DOI 10.4149/BLL_2024_72

Bratisl Med J 2024; 125 (8):
468–471

CLINICAL STUDY

Adaptive post-COVID-19 immune response in female subjects of the Russian arctic region

Lyubov S. SHCHEGOLEVA[1], Mohammad-Sohib KABBANI[1,2], Elizaveta Y. SHASHKOVA[1], Oksana E. FILIPPOVA[1], Ekaterina V. POPOVSKAYA[1], Tatyana B. SERGEEVA[1], Olga S. MOROZOVA[1]

N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia. **kabbani.narfu@bk.ru**

ABSTRACT

The Arctic region's unfavorable living conditions adversely affect the spread of infectious diseases, including COVID-19, This, in turn, can also lead to increased morbidity and mortality rates in the area due to a number of factors such as climate, environment, and high prevalence rate of pre-existing health issues like diabetes, obesity, and respiratory infections. These circumstances adversely affect maintaining the level of working capability. The aim of this paper is to investigate the ratio of immunocompetent cells involved in the adaptive post-COVID-19 immune response. The research includes an immunological assessment of 29 women aged 20-40 years residing in Arkhangelsk, Russia, six months after recovering from COVID-19. The count of leukocytes in the peripheral blood and their differential were evaluated using standard methods to assess the immunological status. To delve deeper into the immunological landscape, phenotypes of lymphocytes ($CD5^+$, $CD8^+$, $CD10^+$, and $CD95^+$) were evaluated using an indirect immunoperoxidase reaction with monoclonal antibodies on dried drop lymphocyte preparations. After incubating blood with latex molecules, the activity and quantity of phagocytes were assessed using a light microscope. The neutrophil/lymphocyte ratio was found to be inverted in the female subjects under investigation. The high concentration of cytotoxic T-lymphocytes ($CD8^+$) and lymphocytes with apoptotic receptors ($CD95^+$) suggests a potential correlation with a concurrent reduction in the expression of the total T-cell marker ($CD5^+$) across all cases. This association was further linked to a decrease in lymphoproliferative activity and a relative decline in phagocytic activity. These findings led us to posit that the total recovery time after COVID-19 might extend beyond six months, indicative of a prolonged impact on the body's protective capacity. Our observations prompt the hypothesis that cellular immunity plays a crucial role in determining the severity of COVID-19 infection. Specifically, individuals with initially robust phagocytic activity may be predisposed to experiencing a milder form of the infection. However, this assumption warrants further investigation and clarification in individuals with moderate and severe disease progression *(Tab. 1, Ref. 17)*. Text in PDF www.elis.sk

KEY WORDS: arctic, COVID-19, cytotoxic t-lymphocytes, apoptosis, lymphoproliferation, cellular immunity, phagocytic activity.

Introduction

Due to a variety of factors, populations residing in the Arctic area have disproportionately higher COVD-19 morbidity and mortality rates. These may include, for example, geographic challenges to obtaining healthcare and a greater prevalence of pre-existing health conditions such as diabetes, obesity, and respiratory infections (Hathaway, 2021).

The Arctic region's extreme climatic and environmental conditions prevent the development of self-regulation processes that return the body systems to their optimal mode of functioning, resulting in the activation and tension of cellular and humoral immunity and, ultimately, a reduction in the body's reserve capacity (Donaldson et al, 2016, Shchegoleva et al, 2016).

The infection by the novel coronavirus SARS-CoV-2 infection leads to the development of an acute infectious disease of the respiratory tract characterized by classic catarrhal symptoms that can clinically present as SARS. In 80% of patients, the condition progresses to a moderate form and typically concludes with spontaneous recovery (Akimkin et al, 2022).

COVID-19 has already infected over 5 million people and killed at least 500 thousand people in over 200 countries. Asymptomatic sickness might progress to severe viral pneumonia, acute respiratory syndrome and sepsis, myocarditis, and renal failure (Troshina et al, 2020).

