



Research article

IMBALANCE IN LYMPHOCYTE COMPOSITION AND CYTOKINE PROFILE AS A RISK FACTOR OF VIBRATION DISEASE

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To provide consistent functioning of the immune and nervous systems, both in normal conditions and in case of any pathology, is among the most significant functions performed by the cytokine system. It seems important to examine mechanisms underlying the well-coordinated working of the immune system since such studies can give grounds for developing certain criteria applied to assess risks of developing vibration disease (VD) and making prognosis for it. Our research goal was to identify peculiarities related to the balance in the phenotypic composition of lymphocytes and cytokines as risk factors of developing vibration disease.

We estimated the phenotypic structure of lymphocytes by indirect immunofluorescence using monoclonal antibodies to CD3⁺, CD4⁺, CD8⁺, CD16⁺, CD20⁺, CD25⁺, and CD95⁺ molecules. IL-2, IL-4, IL-8, and INF- γ cytokines were determined in blood serum with the solid-phase ELISA method.

We analyzed cytokine contents in patients with VD and established a statistically significant growth in levels of IL-8 and INF- γ and a decrease in IL-2 contents against the reference group. We also found certain differences in the correlations between lymphocytes and cytokines in patients with VD and healthy people. Thus, in patients with VD, there was a positive correlation between the absolute number of CD3⁺-lymphocytes and the level of the immune-stimulating IL-2 and a positive correlation between the quantity of CD4⁺-lymphocytes and IL-4 concentration. At the same time, having examined healthy people from the reference group, we detected a negative correlation between INF γ and the absolute quantity of CD3⁺ and CD95⁺-lymphocytes. Therefore, the risk of developing and progressing vibration disease is to a certain extent associated with its pathogenetic peculiarities based, among other things, on complex functional relationships between lymphocytic phenotypes and pro-anti-inflammatory cytokines. In future this will allow substantiating new biomarkers in the additional VD diagnostics.

Key words: vibration disease, workers, lymphocytes, cytokines, immune response, risk of developing pathology, inflammation.

Vibration disease (VD) is a widely-spread occupational pathology which occurs in working population and is medically and socially significant owing to the risk of social and economic losses caused by it. Working people are exposed to a set of harmful occupational factors; therefore, it is vital to perform monitoring over their health and implement timely activities aimed at preventing development of occupational and work-related diseases. All this is extremely important for achieving longer employment periods and life expectancy [1–3].

Neural and vascular disorders are the key elements in the VD clinical picture and peripheral circulatory disorders play a special role among them [4, 5]. Hypoxia and micro-circulatory disorders activate cells which then start to release active substances, notably cytokines. Cytokines are molecules responsible for a type and duration of an immune response. They are synthesized immediately as a response to tissue lesions. Cytokines facilitate interactions between the immune system and organs and tissues in the body at the molecular

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level. The same mechanism mediates regulation of an immune response¹. In case there are no pathological processes, cytokines are not detected in blood under the primary immune response. Their occurrence in blood indicates there are local functional disorders in the cytokine system which are the most frequent in case there are intense inflammatory reactions and create risks of a disease becoming more severe [6, 7]. Interleukins and interferons accumulated in an inflammation focus and produce their effects on most cells participating in this reaction. Such affected cells include granulocytes, macrophages, T- and B-lymphocytes as well as fibroblasts, endotheliocytes, and epithelial cells [8–10].

Examining mechanisms which underlie the well-coordinated working of the immune and nervous system can give grounds for developing criteria applied to assess risks of developing VD.

Our research goal was to estimate changes in the balance and ratio between the phenotypic structure of lymphocytes and cytokines as risk factors causing developing vibration disease.

Materials and methods. 54 men with diagnosed VD took part in our study. The disease was induced by local vibration or combined exposure to local and overall vibration. VD was diagnosed by occupational pathologists at the clinic of the East-Siberian Institute of Medical and Ecological Research according to the International Classification of Diseases, version ICD-10. Patients' average age was 51.45 ± 0.87 years; average working experience, 25.5 ± 2.13 years. The most common occupations in this (test) group were riveters, mechanic assemblers, dozer drivers, timber lorry drivers, crane operators, loader drivers, and tractor drivers. The reference group was compa-

table as per age and sex (51.06 ± 0.95 years, males) and included 27 practically healthy people who were not occupationally exposed to vibration. We used the following criteria to include patients with VD into our research: VD diagnosis was verified, they gave their written informed consent to take part in the research, and their working experience under exposure to vibration exceeded 10 years.

