All participants were divided into 3 groups depending on the severity of the coronavirus disease. Group 1 (mild course) consisted of 12 women; group 2 (moderate course) was the largest and included 30 women, and group 3 (severe course) was the least numerous and covered 11 female patients. The recommendations for treatment and taking medications depending on the symptoms and severity of the disease were provided by the family doctor listed in each participant's healthcare declaration. But additionally, as part of the experiment, each participant took vitamins C (500 mg), D3 (800 IU) and zinc (10 mg) daily during the illness.

Results and Discussion

The first step was to determine the level of immunoglobulins G, M and A in the blood serum of patients with COVID-19 of different disease severity. The obtained data indicate a normal concentration of immunoglobulin M (IgM) in all experimental groups before and after treatment. In patients with a severe form of infection at the time of recovery, a slight decrease in the concentration of IgM was observed, while in the other two groups, an increase in the concentration of this immunoglobulin was recorded after the disease (Fig. 1, *A*).

Although a decrease in IgG was found after recovery in each of the three groups, its concentration was also within the normal range. Dynamics of IgA concentration showed a decrease in patients' blood serum of all three groups after treatment, namely by 15.8% within the mild group, and by 21.2% within the other two groups, compared to the levels measured in the acute phase of the disease (Fig. 1, *A*, *B*, *C*).

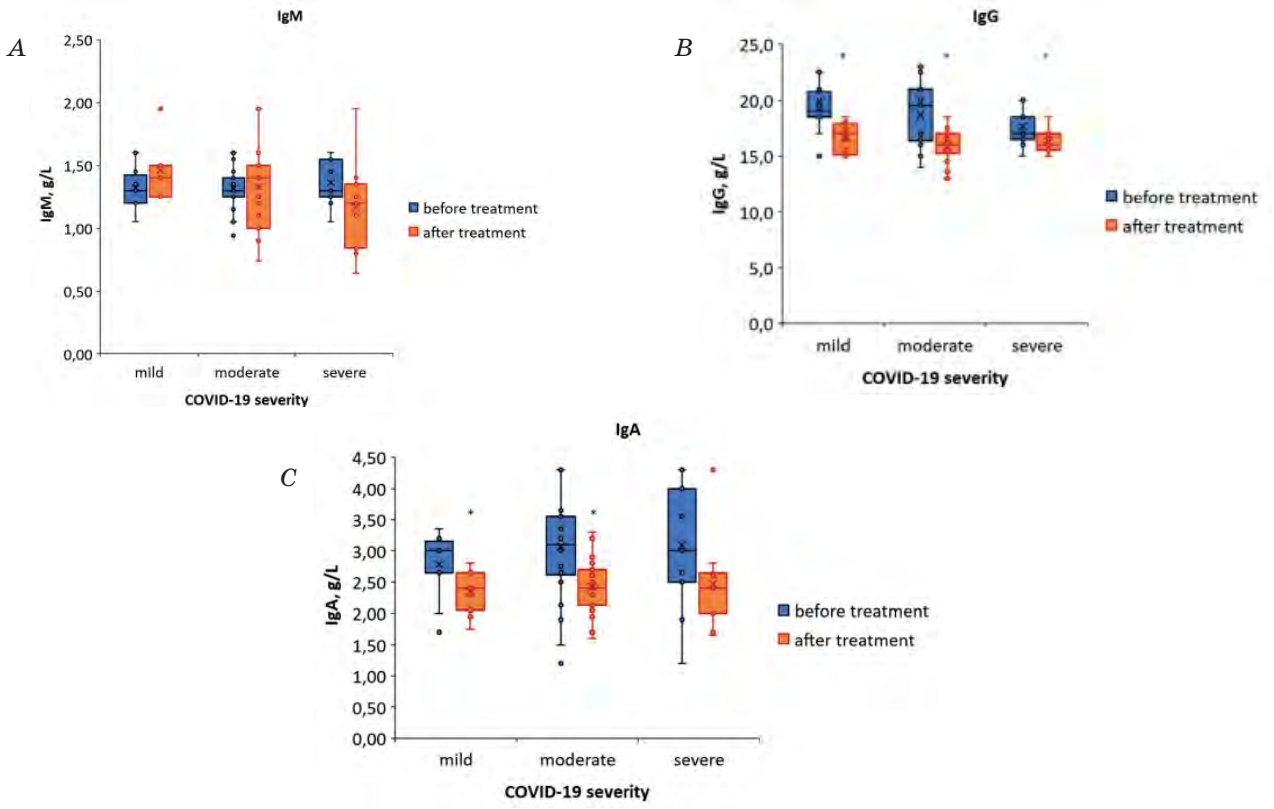

Fig. 1. **Levels of IgM (*A*), IgG (*B*), IgA (*C*) in blood serum of the COVID-19 female patients with different disease severity: mild, *n* = 12; moderate, *n* =30; severe, *n* =11**
* — $P < 0,05$ compared to the values before treatment.

The next step was to determine the number of monocyte populations with classical, intermediate and non-classical phenotypes based on the expression of CD14 and CD16 markers. In the group of patients with a moderate form of the disease, the greatest variability of the classical CD14+CD16- monocytes relative number was revealed: an equal number of patients (13%) had an excess number of this cell population in blood or this index decrease compared to the reference values. A decrease in the number of CD14+CD16- occurred in a third of patients with a mild form of Coronavirus infection. After the treatment, these cells were within the normal values in almost all the examined patients. As for the severe form of the disease, no significant deviations from the reference values were recorded (Fig. 2, *A*).