[1]N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia, and [2]Northern (Arctic) Federal University named after M.V. Lomonosov, Arkhangelsk, Russia

Address for correspondence: Mohammad-Sohib KABBANI, N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, 23 Naberezhnaya Severnoy Dviny, Arkhangelsk, 163000, Russia.

The main publications still include limited information, relying heavily on comparisons with established viral infections and their involvement in disease development (Kaufmann et al, 2018, Zumla et al, 2016).

The long-term implications of the inflammatory processes resulting from SARS-CoV-2 infection remain uncertain. Particularly concerning are the potential alterations to the health of various body systems, including the immune system. The literature frequently deliberates on treatment modalities, especially in individuals with a comorbid background or a history of chronic diseases (Shanmugaraj et al, 2020, Knyazev et al, 2022).

Since acquired immunity after a natural infection is anticipated to require a certain duration to recover contingent upon the severity of the initial infection, reactivation of immune responses due to new infection or vaccination during this recovery period may introduce complications (Van Damme et al, 2020).

There is a scarcity of studies specifically examining the behavior of post-COVID-19 adaptive immune response in individuals residing in the extreme climate conditions of the European North of the Russian Federation

The purpose of this paper is to address this research gap by investigating the ratio of immunocompetent cells involved in the adaptive immune response developed after COVID-19.

Materials and methods

In April and May 2022, a cohort of 29 women aged 20–40 years from Arkhangelsk was examined as part of a descriptive study. Each participant had previously experienced an episode of COVID-19, as evidenced by the clinical diagnosis confirmed through PCR. The evaluation was carried out 6 months post-illness. The participation in the study was contingent upon the volunteers' submission of an informed consent form. Fasting blood samples for the study, totaling 6 ml in volume, were collected in the morning from the cubital vein.

The complex immunological examination of the individuals was conducted, including a complete blood count and the analysis of the content of lymphocytic phenotypes ($CD5^+$, $CD8^+$, $CD10^+$, $CD95^+$) in the peripheral blood. The investigation extended to the assessment of the phagocytic activity and quantification of phagocytic cells. These analyses were carried out at the Laboratory of Physiology of Immunocompetent Cells of the Federal Research Center for Comprehensive Study of the Arctic, Ural Branch of the Russian Academy of Sciences.

The assessment of the complete blood count was carried out using the hematology analyzer Horiba ABX Pentra 60. The leukocyte differential determination was conducted by examining blood smear stained according to Romanowsky-Giemsa.

The absolute content of T-lymphocyte subpopulations was determined by the method of indirect immunoperoxidase reaction using monoclonal antibodies (MedBioSpectr, Moscow) on lymphocyte preparations of the "dried drop" type. The enumeration was performed using a Nicon Eclipse 50i microscope. The activity and quantity of phagocytes were determined by incubating blood with latex particles for 30 minutes at a temperature of 37 °C and subsequent staining according to Romanowsky-Giemsa.

The study's data were statistically analyzed using Microsoft Excel and Statistica 6.0 software (StatSoft, USA). The results were reported as the mathematical mean along the mean error (M±m). The correlation analysis was carried out by calculating the non-parametric Spearman rank correlation coefficient (r). The reliability of correlations (p) was further evaluated with statistical significance set at $p<0.05$.

Results

Protective responses against SARS-CoV-2 infection emerge through the activation of both innate and acquired immunity directed specifically at the virus. However, the immunopathogenesis of COVID-19 is marked by the development of an unbalanced immune response, which in particularly severe cases, leads to impaired lung function (Smirnov and Totolyan, 2020, Toptygina et al, 2022).

In this context, it became crucial to investigate the cellular adaptive immune response in young female subjects six months after a moderate illness.

