

Zabara, D., Kozeretska, I., Deineko, I., Anoshko, Ya., Shapovalenko, N., Stamboli, L., & Dons'koi, B. (2021). Immune factors and health of Antarctic explorers. *Ukrainian Antarctic Journal*, 2, 94–105.  
<https://doi.org/10.33275/1727-7485.2.2021.680>



**D. Zabara<sup>1</sup>, I. Kozeretska<sup>2</sup>, I. Deineko<sup>2</sup>, Ya. Anoshko<sup>1</sup>,  
N. Shapovalenko<sup>1</sup>, L. Stamboli<sup>1</sup>, B. Dons'koi<sup>1,\*</sup>**

<sup>1</sup> Institute of Pediatrics, Obstetrics and Gynecology of the National Academy of Medical Sciences of Ukraine, Kyiv, 04050, Ukraine

<sup>2</sup> State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine, Kyiv, 01601, Ukraine

\* Corresponding author: boris\_donskoy@ukr.net

## Immune factors and health of Antarctic explorers

**Abstract.** The immune system plays a major role in human homeostasis, yet a body's unique individuality complicates the diagnostic forecasting of unfavourable physiological states and diseases. Studying the immunophenotypic features of winterers of the Ukrainian Antarctic Expeditions before, during, and after their assignments might shed some light on the possible place of immune accentuations in the development of certain physiological states. To determine the natural-killer (NK) cytotoxicity and the immunophenotype in 52 applicants who wanted to take part in an expedition and nine participants who had come back, we used flow cytometry. Blood serum samples taken before, during, and after the expeditions were also tested for hormones, anti-infective, anti-parasitic, and autoimmune antibodies. The high absolute and relative numbers of NK lymphocytes, high NK cytotoxicity, and high expression of HLA-DR on the CD3+CD8+ lymphocytes were correlated with a person's unfavorable health status during the expedition. In Antarctica, cortisol levels sharply increased, yet they normalized upon return. In most winterers, there were no significant health complications during the expeditions. Neither reactivated nor primary viral infections were registered, as well as clinical autoimmune ones. Upon return, the winterers had significantly lower leukocytes and lymphocytes and increased expression of activation markers (HLA-DR) on the T-cells. The found risk factors can characterize the polar researchers' immunophenotypes yet require validation on larger samples. The expedition environment causes increased stress, entailing, however, neither clinical manifestations nor elements of immunosuppression. The polar researchers bear the consequences of the prolonged stress that inhibit leucopoiesis as late as six months after their return, which should be considered while reviewing applications for the next season.

**Keywords:** Antarctic expedition, immune accentuations, NK lymphocytes, NK cytotoxicity, prognostic factors

### 1 Introduction

Broadly speaking, the state of a body's immune system determines its health. Certain accentuations in the system's links can shape a favorable answer to the challenges it encounters and create a less favorable environment for other physiological processes. We developed a theory of immune accentuations supported by a model to forecast the complications in the female reproductive process and the severity of infectious

diseases in children. The theory predicts that specific accentuated levels of immune parameters can be unfavorable for certain physiological states and processes (Dons'koi, 2014; Dons'koi et al., 2016).

The health of polar researchers attracts great scientific interest, both fundamental and applied since there is a need to curb the expeditions' adverse effect on well-being. The problem has been attacked from several angles; Strewe et al. (2019) investigated the increase and specifics of secretion of cortisol, catecholamines, and endocan-

nabinoids, as well as higher levels of lymphocytes, platelets, and hemoglobin in men and women during one year in the field, and Reyes et al., (2017) reported reactivation of the Herpes Zoster infection and the Epstein-Barr virus under stressful conditions in Antarctica.

Consequences of long expeditions were traced in the oxidative stress markers, microelements content, the electrical activity of the central nervous system; the changes might themselves contribute to pathological processes in the body (Moiseyenko et al., 2016).

A detailed study of the immunophenotypes of applicants to polar expeditions and a clinical analysis of their health at the research base would reveal risk factors underlying the above-mentioned unfavorable states of health. A complementary study of the explorers upon their return would shed light on the probability of adverse consequences conditional on the immunophenotype variant.

We studied the participants' immunophenotypes and obtained frozen blood serum samples before the 25th and 26th Ukrainian Antarctic Expeditions (UAE). There prevailed normal unaccentuated immunophenotypes, yet there were occasional cases of immune accentuations both single and systemic. Comparing their health to the observed during the expedition would answer the question of which specific accentuations have which risk levels for human well-being in the harsh polar environment. A detailed examination upon their return would reveal the possible impact of the polar expeditions on the state of specific links of the whole immune system.

This paper analyzes the absolute and relative counts of NK lymphocytes, NK cytotoxicity, and expression of HLA-DR on CD3+CD8+ lymphocytes of the UAE participants. We also tested the blood sera and studied the traits of viral infections' reactivation.

## 2 Materials and methods

### 2.1 Study design

We examined the applicants for the seasonal and winter teams ( $n = 52$ ) for the lymphocyte immunophenotype and NK cytotoxicity before the start and upon the return from the expedition (for winterers,  $n = 10$ ). Blood serum samples were frozen ( $n = 30$ ) and compared to samples collected during the expedition ( $n =$

$= 19$ ), and samples collected six months afterwards ( $n = 10$ ). For the control group, we chose the laboratory team ( $n = 14$ ), testing and freezing samples to obtain the baseline trends of the parameters' changes over time. All participants signed the informed consent form before being enrolled in the study (approved by the Biomedical Ethics Committee of Institute of Pediatrics, Obstetrics, and Gynecology, National Academy of Medical Sciences of Ukraine, No. 6, and Clinical Immunology and Allergology Committee of Ministry of Health, No. 11 dated February 14, 2013, according to Declaration of Helsinki). For correct randomization, the association lymphocyte subpopulations and NK-cytotoxicity with health state was analyzed only in men.

### 2.2 Enzyme-Linked Immunosorbent Assay (ELISA)

Specific Ab (IgG) and hormones were measured in the patients' sera by the VitoTest ELISA system (Ukraine) and VectorBest ELISA (Russia) systems according to instructions. The analysis targeted serum concentrations of prostate-specific antigen total, testosterone, and cortisol, anti-ssDNA-IgG, the rheumatoid factor, antibodies IgG, IgM, and IgA to SarsCov2, antibodies IgG to the cytomegalovirus, *Toxoplasma gondii*, *Toxocara canis*, *Ascaris lumbricoides*, *Echinococcus granulosus*, *Chlamydophila pneumoniae*, human herpesvirus 8 (HHV-8), varicella-zoster virus (VZV), Epstein-Barr virus (antigens NA and EA), *Mycoplasma hominis*, and *Mycoplasma pneumoniae*.