We examined peripheral blood taken in the morning on an empty stomach from the ulnar vein. The blood was taken into a vial with heparin (to extract leukocyte suspension) and a vial with a coagulation activator (to obtain serum). Lymphocytes and their populations were quantified in vitro by indirect immunofluorescence using monoclonal antibodies specific to differentiation antigens ($CD3^+$, $CD4^+$, $CD8^+$, $CD9^+$, $CD16^+$, $CD20^+$, $CD21^+$, $CD23^+$, $CD25^+$, $CD95^+$) (“Klonospektr”, Moscow). We calculated a relative quantity of CD-positive cells in the total lymphocytes numbers using “Olimpus CX-41” microscope (Japan). IL-2, IL-4, IL-8, $INF\gamma$ cytokines were determined in blood serum with the solid-phase ELISA method using relevant test-systems (“Vector-Best”, Novosibirsk). All the data were statistically analyzed with Statistica 6.0 applied software package (StatSoft, USA). We applied non-parametric Mann-Whitney U test to compare different samplings. The samplings were described with median and interquartile range (Me (Q25–Q75)). We applied Spearman's correlation analysis to establish correlations between indicators. The critical level of significance (p) was taken at 0.05 when statistical hypotheses were tested. Our research is in full conformity with the ethical standards stipulated by the Helsinki Declaration (2000)² and the Order No. 200n issued by the RF Public Healthcare Ministry on April 01, 2016³.

¹ Paltsev M.A., Kvetnoi M.I. Rukovodstvo po neuroimmunoendokrinologii [The guide on neural and immune endocrinology]. Moscow, Meditsina, 2008, 512 p. (in Russian).

² Declaration of Helsinki. Ethical principles for medical research involving human subjects. *The WMA, Inc.* Available at: <https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2008.pdf> (July 18, 2021).

³ Ob utverzhdenii pravil nadlezhashchei klinicheskoi praktiki: Prikaz Ministerstva zdravookhraneniya RF ot 1 aprelya 2016 g. № 200n [On Approval of the rules for good clinical practice: The Order by the RF Public Healthcare Ministry issued on April 01, 2016 No. 200n]. *GARANT: the information and legal support.* Available at: <https://base.garant.ru/71473446/> (July 18, 2021) (in Russian).

Results and discussion. In our previous study we analyzed the sub-population structure of lymphocytes in the same patients with VD. The study established changes in the immune response, notably growing quantities of CD3⁺, CD4⁺, CD8⁺-lymphocytes and declining levels of CD20⁺, CD25⁺-lymphocytes in the patients with VD; the changes were caused by exposure to local vibration. We also revealed growing quantities of CD3⁺, CD4⁺, CD8⁺, CD16⁺, CD20⁺, CD25⁺, CD95⁺-lymphocytes in the patients with VD caused by combined exposure to local and overall vibration, All this indicated that the immune system was apparently activated owing to this occupational pathology [11]. Bearing in mind that lymphocytes sub-populations produced cytokines [6, 12], we analyzed levels of those cytokines which reflected how active lymphocytic cells were.

Our analysis of cytokine contents in patients with VD (Table 1) established a statistically significant increase in contents of pro-inflammatory cytokines IL-8 and INFγ in the test group against the reference one ($p = 0.007$ and $p = 0.016$ respectively). As for the contents of regulatory IL-2, we detected a decline in it in the test group in contrast to the reference group ($p = 0.0003$).

Interleukins and interferons in Table 1 create a whole network of interactions where they have crossing functions and can act in synergy with other cytokines and lymphocytes [13]. Therefore the next stage in our research involved analyzing possible correlations between the phenotypic structure of lymphocytes and cytokines levels in the examined people. We established that those correlations in patients with VD were different from those detected for healthy people. This can be due to risks of more severe vibration disease. Significantly, all those correlations were positive.

Thus, the patients with VD had a positive correlation between the absolute quantity of the overall CD3⁺ lymphocytes population ($r = 0.46$, $p = 0.029$) and the level of immune-stimulating IL-2 (Table 2). Also there was a positive correlation between the quantity of T-helpers CD4⁺ and IL-4 contents in the examined patients with VD ($r = 0.39$, $p = 0.027$).