The investigation revealed that the average concentration of leukocytes, lymphocytes, and neutrophils among the examined women six months post-COVID-19 episode fell within the generally accepted physiological reference intervals: 5.55±0.01, 2.81±0.02, and 1.99±0.02x10^9 cells/l, respectively (Tab. 1). How-

Tab. 1. The immune status of female subjects from Arkhangelsk aged 20–40 years (n=29) 6 months after COVID-19.

Cell type	Level in the blood (M±m)	Reference interval	Imbalance registration frequency, %	
			Low	High
Leukocytes, x10^9 cell/l	5.55±0.13	4–10	10.34	48.27
Lymphocytes, x10^9 cell/l	2.81±0.08	1.5–3.5	–	13.79
Neutrophils, x10^9 cell/l	1.99±0.06	1.5–5.5	–	10.34
$CD5^+$, x10^9 cell/l	0.45±0.01	1.5–2.5	100	–
$CD8^+$, x10^9 cell/l	0.42±0.03	0.2–0.4	–	48.27
$CD10^+$, x10^9 cell/l	0.43±0.04	0.5–0.6	44.83	6.90
$CD95^+$, x10^9 cell/l	0.60±0.05	0.45–0.55	20.69	51.72
Phagocytic number	6.00±0.20	1–8	–	27.59
Phagocytic activity, %	50.40±1.12	>50	37.93	–

Compiled by the authors

ever, the lymphocyte content was found to be closer to the upper limit of reference interval and notably, the neutrophil/lymphocyte ratio exhibited a reversal.

The investigation revealed a remarkably low level of the general population of all T-cells ($CD5^+$), being three times below the lower limit of the established physiological norm ($1.5–2.5x10^9$ cells/l). The average concentration of CD5+ T-cells was recorded at $0.45±0.01x10^9$ cells/l.

The concentration of cytotoxic cells (CD8+) is relatively high, with an average of $0.42±0.03x10^9$ cells/l, compared to the established norm ($0.2–0.4x10^9$ cells/l).

It is important to emphasize that the mean concentration of T-lymphocytes with the $CD10^+$ marker, representing lymphoproliferative activities, was recorded at $0.43±0.04x10^9$ cells/l, which falls below the physiological reference interval ($0.5–0.6x10^9$ cells/l)

It was observed that in the examined young female subjects, six months following COVID-19 episode, the count of lymphocytes with $CD95^+$ marker, indicative of apoptotic process, was $0.60±0.05x10^9$ cells/l which narrowly exceeds the normal reference range ($0.45–0.55x10^9$ cells/l).

Thus, in the investigated individuals, six months following COVID-19 episode, the count of T-cells is extremely low, with the exception of cytotoxic lymphocytes ($CD8^+$) and lymphocytes with apoptotic receptors ($CD95^+$).

The highest correlation values were found between $CD8^+$ and $CD95^+$ cells ($r=0.81$, $p<0.001$).

The number of phagocytic cells and the level of phagocytic activity are within the physiological norm; on average, the quantity of phagocytic cells in the investigated women was 6±0.20, and the phagocytic activity was registered at 50.40±1.12%, which is at the lower limit of the reference values.

The frequency of occurrence of immunological abnormalities in the investigated individuals raises concerns. In our research, leukopenia was about 5 times less prevalent than leukocytosis, occurring in 10.34% and 48.27% of young female individuals, respectively.

Furthermore, neither lymphocytopenia nor neutropenia were observed among the cases. On the contrary, 13.79% of the volunteers demonstrated mild lymphocytosis, and 10.34% had a slight increase in total neutrophils.

Moreover, the concentration of cytotoxic cells was established at a minimum range of $0.20–0.22x10^9$ cells/l in 41.38% of individuals with relatively high phagocytic activity (within the range of 90 to 100%).

In 100% of the studied cases, there was a deficiency in the concentration of the total T-cell population ($CD5^+$). It is important to note that this low T cell content was found to be associated with the low level of cells representing lymphoproliferation processes ($CD10^+$; $r=0.78$, $p>0.001$).