### 2.3 Flow Cytometry of Lymphocyte Subsets

Lymphocyte subsets were identified by three-color flow cytometry using erythrocyte-lysing whole blood method of lymphocyte staining by FITC-, PE- or PE-Cy5-conjugated monoclonal antibodies (BD Bioscience, San Jose, USA). Stained samples were lysed, washed, and analyzed by FACScan flow cytometer using CellQuest software (BD Bioscience, San Jose, USA) as described in (Dons'koi et al., 2021). We analyzed absolute and relative (%) levels of leucocytes, monocytes CD14+, lymphocytes and lymphocytes subsets CD3+, CD3+CD8+, CD3+CD8+, and HLA-DR and CD158a expression on T lymphocytes. NK

lymphocytes (CD3-CD56+) subsets CD3-CD56 + HLA-DR+, CD3-CD56 + CD8+, CD3-CD56 + CD158a+, CD3-CD56++ and B-lymphocytes CD19+, and CD19+CD5+.

## 2.4 NK cytotoxicity assays

Flow cytometry NK cytotoxicity assay (FCA) was described previously (Dons'koi et al., 2020a). We used Calcein-AM (Sigma), labeling K562 as target cells. Dead cells after 4h incubation were labeled by propidium iodide. NK cytotoxicity levels were analyzed by FACScan flow cytometer using CellQuest software (BD Bioscience, San Jose, USA)

## 2.5 Health conditions

Health was evaluated according to the monthly reports of the expedition's doctor. Health was considered unfavorable if a participant complained on average more than thrice a month (malaise, headache, disrupted sleep, fatigue, arterial hypertension). Colds, traumas, and sunburns were very rare and not taken into account. The "no health complaints" group included participants who had them less frequently than twice a month.

## 2.6 Statistical Analysis

The statistical analysis of the results was performed using approximation of Woolf for OR (Odds Ratio) and 95% CI (Confidence Intervals), Fisher's Exact Test (unpaired, non-parametric, two-sided P value), and the Spearman correlation (In Stat version 3.0 for Windows Graph Pad Software Inc., San Diego, CA, USA) (<https://www.graphpad.com/support/instat-3-updates/> <https://www.graphpad.com/>).

# 3 Results

## 3.1 Immunophenotype characteristics of the applicants and participants of the expeditions

Immediately before the expedition, five out of 52 applicants were rejected on health grounds. We compared the immunophenotype parameters for the applicants who passed the medical examination ( $n = 47$ ) and those who did not ( $n = 5$ ).

The rejected applicants had higher absolute and relative counts of the NK lymphocytes ( $780/\mu\text{l}$  ( $SD = 240$ ) and 26.4% ( $SD = 6.9$ )) compared to the selected ( $393/\mu\text{l}$  ( $SD = 268$ ) and 17.3% ( $SD = 3.6$ )) ( $p < 0.02$ ). All five rejected applicants had the relative count of NK lymphocytes over 22%, while only 29.7% of accepted applicants ( $n = 14$ ) had such an accentuated level.

Thus, the accentuated NK lymphocytes level might be an unfavourable factor of the overall health (Odds Ratio = 25.414,  $p = 0.0045$ ).

## 3.2 Health in Antarctica and the immunophenotype

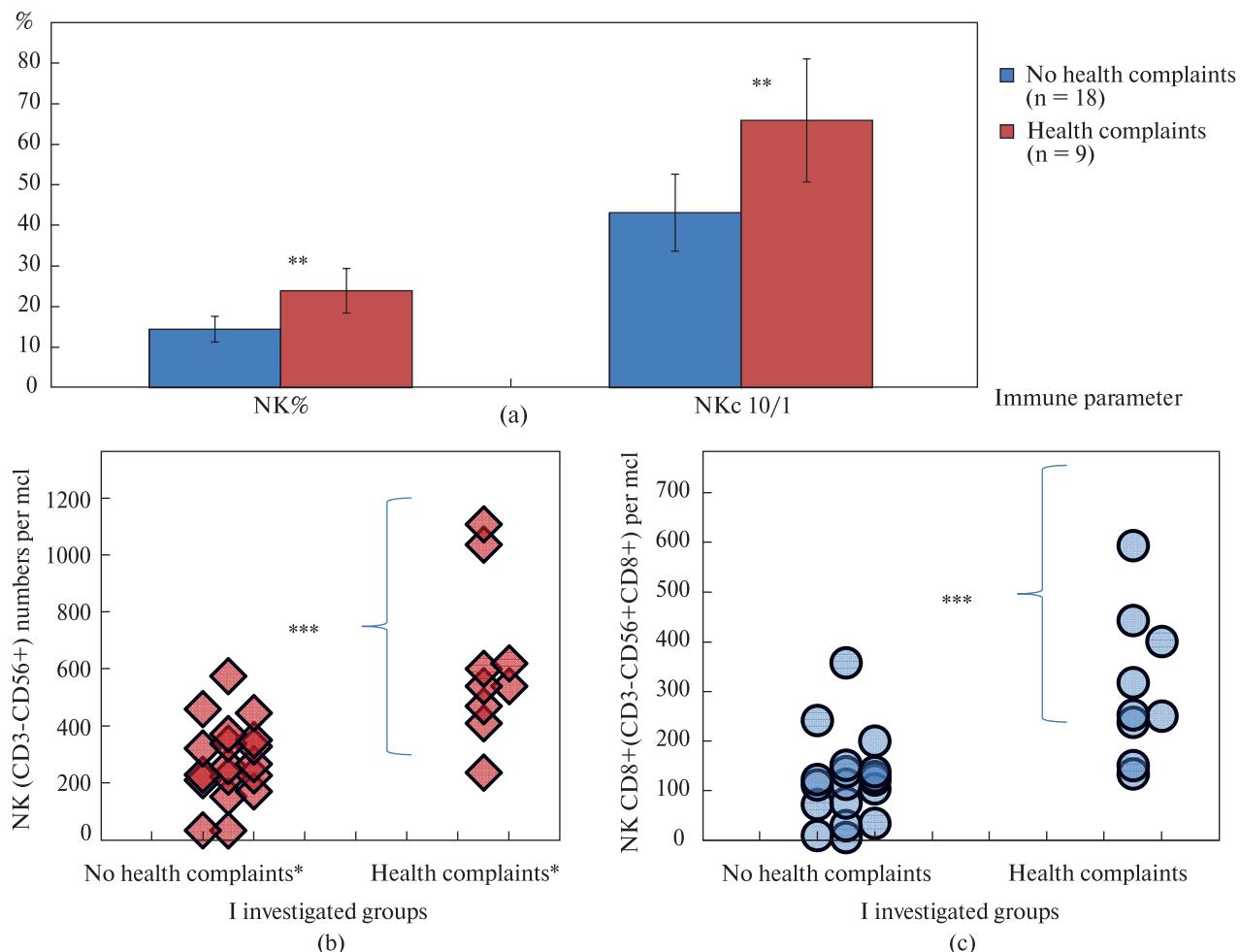
We analyzed the medical reports on the researchers' health (for UAE-25 — over 12 months, UAE-26 — over three months). Mostly, the complaints were episodic, and no participant asked for medical help more than twice. No complaints were caused by infectious disease, skin problems, or the digestive tract.