On the contrary, our examination of correlations between cellular immunity parameters and cytokines concentrations in the healthy people from the reference group established negative correlations between INFγ and absolute quantities of CD3⁺ ($r = -0.68$, $p = 0.029$)

Table 1

Cytokine contents in blood serum in patients with vibration disease, *Me* (Q25–Q75)

| Indicators, pg/ml | Patients with VD, $n = 54$ | Reference group, $n = 27$ | p |
|-------------------|----------------------------|---------------------------|--------|
| IL-2 | 4.16 (1.97–7.55) | 6.01 (5.68–8.32) | 0.0003 |
| IL-4 | 0.01 (0.01–2.71) | 0.01 (0.01–0.32) | |
| IL-8 | 15.84 (0.94–37.38) | 10.3 (0.01–14.6) | 0.007 |
| INFγ | 1.63 (0.01–38.96) | 0.23 (0.01–9.49) | 0.016 |

Note: p shows the level of statistical significance for differences between the groups.

Table 2

Results produced by analyzing correlations between the quantity of lymphocytes and cytokines in the patients with VD and the reference group

| Correlation pairs lymphocytes – cytokines | Patients with VD | Reference group |
|---|------------------|-----------------|
| CD3 ⁺ – IL-2 | 0.459 | – |
| CD4 ⁺ – IL-4 | 0.389 | – |
| CD3 ⁺ – INFγ | – | -0.681 |
| CD95 ⁺ – INFγ | – | -0.633 |

Note: the given values of r (Spearman’s rank correlation coefficient) correspond to $p < 0.05$; dashes mean there are no statistically significant correlations, $p > 0.05$.

and CD95⁺-lymphocytes ($r = -0.68, p = 0.047$) (Table 2). Given that there were no correlations between IFN γ and lymphocytes in the patients with VD, we can conclude that this interferon was completely excluded from the immune regulation in their bodies.

The basic function performed by the cytokines system is to provide well-coordinated working of the immune and nervous systems as a response to stress [14]. Our analysis of cytokines contents in the patients with VD allowed establishing hyperactivity of pro-inflammatory cytokines IL-8 and INF γ . It should be noted that IL-8 has a specific property which is its ability to regulate fast secretion of IL-8 protein product due to its release from neutrophilic granulocytes (they contain preformed IL-8) migrating after cells activation. In its turn, following the positive feedback principle, a drastic IL-8 ejection can induce its synthesis by the same cells and activate its functional properties and block up apoptosis of granulocytes [15–17]. We can assume that this mechanism for self-regulating the production of the aforementioned interleukin can underlie chronic inflammation supporting its acute phase in tissues in patients with VD. Undoubtedly, this creates a risk that the disease will last longer and be more severe. A significant growth in INF γ level is also related to the process becoming chronic when its production by type I T-helpers is enhanced [18]. This can explain a situation when IL-4 was determined within its reference values in patients with VD. Elevated concentrations of pro-inflammatory cytokines which we detected in such patients indicate that the inflammation process is highly active in them and has already become systemic. However, the examined patients had reduced IL-2 levels. We should note that they had lower contents of CD25⁺ lymphocytes which expressed the receptor to IL-2 (IL-2R). Reduced IL-2 contents in blood don't properly support IL-2 binding to IL-2R thus disrupting T-helpers proliferation¹. Obviously, prolonged VD caused by exposure to vibration results in depleting secretion of the aforementioned cytokine and the latter in its turn leads to apparent disorders in immune responses.

We performed the correlation analysis with its focus on correlations between lymphocytes sub-population and cytokines in the examined people. The analysis established a positive correlation between the absolute quantity of CD3⁺-lymphocytes and the level of immune-stimulating IL-2. We can assume that lower IL-2 concentration in blood of the patients with VD occurred against an imbalance between the structure of lymphocytes population and sub-population which developed due to immune cells differentiating and ripening improperly as well as their migration into an inflammation focus. The number of CD4⁺-lymphocytes in the examined patients with VD was associated with the level of IL-4 which was mainly produced by Th2-lymphocytes. Besides, mast cells, basophils and D-lymphocytes also participate in synthesizing this interleukin. IL-4 induces T-lymphocytes differentiating into Th-2 cells thus inhibiting Th-1 lymphocytes development, activates macrophages, and evokes proliferation of activated mature T-cells [6, 19]. All this becomes obvious due to detecting positive correlations between concentrations of this cytokine and the quantity of T-helpers (CD4⁺). Later this can be used in developing informative criteria showing severity of the pathological process. People in the reference group had negative correlations between the absolute quantity of CD3⁺ and CD95⁺-lymphocytes and IFN γ . This is easily explained since CD3⁺-cells produce IFN γ which simultaneously activates CD3⁺-lymphocytes whereas CD95⁺-cells promote its apoptosis [20, 21].