Additionally, the high level of cytotoxic activity ($CD8^+$) observed in 48.27% of the investigated individuals, was found to be inversely correlated with phagocytic activity ($r=–0.77$; $p>0.001$) and, conversely, highly correlated with cells expressing apoptotic receptors ($CD95^+$; $r=0.78$; $p>0.001$.

Discussion

According to (Toptygina et al, 2022), individuals who recovered from COVID-19 exhibited a balanced distribution of high and low levels of cellular immunity in a ratio of 50 to 50%. These results align with analogous findings reported in the works of Dan et al (2021), Stephens and McElrath (2020) and Stephens et al, where the level of specific cellular response in recovering COVID-19 patients was found to vary according to the patients' functional systems and their capability, and to be affected by the severity of the disease. Antiviral cytotoxic activity tended to be low after mild or asymptomatic illness, a trend consistent with the results obtained in this report. In this context, the current study mirrors these patterns, as evidenced by the particular fact that almost 40% of the examined patients had a decreased phagocytic activity alongside relatively low neutrophil content. Additionally, an increase in the frequency of high concentrations of cytotoxic cells ($CD8^+$) and the expression of apoptosis receptors ($CD95^+$) reinforce the notion that immune responses in individuals recovering from COVID-19 may vary based on disease severity.

The study conducted by Grifoni et al. (2020) revealed the presence of specific immune responses in individuals recovered from COVID-19. According to their findings, specific $CD4^+$ T-helpers and $CD8^+$ cytotoxic T-lymphocytes in COVID-19 were discovered in the peripheral blood of 100% and 70% of patients recovered from COVID-19, respectively, particularly within the timeframe of 20–35 days post COVID-19 recovery. However, $CD8^+$ cytotoxic lymphocytes are directly responsible for the elimination of virus-infected cells.

The persistence of Elevated levels of cytotoxic T-lymphocytes and increased apoptosis observed in individuals six months post COVID-19 recovery may suggest an ongoing process of cellular attenuation. It is conceivable that, even at this juncture, the immune system is actively moderating cells that played a role in the antiviral response during the infection. This moderation is facilitated by expressing the receptor $CD95^+$ and triggering apoptosis.

Moreover, previous studies (Alotaibi et al, 2020, Freitas et al, 2018) demonstrated that a decrease in $CD5^+$expression contributes to an increase in the concentration of lymphocytes with the marker $CD8^+$ and activation of apoptosis. This suggests that a decrease in the concentration of the receptor ($CD5^+$) could explain the elevation in the cytotoxic cell content. At the same time, it triggers hyperactivation of the lymphoproliferation process, which contradicts the rise in the frequency of registering low levels of cells with a marker $CD10^+$, representing the lymphoproliferation process, as observed in our research.

It is also reasonable to presume that during the recovery from COVID-19 infection, the low lymphoproliferation activity is a consequence of the immune system's efforts to restore its reserve capacity.

Conclusion

Consequently, it was determined that six months after mild COVID-19, the adaptive immune response in young female indi-

viduals is characterized by low levels of the entire T-cell population ($CD5^+$) and lymphoproliferation processes ($CD10^+$), alongside a modest increase in the total leukocytes, cytotoxic activity ($CD8^+$), and activity of apoptosis processes ($CD95^+$).

The immune imbalances identified in this investigation may predispose individuals to the development of secondary environmentally dependent immunodeficiencies, particularly in those exhibiting elevated cytotoxic activity coupled with increased apoptotic activity. The lowest concentrations of cytotoxic cells were detected in individuals with high phagocytic activity, which, in our interpretation, may serve as a positive prognostic indicator, potentially reducing the risk of complications.

These findings suggest that cellular immunity plays a pivotal role in determining the development of a mild form of COVID-19 infection in individuals with initially robust phagocytic activity. This proposition necessitates further investigation aimed at deeper understanding of the intricate dynamics influencing immune responses and disease severity, especially in individuals with moderate or severe disease development.