However, five people from UAE-25 complained of general malaise (fatigue, unstable blood pressure, headaches, insomnia). These participants constitute the Unfavorable Health conditions group (UH), while the rest ( $n = 18$ ) constitute the Favorable Health conditions group (FH).

The UH group had higher relative and absolute NK lymphocytes (22% ( $SD = 7.2$ );  $451/\mu\text{l}$  ( $SD = 140$ )) compared with FH were NK % was (14.2% ( $SD = 5.8$ )) and absolute NK lymphocytes  $275/\mu\text{l}$  ( $SD = 139$ )) ( $p < 0.05$ ); both the relative NK lymphocyte level of > 20% and the absolute NK lymphocyte level of >  $400/\mu\text{l}$  were associated with UH risk (Relative Risk = 7.500,  $p = 0.0329$  and RR = 9.143,  $p = 0.0173$ , respectively).

Accentuatedly high expression of activation marker HLA-DR on CD8<sup>+</sup>T-cytotoxic lymphocytes (>30%) was associated with UH risk (RR = 7.000,  $p = 0.0395$ ).

The comparison of immunophenotype parameters typical for the rejected applicants taken together with the UH researchers and for the accepted applicants not in the UH group permitted us to pinpoint prognostic factors for unfavourable health. To these belong the accentuated elevated levels of NK lymphocytes (relative >21% and absolute > $400/\mu\text{l}$ ), accentuated el-



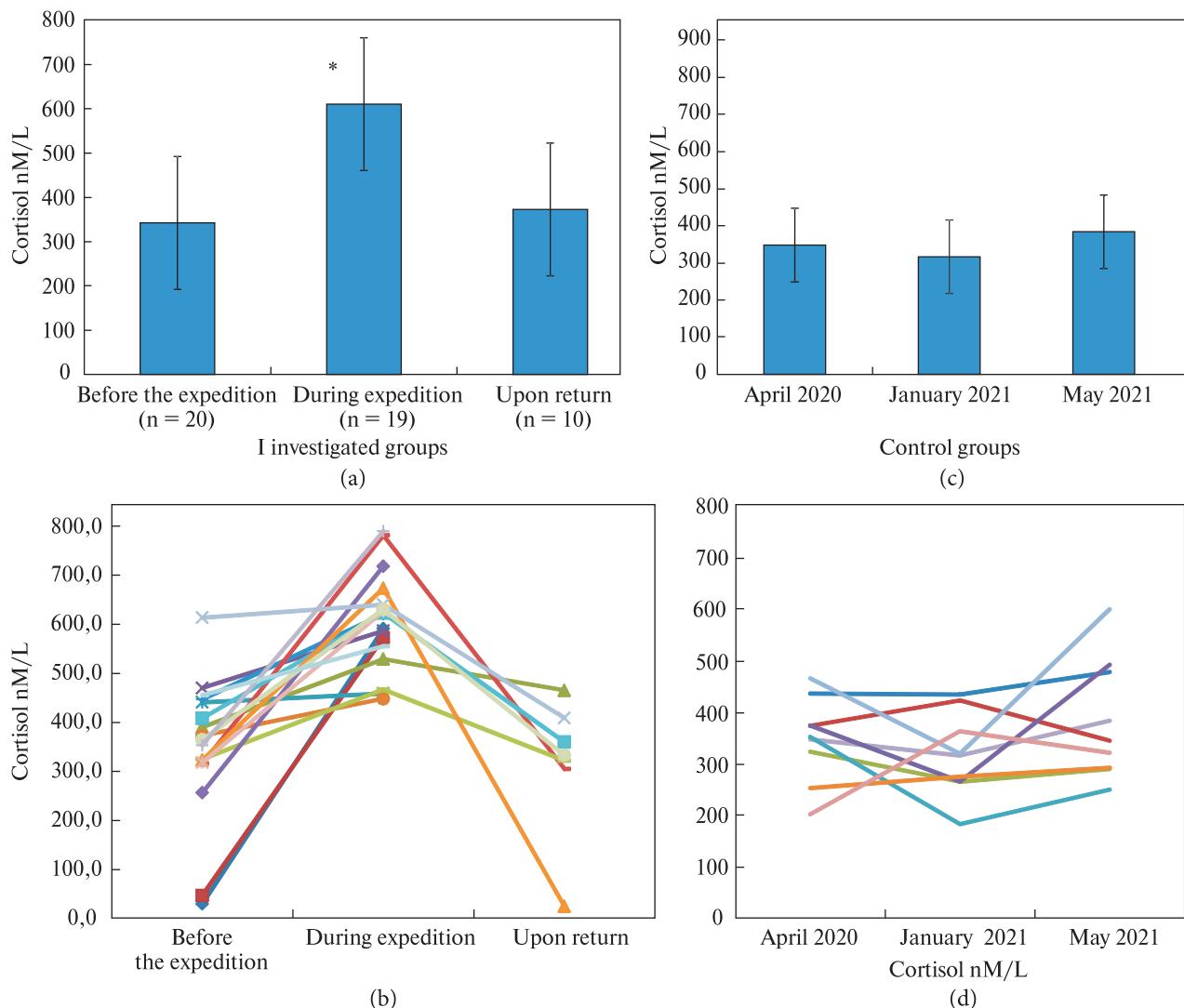
**Figure 1.** Average levels of the NK cells (CD56+CD3-) and cytotoxic activity of the NK cells (E/T 10/1) in the Favorable Health conditions group (no health complaints) and the Unfavourable Health conditions (over three complaints per month or had been rejected from participation on health grounds) (a). Absolute levels of NK lymphocytes (b) and CD8+NK cells (c)  
\*\* p < 0.05 compared with Favorable Health conditions group, \*\*\* statistically significant association with UH risk, p < 0.05

evated levels of expression of HLA-DR on CD8<sup>+</sup>T-cytotoxic lymphocytes (>30%), high levels of cytotoxic activity of the NK lymphocytes (Target lysis>70%, at the effector to target cell ratio of 10/1) (Fig. 1).