An increased number of intermediate monocytes was observed in all groups after treatment compared with those registered in the acute phase of the infection, but they did not exceed the reference range of values in both time points of the survey. In particular, the number of CD14+CD16+ monocytes increased almost twice and was at the upper limit of the reference range among women with moderate disease severity. Among the patients of the third group, who suffered from severe COVID-19, the number of cells with intermediate phenotype increased statistically insignificantly (Fig. 2, *B*). The relative number of non-classical monocytes with CD14+-CD16++ phenotype in the majority of patients from all examined groups, both before and after treatment, was lower than the reference values. Also, a decrease in these cells' index after treatment was registered in patients from each group: by 3.3 times — in the mild severity group, by twice — in the moderate severity group, and by 1.6 times – in the third group with the highest severity level. It is important to note that the number of CD14+-CD16++ cells was less than 1% in about 90% of those patients examined after treatment, which may indicate a persistent dependence of this subpopulation on the COVID-19 course and severity (Fig. 2, ***C***).

Conclusions

The results indicate that the SARS-CoV-2 infection is associated with prominent quantitative changes in the monocyte-macrophage lineage in female patients

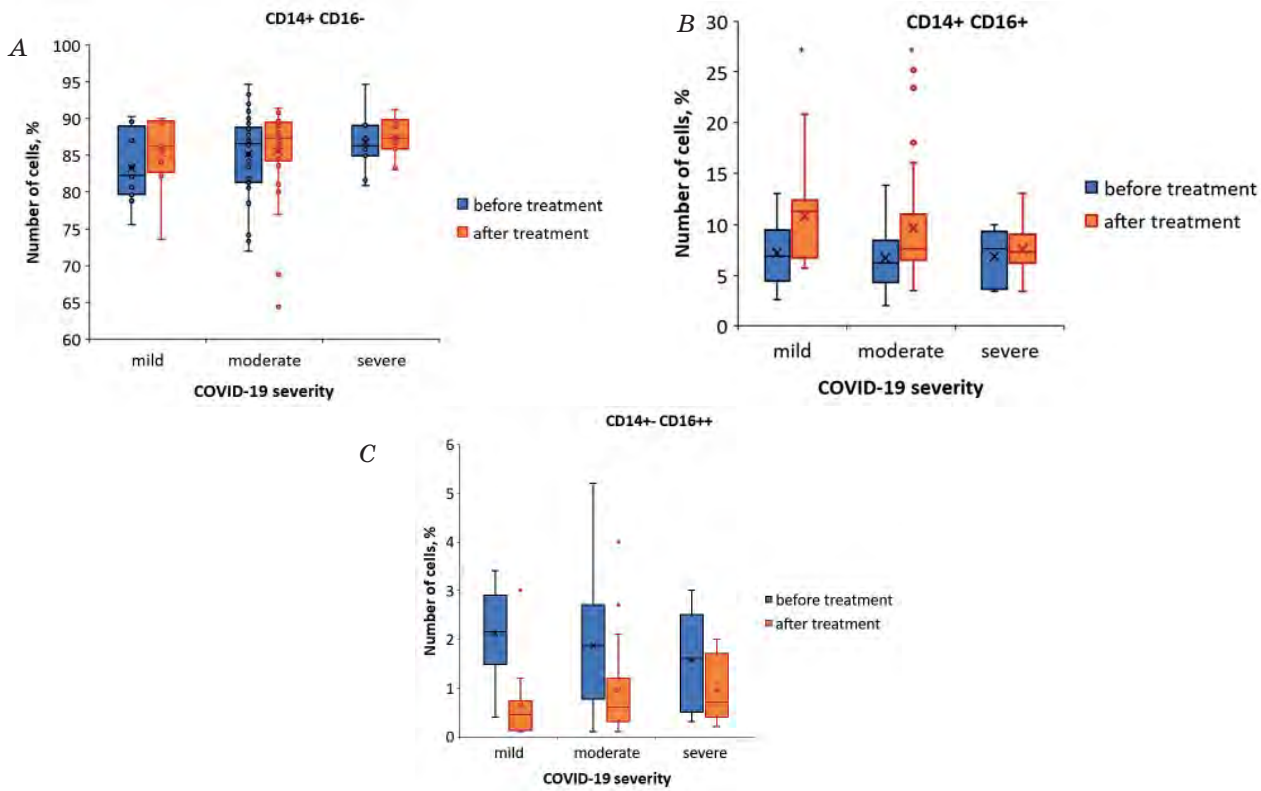

Fig. 2. **The relative number of classical CD14+CD16- monocytes (*A*), intermediate CD14+CD16+ monocytes (*B*), and non-classical CD14+-CD16++ monocytes (*C*) in female patients with different COVID-19 severity: mild, $n = 12$; moderate, $n = 30$; severe, $n = 11$**
*— $P < 0.05$ compared to the values before treatment.

with different disease severity. Significant severity-related decrease of the non-classical monocyte number in patient blood can indicate their important role in the resolution of inflammation during COVID-19. A noticeable decrease in the levels of immunoglobulins after recovery was typical for IgG and IgA, however, it was statistically significant for the female patients with mild and moderate COVID-19.

Based on the obtained results, the tendency to decrease the concentrations of immunoglobulins of all classes after the illness may indicate that after recovery, the level of virus-specific antibodies decreases over time. Also, a decrease may prove a deterioration of the functional properties of the immunity humoral link, which is expressed in a violation of their synthesis or an increase in catabolism and adsorption on immune complexes. Additional studies of the functional characteristics of monocytes are needed for their use as diagnostic markers of disease severity.

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