References

1. Akimkin VG et al. COVID-19: the evolution of the pandemic in Russia. Report I: manifestations of the COVID-19 epidemic process. J Microbiol Epidemiol Immunobiol 2022; 99 (3): 269–286. DOI: 10.36233/0372-9311-276.

2. Alotaibi F et al. CD5 blockade enhances ex vivo CD8+ T cell activation and tumour cell cytotoxicity. Eur J Immunol 2020; 50 (5): 695–704. DOI: 10.1002/eji.201948309.

3. Dan JM et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021; 371 (6529): eabf4063. DOI: 10.1126/science.abf4063.

4. Donaldson S et al. Overview of human health in the Arctic: conclusions and recommendations. Internat J Circumpolar Health 2016; 75 (1): 33807. DOI: 10.3402/ijch.v75.33807.

5. Freitas CMT, Johnson DK, Weber KS. T cell calcium signaling regulation by the co-receptor CD5. Internat J Mol Sci 2018; 19 (5): 1295. DOI: 10.3390/ijms19051295.

6. Grifoni A et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 2020; 181 (7): 1489–1501. DOI: 10.1016/j.cell.2020.05.015.

7. Hathaway ED. American Indian and Alaska native people: Social vulnerability and COVID-19. J Rural Health 2021; 37 (1): 256–259. DOI: 10.1111/jrh.12505.

8. Kaufmann SH, Dorhoi A, Hotchkiss RS, Bartenschlager R. Host-directed therapies for bacterial and viral infections. Nature Rev Drug Discov 2018; 17 (1): 35–56. DOI: 10.1038/nrd.2017.162.

9. Knyazev et al. Unlocking capacities of genomics for the COVID-19 response and future pandemics. Nature Methods 2022; 19 (4): 374–380. DOI: 10.1038/s41592-022-01444-z.

10. Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asian Pacific J Allergy Immunol 2020; 38 (1): 10–18. DOI: 10.12932/AP-200220-0773.

11. Shchegoleva LS, Sergeeva TB, Shashkova EJ, Filippova OE, Popovskaya EV. Peculiarity of Immunological Activity of Peripheral Blood in Persons of Different Age Groups in Polar Regions. Ekologiya cheloveka (Human Ecology) 2016; 8 (8): 15–20. DOI: 10.33396/1728-0869-2016-8-15-20.

12. Smirnov VS, Totolyan AA. Innate immunity in coronavirus infection. Russian J Infection Immunity 2020; 10 (2): 259–268. https://doi.org/10.15789/2220-7619-III-1440.

13. Stephens DS, McElrath MJ. COVID-19 and the Path to Immunity. Jama 2020; 324 (13): 1279–1281. DOI: 10.1001/jama.2020.16656.

14. Toptygina AP, Semikina EL, Zakirov RS, Afridonova ZE. Comparison of the humoral and cellular immunity in COVID-19 convalescents. Russian J Infection Immunity 2022; 12 (3): 495–504. https://doi.org/10.15789/2220-7619-COT-1809.

15. Troshina EA, Melnichenko GA, Senyushkina ES, Mokrysheva NG. Adaptation of the hypothalamo-pituitary-thyroid and hypothalamo-pituitary-adrenal systems to a new infectious disease-COVID-19 in the development of COVID-19 pneumonia and/or cytokine storm. Clin Exp Thyroidol 2020; 16 (1): 21–27. DOI: 10.14341/ket12461.

16. Van Damme W et al. The COVID-19 pandemic: diverse contexts; different epidemics – how and why? BMJ Global Health 2020; 5 (7): e003098. DOI: 10.1136/bmjgh-2020-003098.

17. Zumla A et al. Host-directed therapies for infectious diseases: current status, recent progress, and future prospects. Lancet Infectious Dis 2016; 16 (4): e47–e63. DOI: https://doi.org/10.1016/S1473-3099(16)00078-5.

Received August 4, 2023.
Accepted February 14, 2024.