### 3.3 Serological assay

Frozen blood sera samples of the UAE participants were collected before, during, and six months after the expedition. Seropositive for *Helicobacter pylori* were 35% samples (7 out of 20), to Epstein-Barr virus (NA antigen) — 90% (18 out of 20), to *T. gondii* — 50% (10 out of 20), herpes simplex virus type I and II —

90% (18 out of 20), to cytomegalovirus — 75% (15 out of 20), antibodies to varicella-zoster were found in 75% samples (15 out of 20). Three UAE participants had trace levels of antibodies to *Mycoplasma pneumoniae*. Detectable levels of antibodies to parasitic agents *Ascaris lumbricoides*, *C. pneumoniae*, *Trichinella*, *Echinococcus* were not found in any UAE participant, while there were some positive cases in the control group. No UAE participant had the marker of acute humoral response to the Epstein-Barr virus (EBV-Antigen). There was found reactivation of the EBV infection for one participant in the control group.



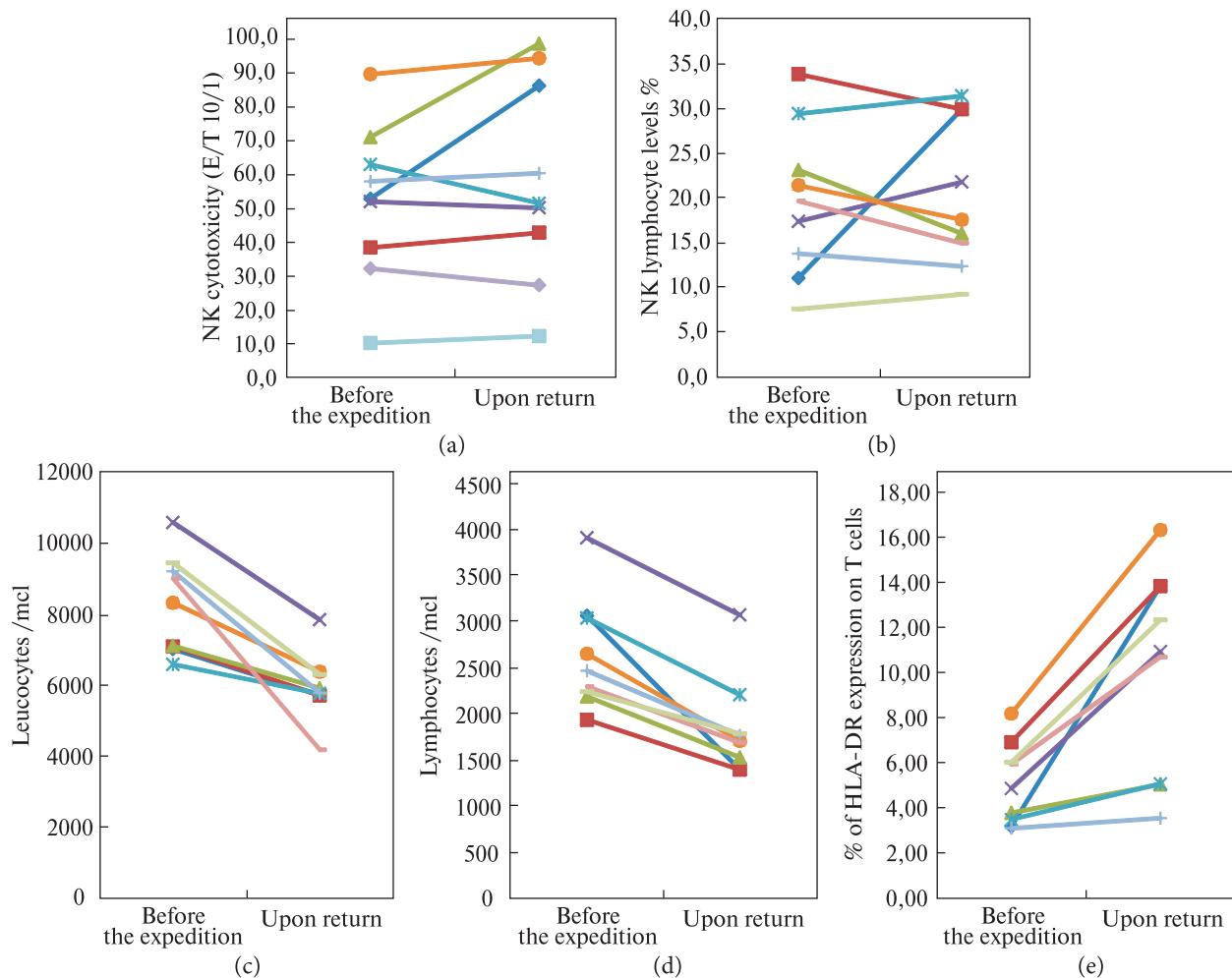
**Figure 2.** Changes in the average cortisol levels in the UAE participants (a) and the control group (c). \* statistically significant difference between the values before the expedition and upon return ( $p < 0.05$ ). Individual changes in cortisol levels among the UAE participants (b) and in the control group (d)

The UAE participants were also tested for cortisol, testosterone, autoimmune disease markers (ssDNA, the rheumatoid factor). Analysis showed that not one tested serological parameter was a reliable prognostic factor for the unfavorable health conditions. Thus, for two UAE participants were found positive levels of antibodies to single-spiral DNA and subpositive levels — to the rheumatoid factor; however, this was in no way associated with the favorability of health dur-

ing the UAE. In general, the serologic report was comparable to the control values.

### 3.4 Comparison of immunophenotypic and serologic parameters before and during the expedition

The results for the expedition participants ( $n = 19$ ) during their stay at the station, compared to samples collected before the expedition, were not significantly elevated in the parameters, which would be evidence



**Figure 3.** The levels of individual immune parameters in the UAE in seasonal-team participants before the expedition and upon their return. Levels of NK cytotoxicity (a) and the relative counts of the NK cells (b) did not statistically differ from the control. Levels of leukocytes (c) and lymphocytes (d) decreased in all UAE participants upon return, and the expression of HLA-DR on T-lymphocytes (e) grew in all UAE participants after their return, unlike the control values

of reactivation of the opportunistic infections in their carriers. The antibody titers also did not change for *H. pylori*, the Epstein-Barr virus, *T. gondii*, herpes simplex virus type I and II, and for the cytomegalovirus.