A change in the nature of interrelations between lymphocytes sub-populations and cytokines contents can be due to a change in cytokine producers as well as a less sensitive cellular response to cytokines [20, 22]. This creates elevated risks of developing immune pathologic reactions playing an important role in VD pathogenesis.

Conclusions. Therefore, risks of developing and progressing vibration disease are to a certain extent associated with its pathogenetic peculiarities. These peculiarities are related to, among other things, an imbalance of lympho-

cytic phenotypes and pro-anti-inflammatory cytokines characterized with the direct correlation between CD3⁺ and IL-2, CD4⁺ and IL-4 against activated pro-inflammatory cytokines IL-8 and INF γ and reduced levels of IL-2. Our research data provide evidence that there are complex functional interrelations in the immune system. These data can be used in future research for substantiating new biomarkers in the additional VD diagnostics. To preserve workers' health, it seems advisable to draw on

experience in examining the immune status during periodical medical examinations aimed at minimizing risks of developing occupational diseases.

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References

1. Popova A.Yu. Working conditions and occupational morbidity in the Russian Federation. *Meditsina truda i ekologiya cheloveka*, 2015, no. 3, pp. 7–13 (in Russian).
2. Onishchenko G.G. Actual problems of hygiene science and practice in the preservation of public health. *Gigiya i sanitariya*, 2015, vol. 94, no. 3, pp. 5–9 (in Russian).
3. Dolgikh O.V., Starkova K.G., Krivtsov A.V., Bubnova O.A. Variability of immunoregulatory and genetic markers in conditions of the combined effects of industrial environmental factors. *Gigiya i sanitariya*, 2016, vol. 95, no. 1, pp. 45–48. DOI: 10.18821/0016-9900-2016-95-1-45-48 (in Russian).
4. Zheglava A.V., Fedina I.N. Modern approaches to carrying out preventive examinations of workers of vibration-dangerous occupations. *Gigiya i sanitariya*, 2016, vol. 95, no. 11, pp. 1048–1064. DOI: 10.18821/0016-9900-2016-95-11-1048-1051 (in Russian).
5. Saarkoppel' L.M., Kir'yakov V.A., Oshkoderov O.A. Role of contemporary biomarkers in vibration disease diagnosis. *Meditsina truda i promyshlennaya ekologiya*, 2017, no. 2, pp. 6–10 (in Russian).
6. Simbirtsev A.S. Tsitokiny v patogeneze i lechenii zabolevaniy cheloveka [Cytokines in the pathogenesis and treatment of human diseases]. St. Petersburg, Foliant, 2018, 512 p. (in Russian).
7. Maslyanskiy A.L., Penin I.N., Cheshuina M.D., Trichina I.N., Novikova A.N., Kolesova E.P., Lazareva N.M., Mazing A.V. [et al.]. Common consistent patterns of the cytokine and chemokine production in patients with diffuse connective tissue diseases, inflammatory arthropathies and atherosclerosis]. *Tsitokiny i vospalenie*, 2014, vol. 13, no. 3, pp. 9–21 (in Russian).
8. Aksu I., Topcu A., Camsari U.M., Acikgoz O. Effect of acute and chronic exercise on oxidant-antioxidant equilibrium in rat hippocampus, prefrontal cortex and striatum. *Neurosci. Lett.*, 2009, vol. 452, no. 3, pp. 281–285. DOI: 10.1016/j.neulet.2008.09.029
9. Savchenko A.A., Zdzitovetsky D.E., Borisov A.G., Luzan N.A. Peculiarities of the cellular and humoral immunity status and the levels of concentrations of cytokines in patients with extensive purulent peritonitis. *Sibirskoe meditsinskoe obozrenie*, 2013, no. 1, pp. 24–28. DOI: 10.20333/25000136-2013-1-24-28 (in Russian).
10. Petrov R.V., Khaitov R.M., Chereshnev V.A. Physiology of the immune system: cellular and molecular-biological mechanisms. *Vestnik Rossiiskogo fonda fundamental'nyh issledovaniy*, 2017, no. S1, pp. 96–119. DOI: 10.22204/2410-4639-2017-094-02S-96-119 (in Russian).
11. Kurchevenko S.I., Bodienkova G.M., Lakhman O.L. Comparative characteristics of the subpopulation composition of lymphocytes and heat shock protein in patients with vibration disease. *Rossiiskii immunologicheskii zhurnal*, 2019, vol. 13, no. 2–2, pp. 846–848. DOI: 10.31857/S102872210006677-9 (in Russian).
12. Zakharenkov V.V., Kazitskaya A.S., Mikhailova N.N., Romanenko D.V., Zhdanova N.N., Zhukova A.G. Influence of occupational hazards on human immune state. *Meditsina truda i promyshlennaya ekologiya*, 2017, no. 12, pp. 19–23 (in Russian).
13. Koval'chuk L.V., Gankovskaya L.V., Khoreva M.V., Sokolova E.V. Sistema tsitokinov, komplementa i sovremennye metody immunnogo analiza [The system of cytokines, complement and modern methods of immune analysis]. Moscow, RGMU Publ., 2001, 158 p. (in Russian).