In all UAE participants who were seronegative before the expedition, there were still no antibodies to the infectious agents. This is evidence of no cross-infection between the participants (or the reactivation of infections).

Changes in the antibodies to ssDNA and rheumatoid factor also did not occur, evidence of no unfavo-

rable dynamics of the autoimmune processes during a UAE.

However, a comparison of cortisol levels demonstrated statistically significant growth in all expedition participants, on average 1.6 times regardless of the initial level ( $p < 0.005$ ).

The testosterone levels also grew on average 1.2 times ( $p < 0.05$ ) (Fig. 2a, b).

For the seasonal team (not winterers), every participant's testosterone and cortisol returned to the initial levels upon return from Antarctica. The pros-

tate-specific antigen total, slightly elevated in two explorers before they went overseas, was normalized at the station and remained so. The control group did not exhibit similar changes in cortisol (Fig. 2c, d).

### 3.5 Immunophenotype parameters of the polar researchers upon return

The seasonal team explorers ( $n = 10$ ) were given an immunophenotype assay six months after coming back; we analyzed the changes compared to the pre-expedition data.

In all participants, there was a statistically significant decrease of the absolute leukocyte counts, on average 1.4 times ( $p < 0.001$ ), with an even decrease in the subpopulations of lymphocytes and granulocytes (Fig. 3).

There was also found a statistically significant level of expression of the activation marker HLA-DR on T-lymphocytes ( $p < 0.05$ ), in particular, due to the increased expression of HLA-DR on CD4<sup>+</sup>T-lymphocytes. Levels of the NK lymphocytes either did not undergo significant changes or recovered to the initial values. In the control group, there was no change in the parameters aligned with the trends in the majority.

## 4 Discussion

The state of an immune system determines the body's ability to resist acute and chronic infections and invasions as well as tumors. However, the common procedure of counting leukocytes, lymphocytes, and lymphocyte subpopulations, cannot yield unambiguous prognostic knowledge (Dons'koi, 2019) except for the primary and secondary immunodeficiencies (Farmer & DeLelys, 2019).

A functionally healthy immune system has a highly individual phenotype formed under many exogenic and endogenic factors. The immune accentuations theory explains that the same immunophenotype can be highly favorable for a particular physiological state and unfavorable for a different one (Dons'koi et al., 2014).

Thus, in our previous studies, we found a number of immunophenotype features (immune accentuations)

to be unfavorable for embryo implantation and successful pregnancy (Dons'koi et al., 2013; Dons'koi et al., 2014). We also described immunophenotypes associated with latent cytomegalovirus infection (Dons'koi et al., 2020b) and common diseases in children (Osypchuk et al., 2016). Therefore, the accentuated immunophenotype may predict a specific unfavorable physiological process with some probability.

An Antarctic expedition lasts for more than a year (for winterers) or less than three months (for seasonal explorers). The participants live in isolated and unfavorable conditions without easy access to all-encompassing medical aid. Selection of the UAE participants based on immunophenotype parameters can be a valuable prognostic tool to prevent worsening of some already established or de-novo development of infectious, autoimmune, and oncological diseases in the expedition participants and complications of adaptation to the new environment.

Based on the immunophenotype analysis, five out of 52 applications for the UAE were declined. According to the immunophenotype parameters of the accepted and the rejected applicants, the elevated count of NK lymphocytes was an unfavorable factor of the overall health. The relative NK lymphocyte count of over 22% is an accentuated parameter and can be used as a prognostic marker of an unfavorable phenotype.

Further analysis confirmed the predictive value of the accentuated levels of NK lymphocytes for unfavorable health. Thus, analyzing the medical reports on the well-being of the UAE participants showed that the UH group (over three complaints of general malaise per month) had elevated NK lymphocytes. The relative NK lymphocytes count of over 20% and the absolute count of over 400/ $\mu$ l were associated with UH risk. Our previous study showed that the same NK accentuation in women was associated with an increased risk of pregnancy failures (Chernyshov et al., 2014).

The number and function of the NK lymphocytes are essential for resistance to viral and oncological diseases (Ogata et al., 2001; Kutukculer et al., 2015) yet, on the other hand, unfavorable for gestation (Chernyshov et al., 2014; 2016), recovery after a heart attack (Mandal & Viswanathan, 2015), or transplant grafting (Sun et al., 2015).

NK cells shape an adaptive immune response by secreting cytokines and chemokines or interacting with other immune cells such as T- and B-cells and dendrite cells (DC). Thus, hyperactivation or dysfunction of the NK cells is connected to the pathogenesis of various inflammatory, infectious and autoimmune diseases (Jiao & Wang, 2016; Liu et al., 2021).

Considering the role of the NK lymphocytes in several processes in the body, such an accentuated level of NK lymphocytes might be necessary for increased control over the infectious or autoimmune process; it can also be evidence of an inefficient balance of KIR receptors and HLA repertoire (Penman et al., 2016). Another piece of evidence for the possible inflammatory process in the UH group is the accentuatedly high level of expression of the HLA-DR activation marker on CD8<sup>+</sup>T-cytotoxic lymphocytes (over 30%).

HLA DR is a marker of activation and inflammation; a number of studies traced the connection of its elevated expression on T-lymphocytes with autoimmune and infectious diseases, in particular, as a marker of an early stage of non-surgical sepsis, etc. (Fernández-Grande et al., 2019; Du et al., 2021).

Thus, a study of immunophenotype parameters at this stage pinpointed the accentuatedly high levels of NK lymphocytes and high levels of expression of HLA-DR on CD8<sup>+</sup>T-cytotoxic lymphocytes as possible immunophenotype risk factors to be used for applications review. However, regardless of whether the immunophenotype was connected to a latent infectious or an autoimmune disease, the serologic data analysis showed that not one of the tested parameters (such as seropositivity to opportunists and markers of immune processes) was a statistically significant predictor for the development of some unfavorable health state. Further research is necessary to find the possible reasons for such an unfavorable immunophenotype.

Participants of an Antarctic expedition are under significant stress because of the frigid and harsh climate amidst a largely unvarying landscape of snow and ice, fully isolated from most of the world and a disrupted natural rhythm of day and night. Studies describe hormonal changes due to shifts in the biological rhythm (Arendt & Middleton, 2018), which

directly influence the regime and quality of sleep (Arendt, 2012; Steinach et al., 2015) and psychosocial behavior (Palinkas et al., 2004). Moreover, these unfavorable states are linked to endocrine and metabolic dysfunctions and negative mood models (Palinkas et al., 2010); some changes in the functions of innate and adaptive immunity (Yadav et al., 2012; Crucian et al., 2018) were found to be connected to reactivation of latent infections (Mehta et al., 2000; Reyes et al., 2017).

The serological data revealed a statistically significant increase in cortisol and testosterone (1.6- and 1.2-fold, respectively) in all expedition participants during their stay at the station. Our data align with previous research describing increasing cortisol levels in men and women during one year of expedition (Strewe et al., 2019). The increase most probably is caused by substantial psychological and physical stress as the parameters recovered six months upon the explorers' return, which is also in agreement with the cited study.

The literature contains numerous descriptions of functional and quantitative changes in the innate and adaptive immunity in expedition participants during their stay at the base, which might favor the reactivation of latent infections (Mehta et al., 2000; Reyes et al., 2017). However, our study showed no statistically significant changes in the titers of antibodies against infectious agents, which could signify activation of an infection. Notably, while no infection reactivation was found, neither were the seronegative participants infected despite sharing their living space with the seropositive ones for a long time. Thus, there were no significant changes in the functioning of their immune systems regardless of the elevated stress hormone. Even a risk factor for systemic autoimmune diseases (antibodies to ssDNA) in two UAE participants did not develop into unfavorable clinical manifestations and had no such dynamics afterwards. By their general health and lack of serologic changes, most UAE participants possessed the overall immunoregulatory potential and immunoresistance to withstand the Antarctic environment.

Results of the immunophenotype study of the participants half a year upon their return revealed a sig-

nificant decrease in the absolute leukocyte counts with proportional changes in every subpopulation of lymphocytes and granulocytes compared to before the expedition. There was also a statistically significant level of expression of the activation marker HLA-DR on T-lymphocytes, but its levels were still within the normal range in all participants. It is possible that lymphocyte downregulation results in increased T-activation upon their return.

Unfortunately, we could not evaluate the immunophenotype parameters during the explorers' stay at the station; according to Strewe et al. (2019), leukocytes' levels significantly rose during the whole expedition period and recovered upon the participants' return. Such a quantitative change is a reaction to physiological and psychological stress and leads to immune cells mobilization in the peripheral bloodstream. However, prolonged action of stress hormones and chronic stress causes a decrease in the circulating leukocytes counts and negatively impacts their ability to migrate (Dhabhar et al., 2013). Taken together, these developments can be a reason for transient leukopenia, which makes the body more susceptible to infections. Although no participant had critically low leukocyte counts (below 3000/ $\mu$ l), it is important to control how this parameter recovers to the baseline levels.

Thus, we did a detailed immunophenotype study of the samples collected from applicants to the Antarctic expedition and the participants who came back. The data, taken in the context of health parameters observed at the Antarctic station, allowed us to find the immunophenotype parameters which can be considered solid predictors of unfavorable health conditions. The data can be used to build a clinical and diagnostic approach to draft the expedition team and decrease the adverse effect on its health; further research is required to formulate practical recommendations for the application review process and to predict developments in the explorers' health.

The study's limitations include the small samples for the immunophenotype analysis, and the low number of women (only three). The female immunophenotype cannot be adequately compared with the male one. Thus, our conclusions hold only for men, and a

larger and more balanced sample is needed to test them for women.

## 5 Conclusions

The immunophenotype of applicants to study in the UAE generally corresponds to the normal reference values of a healthy adult immune system with isolated cases of immunoaccentuated conditions. Most individuals with such conditions were eliminated at the stage of the medical commission. Accentuated high levels of NK lymphocytes, cytotoxic activity of NK cells and the level of CD8aa expression on NK together with high levels of HLA-DR expression on T-cytotoxic cells may be markers of adverse health conditions in UAE. To develop a clinical diagnostic algorithm, these parameters need further study in a larger research group. Conditions of UAE lead to an increase in stress markers, but as a result do not lead to immunosuppressive states with reactivation of latent chronic viral infections and negative dynamics of autoimmune markers. No cases of primary infection were detected. However, the study should be continued with bigger study group. The immunophenotype of UAE participants (upon return) shows signs of depletion and requires further research to prevent deterioration of health, as well as to develop rehabilitation measures.

*Acknowledgments:* We thank the State Institution National Antarctic Scientific Center and the participants of the UAE 25 and 26 for their cooperation and voluntary participation in the study as well as for funding the research (registration number NDKKR 0121U112689).

*Author contributions.* DZ, YaA, NS, LS, BD — laboratory investigation, analysis and manuscript preparation. IK — idea of investigation, moderation of investigation and group formation. ID — collection of clinical data and blood samples in UAE.

*Funding.* Grant from State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine "Study of immunophenotypic features of participants in Antarctic expeditions and the impact of long stay at the Antarctic station on

the immune system and their general health" (H/30-2021).