14. Chernii V.I., Nesterenko A.N. Narusheniya immuniteta pri kriticheskikh sostoyaniyakh: osobenosti diagnostiki [Immune disorders in critical states: diagnostic features]. *Vnutrennyaya meditsina*, 2007, vol. 3, no. 3, pp. 11–14 (in Russian).
15. Serebrennikova S.N., Seminsky I.Zh. The role of cytokines in the inflammatory process (Part 1). *Sibirskii meditsinskii zhurnal (Irkutsk)*, 2008, vol. 81, no. 6, pp. 5–8 (in Russian).
16. Voronina M.S., Shilkina N.P., Vinogradov A.A., Butusova S.V. Interleukins 4, 6, 8 in the pathogenesis of rheumatoid arthritis and its complications. *Tsitokiny i vospalenie*, 2014, vol. 13, no. 1, pp. 5–10 (in Russian).
17. Ketlinskii S.A., Simbirtsev A.S. Tsitokiny [Cytokines]. St. Petersburg, Foliant, 2008, 552 p. (in Russian).
18. Eivazi S., Bagheri S., Hashemzadeh M.S., Ghalavand M., Qamsari E.S., Dorostkar R., Yasemi M. Development of T follicular helper cells and their role in disease and immune system. *Biomed. Pharmacother.*, 2016, vol. 84, pp. 1668–1678. DOI: 10.1016/j.biopha.2016.10.083
19. Jiménez E., Sacedón R., Vicente A., Hernández-López C., Zapata A.G., Varas A. Rat peripheral CD4⁺CD8⁺ T-lymphocytes are partially immunocompetent thymus-derived cells that undergo post-thymic maturation to become functionally mature CD4⁺ T lymphocytes. *J. Immunol.*, 2002, vol. 168, no. 10, pp. 5005–5013. DOI: 10.4049/jimmunol.168.10.5000
20. Singh R., Kumar A., Creery W.D., Ruben M., Giulivi A., Diaz-Mitoma F. Dysregulated expression of IFN γ and IL-10 and impaired IFN γ mediated responses at different disease stages in patients with genital herpes simplex virus-2 infection. *Clin. Exp. Immunol.*, 2003, vol. 133, no. 1, pp. 97–107. DOI: 10.1046/j.1365-2249.2003.02183.x
21. Drannik G.N. Klinicheskaya immunologiya i allergologiya [Clinical immunology and allergology]. Moscow, MIA, 2003, 604 p. (in Russian).
22. Cousins W.S., Espinosa-Heidmann D.G., Miller D.M., Pereira-Simon S., Hernandez E.P., Chien H., Meier-Jewett C., Dix R.D. Macrophage activation associated with chronic murine cytomegalovirus infection results in more severe experimental choroidal neovascularisation. *PLoS Pathog.*, 2012, vol. 8, no. 4, pp. e1002671. DOI: 10.1371/journal.ppat.1002671

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