**Conflict of Interest.** Authors declare no conflict of interest.

## References

- Arendt, J. (2012). Biological Rhythms during Residence in Polar Regions. *Chronobiology International*, 29(4), 379–394. <https://doi.org/10.3109/07420528.2012.668997>
- Arendt, J., & Middleton, B. (2018). Human seasonal and circadian studies in Antarctica (Halley, 75° S). *General and Comparative Endocrinology*, 258, 250–258. <https://doi.org/10.1016/j.ygenc.2017.05.010>
- Chernyshov, V. P., Dons'koi, B. V., Sudoma, I. O., & Goncharova, Y. O. (2014). Favorable immune phenotype predicts successful implantation and pregnancy. *Immunology Letters*, 162(2), 217–221. <https://doi.org/10.1016/j.imlet.2014.10.022>
- Chernyshov, V., Dons'koi, B., Sudoma, I., & Goncharova, Y. (2016). Multiple immune deviations predictive for IVF failure as possible markers for IVIG therapy. *Immunology Letters*, 176, 44–50. <https://doi.org/10.1016/j.imlet.2015.12.010>
- Crucian, B. E., Choukèr, A., Simpson, R. J., Mehta, S., Marshall, G., Smith, S. M., Zwart, S. R., Heer, M., Ponomarev, S., Witmire, A., Frippiat, J. P., Douglas, G. L., Lorenzi, H., Buchheim, J.-I., Makedonas, G., Ginsburg, G. S., Ott, C. M., Pierson, D. L., Krieger, S. S., ... & Sams, C. (2018). Immune system dysregulation during spaceflight: potential countermeasures for deep space exploration missions. *Frontiers in Immunology*, 9, 1437. <https://doi.org/10.3389/fimmu.2018.01437>
- Dhabhar, F. S., Malarkey, W. B., Neri, E., & McEwen, B. S. (2013). Stress-induced redistribution of immune cells — from barracks to boulevards to battlefields: a tale of three hormones — Curt Richter Award Winner. *Psychoneuroendocrinology*, 37(9), 1345–1368. <https://doi.org/10.1016/j.psyneuen.2012.05.008>
- Dons'koi, B. V., Chernyshov, V. P., Sudoma, I. A., Honcharova, J., & Osypchuk, D. V. (2013). Qualitative analysis is preferable under average value comparison in case of bilateral parameter distribution: NK-lymphocyte CD158a expression in patients with reproductive failures. *Likars'ka Sprava*, (1), 86–93. (In Ukrainian)
- Dons'koi, B. V. (2014). Immune factors in reproductions. Immune accentuations theory and prognosis of reproductive success. *Medical aspects of women's health*, 4 (79). (In Ukrainian)
- Dons'koi, B. V., Chernyshov, V. P., Sirenko, V. Iu., Strelko, H. V., & Osypchuk, D. V. (2014). Effect of hypo- and hyper-accentuated NK cell activity on embryo implantation. *Fiziologichnyi Zhurnal*, 60(1), 56–63. <https://doi.org/10.15407/fz.60.01.056> (In Ukrainian)
- Dons'koi, B., Chernyshov, V., Sudoma I., & Goncharova, Y. (2016). Theory of immune accentuation: clinical background. *Obstetrics. Gynecology. Genetics*, 2(2). (In Ukrainian)
- Dons'koi, B. V. (2019). Numbers of natural killers lymphocytes do not determine their cytotoxicity. *Biologichni studii*, 13(2), 11–20. <https://doi.org/10.30970/sbi.1302.599>
- Dons'koi, B. V., Chernyshov, V. P., Osypchuk, D. V., Sudoma, I., Khazhylenko, K. G., Strelko, G. V., & Sirenko, W. J. (2020a). Natural killer frequency determines natural killer cytotoxicity directly in accentuated zones and indirectly in "moderate-to-normal frequency" segment. *Central European Journal of Immunology*, 45(3), 315–324. <https://doi.org/10.5114/ceji.2020.101263>
- Dons'koi, B. V., Tutchenko, T. M., Chernyshov, V. P., & Stepaniuk, K. S. (2020b). HCMV seropositivity is associated with specific proinflammatory immune phenotype in women with implantation failure. *Immunology Letters*, 217, 84–90. <https://doi.org/10.1016/j.imlet.2019.11.008>
- Dons'koi, B. V., Osypchuk, D. V., Chernyshov, V. P., & Khazhylenko, K. G. (2021). Expression of natural cytotoxicity receptor NKp46 on peripheral blood natural killer cells in women with a history of recurrent implantation failures. *Journal of Obstetrics and Gynaecology Research*, 47(3), 1009–1015. <https://doi.org/10.1111/jog.14631>
- Du, J., Wei, L., Li, G., Hua, M., Sun Y., Wang, D., Han, K., Yan, Y., Song, C., Song, R., Zhang, H., Han, J., Liu, J., & Kong, Y. (2021). Persistent high percentage of HLA-DR+ CD38high CD8+ T cells associated with immune disorder and disease severity of COVID-19. *Frontiers in Immunology*, 12, 3455. <https://doi.org/10.3389/fimmu.2021.735125>
- Farmer, J. R., & DeLelys, M. (2019). Flow cytometry as a diagnostic tool in primary and secondary immune deficiencies. *Clinics in Laboratory Medicine*, 39(4), 591–607. <https://doi.org/10.1016/j.cll.2019.07.007>
- Fernández-Grandea, E., Cabrera, C. M., González, B., Varela, C., & Urra, J. M. (2019). Enhanced HLA-DR expression on T-lymphocytes from patients in early stages of non-surgical sepsis. *Medicina Clinica*, 152(9), 346–49. <https://doi.org/10.1016/j.medcli.2018.07.007>
- InStat version 3.0 for Windows Graph Pad Software Inc., San Diego, CA, (USA). <https://www.graphpad.com/support/instat-3-updates/>
- Jiao, G., & Wang, B. (2016). NK Cell Subtypes as regulators of autoimmune liver disease. *Gastroenterology Research and Practice*, 2016, 6903496. <https://doi.org/10.1155/2016/6903496>
- Kutukuler, N., Azarsiz, E., Karaca, N. E., Ulusoy, E., Koturoglu, G., & Aksu, G. (2015). A clinical and laboratory approach to the evaluation of innate immunity in pediatric CVID patients. *Frontiers in Immunology*, 6, 145. <https://doi.org/10.3389/fimmu.2015.00145>
- Liu, M., Liang, S., & Zhang, C. (2021). NK Cells in Autoimmune Diseases: Protective or Pathogenic? *Frontiers in Immunology*, 12, 624687. <https://doi.org/10.3389/fimmu.2021.624687>
- Mandal, A., & Viswanathan, C. (2015). Natural killer cells: In health and disease. *Hematology / Oncology Stem Cell Therapy*, 8(2), 47–55. <https://doi.org/10.1016/j.hemonc.2014.11.006>

- Mehta, S. K., Pierson, D. L., Cooley, H., Dubow, R., & Lugg, D. (2000). Epstein-Barr virus reactivation associated with diminished cell-mediated immunity in Antarctic expeditioners. *Journal of Medical Virology*, 61(2), 235–240.
- Moiseyenko, Y. V., Sukhorukov, V. I., Pyshnov, G. Yu., Mankovska, I. M., Rozova, K. V., Miroshnychenko, O. A., Kovalevska, O. E., Madjar, S-A. Y., Bubnov, R. V., Gorbach, A. O., Danylenko, K. M., & Moiseyenko, O. I. (2016). Antarctica challenges the new horizons in predictive, preventive, personalized medicine: preliminary results and attractive hypotheses for multi-disciplinary prospective studies in the Ukrainian ‘Akademik Vernadsky’ Station. *EPMA Journal*, 7, 11. <https://doi.org/10.1186/s13167-016-0060-8>
- Ogata, K., An, E., Shioi, Y., Nakamura, K., Luo, S., Yokose, N., Minami, S., & Dan, K. (2001). Association between natural killer cell activity and infection in immunologically normal elderly people. *Clinical and Experimental Immunology*, 124(3), 392–397. <https://doi.org/10.1046/j.1365-2249.2001.01571.x>
- Osypchuk, D. V., Chernyshov, V. P., Chernysheva, L. I., Kisiel, N. P., Dons'koi, B. V., Matvienko, I. M., Rodionov, V. P., & Makovs'ka, Yu. U. (2016). Reduced response of natural killer lymphocytes to toll-like receptor 3 stimulation in children with recurrent infections. *Fiziologichnyi Zhurnal*, 62(4), 12–17. <https://doi.org/10.15407/fz62.04.012> (In Ukrainian)
- Palinkas, L. A., Glogower, F., Dembert, M., Hansen, K., & Smullen, R. (2004). Incidence of psychiatric disorders after extended residence in Antarctica. *International Journal of Circumpolar Health*, 63(2), 157–168. <https://doi.org/10.3402/ijch.v63i2.17702>
- Palinkas, L. A., Reedy, K. R., Shepanek, M., Reeves, D., Case, H. S., Do, N. V., & Reed, H. L. (2010). A randomized placebo-controlled clinical trial of the effectiveness of thyroxine and triiodothyronine and short-term exposure to bright light in prevention of decrements in cognitive performance and mood during prolonged Antarctic residence. *Clinical Endocrinology*, 172(4), 543–550. <https://doi.org/10.1111/j.1365-2265.2009.03669.x>
- Penman, B. S., Moffett, A., Chazara, O., Gupta, S., & Parham, P. (2016). Reproduction, infection and killer-cell immunoglobulin-like receptor haplotype evolution. *Immunogenetics*, 68(10), 755–764. <https://doi.org/10.1007/s00251-016-0935-9>
- Reyes, D., Brinley, A., Blue, R., Gruschkus, S., Allen, A., & Parazynski, S. (2017) Clinical herpes zoster in Antarctica as a model for spaceflight. *Aerospace Medicine and Human Performance*, 88(8), 784–788. <https://doi.org/10.3357/AMHP.4450.2017>
- Steinach, M., Kohlberg, E., Maggioni, M. A., Mendt, S., Opatz, O., Stahn, A., Tiedemann, J., & Gung, H.-C. (2015). Changes of 25-OH-Vitamin D during Overwintering at the German Antarctic Stations Neumayer II and III. *PLoS ONE*, 10(12), e0144130. <https://doi.org/10.1371/journal.pone.0144130>
- Strewe, C., Moser, D., Buchheim, J.-I., Gunga, H.-C., Stahn, A., Crucian, B. E., Fiedel, B., Bauer, H., Gössmann-Lang, P., Thieme, D., Kohlberg, E., Choukèr, A., & Feuerer, M. (2019). Sex differences in stress and immune responses during confinement in Antarctica. *Biology of Sex Differences*, 10(1), 20. <https://doi.org/10.1186/s13293-019-0231-0>
- Sun, C., Sun, H.-Y., Xiao, W.-H., Zhang, C., & Tian, Z.-C. (2015). Natural killer cell dysfunction in hepatocellular carcinoma and NK cell-based immunotherapy. *Acta Pharmacologica Sinica*, 36(10), 1191–1199. <https://doi.org/10.1038/aps.2015.41>
- Yadav, A. P., Mishra, K. P., Ganju, L., & Singh, S. B. (2012). Wintering in Antarctica: impact on immune response of Indian expeditioners. *Neuroimmunomodulation*, 19(6), 327–333. <https://doi.org/10.1159/000339512>

Received: 30 October 2021

Accepted: 16 December 2021

Д. Забара<sup>1</sup>, І. Козерецька<sup>2</sup>, І. Дейнеко<sup>2</sup>, Я. Аношко<sup>1</sup>, Н. Шаповаленко<sup>1</sup>, Л. Стамболі<sup>1</sup>, Б. Донської<sup>1, \*</sup>

<sup>1</sup> Інститут педіатрії, акушерства і гінекології імені академіка О. М. Лук'янової НАМН України, м. Київ, 04050, Україна

<sup>2</sup> Державна установа Національний антарктичний науковий центр МОН України, м. Київ, 01601, Україна

\* Автор для кореспонденції: boris\_donskoy@ukr.net

#### Імунні фактори та стан здоров'я учасників антарктичних експедицій

**Реферат.** Імунна система відіграє ключову роль у підтриманні гомеостазу в організмі людини, однак її індивідуальна надунікальність ускладнює можливість діагностичного прогнозування розвитку несприятливих фізіологічних станів та хвороб. Дослідження імунофенотипових особливостей учасників Українських антарктичних експедицій та аналіз цих характеристик до, під час та після зимівлі може дозволити з'ясувати можливу роль специфічних імунних акцентуацій у розвитку певних фізіологічних станів. Визначення НК цитотоксичності та імунофенотипу у 52 претендентів на участь у експедиціях та у 9 учасників, які повернулися з неї, проведено методом проточного цитофлюориметрії.

Зразки сироваток крові взяті до, під час та після експедицій аналізувались також на присутність гормонів, анти-інфекційних, антипаразитарних та автоімунних антитіл. Висока абсолютна та відносна кількість НК лімфоцитів, висока НК цитотоксичність та висока експресія HLA-DR на CD3+CD8+ достовірно асоціювалися із несприятливим станом здоров'я учасників під час експедицій. Протягом перебування в експедиціях різко зростали рівні кортизолу, проте вони відновлювались після повернення. У переважної більшості полярників не було виявлено серйозних ускладнень здоров'я під час експедицій. А також, не виявлено реактивації вірусних інфекцій та первинного зараження, як і клінічних автоімунних. Встановлено достовірне зниження лейкоцитів і лімфоцитів та зростання експресії маркерів активашії (HLA-DR) на Т клітинах у учасників експедицій після повернення. Досліжені фактори ризику несприятливого стану є перспективними для вивчення імунофенотипу полярників, проте потребують детального подальшого дослідження на більших групах. Умови перебування в експедиції призводять до зростання стресу, проте цей факт не тягне за собою клінічних проявів та ознак імуносупресії. Після повернення з експедиції у полярників спостерігаються наслідки тривалого стресу, які негативно впливають на лейкопоез навіть через 6 місяців по поверненню, що варто враховувати для відбору претендентів на участь у експедиціях.

**Ключові слова:** антарктична експедиція, імунні акцентуації, НК лімфоцити, НК цитотоксичність, прогностичні фактори