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# IMMUNOLOGICAL CRITERIA OF THE COURSE OF NON-HODGKIN'S LYMPHOMAS

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**SUMMARY**

The purpose of this study was to study the main cellular and humoral criteria for the course of non-Hodgkin's lymphomas. An analysis of the results revealed significant changes in the cellular immunity that manifested themselves by suppressing the expression of CD3, CD3, CD4, IRI, against this background, an increase in the expression of CD3 CD8, CD16 and CD20 B cells, as well as increased expression of activation molecular markers of lymphocytes CD38, CD95. A deep T-cell immunodeficiency was detected against the background of pathological activation of lymphocytes, which is clinically often reflected by frequent relapses, an unfavorable course of the disease and the results of therapy. Serum concentrations of the main immunoglobulins IgG, IgA, IgM in NHL were analyzed. The highest serum content of IgG and IgA was revealed in the groups of patients with virus carrier. It has been established that one of the most important humoral markers of immunity is the circulating immune complexes (CICs). It has been established that one of the most important biological functions of immunoglobulins is antigen binding and the formation of CIC. A high level of CIC in NHL may be due not only to the activation of the immune response, but also to the suppression of the mechanisms of their elimination. Therefore, based on the results obtained, with NHL there is a pronounced imbalance of humoral immunity. CIC of large and small sizes are also increased, however, the highest increase in CIC was observed in groups of patients before chemotherapy with virus-bearing. An increase in the main immunoglobulins indicates the presence of humoral imbalance, and an increase in the CIC indicates the presence of intoxication of the body either due to the decay of the tumor cells and infected cells themselves, which almost always indicates the progression of the pathological process.

**KEYWORDS:** Immune system, adaptive immunity, polychemotherapy, immunocompetent cells, lymphomas, non-Hodgkin lymphoma.

An analysis of modern literature shows that despite a sufficient amount of clinical material regarding virus-associated NHL, many issues remain unresolved.

Non-Hodgkin's lymphomas (NHL) - a heterogeneous group of tumors of the blood system, characterized by various clinical course, localization, morphological, immunological; cytogenetic features, and results of therapy.[2,6] In recent years, the incidence of NHL has increased.[1,2,5,6] Men get sick about 3 times more often than women.[5,6,9,11]

Non-Hodgkin's lymphomas are a combination of neoplasms in the occurrence of which various agents participate. These agents, depending on the mechanism of their action, can be assigned to three different groups.[12,14] These tumors, with few exceptions, are of B- or T-cell origin, and their heterogeneity, at least in part, is due to many stages of normal differentiation and maturation of these cells.[3,6] Currently, according to the WHO classification adopted in 2008, 36 variants of a tumor are distinguished among NHL: 21 - of B-cell origin and 15 - of T-cell origin (excluding neoplasms of unclear origin). According to this classification, chronic lymphocytic leukemia, together with its non-leukemic variant (small lymphocytic lymphoma) belong to the NHL group. Plasma cell tumors belong to the same group.[3,5,9,11]

In lymphoma cells, the expression of cellular genes (as well as viral ones) is significantly reduced, probably as a result of the inhibitory effect of viral proteins. Nevertheless, malignant cells constantly express B-cell markers such as CD19, CD20 and CD22, surface immunoglobulins, most often IgM, less often IgG and IgA. These cells are also characterized by a low level of expression of class I HLA antigens, cell activation markers, and adhesion molecules[12], which allows the only protein among EBNA-1 viral markers not to be targeted by EBV-activated cytotoxic lymphocytes. In this

case, the expression of EBNA-1 in EBV-infected B cells provides them with a selective advantage (compared with uninfected cells) and avoids immune surveillance by the body.[12]

At present, great attention is paid to the study of immunological mechanisms in the development and progression of lymphomas. An important issue remains the immunodiagnostics of cellular and humoral immunity. As the literature shows, publications devoted to the study of immunity are sporadic. Basically, articles are devoted to clinical study and the description of the results of treatment methods, while there are no works of a pathogenetic nature. Reliable data can only be obtained by analyzing a sufficiently large amount of clinical material, which makes it possible to study the frequency of occurrence of various NHL variants; comparison of clinical, morphological and immunological data, as well as treatment results for T- and B-cell lymphomas, which opens up the possibility of improving the diagnosis and individualization of chemotherapy programs depending on the immunophenotypic characteristics of tumor cells.

**The aim of the study**. To study the basic cellular and humoral criteria for the course of non-Hodgkin's lymphomas.

## MATERIALS AND RESEARCH METHODS

In our study, patients with non-Hodgkin's lymphomas (NHL) were divided into 2 groups: group I - patients with established virus carriers; group II - patients in the control group with no virus carrier. In all patients, the diagnosis was established on the basis of data and the results of a comprehensive study (clinical, biochemical, radiological, ultrasound, CT, myelogram, morphological). The lesion areas for non-Hodgkin lymphomas in patients with viral infection (group I) most often occurred in the cervical and axillary l / nodes (more than 50%), then in the mediastinal, supraclavicular, retroperitoneal and inguinal (25-45%), the lesion was least noted. iliac l / nodes, spleen, Valdeyer (20% or less).

In control group II, the picture of lymph node lesions differed from the experimental group — patients with affected cervical l / nodes were more than 70%, while the remaining lesion zones were less common: axillary, supraclavicular, inguinal l / nodes malignantly transformed in 30-40% of cases; mediastinal, retroperitoneal, iliac - in less than 20% of cases; Valdeyer ring damage was observed in more than 60% of patients. Viral infection changes the picture of lymph node damage in patients with NHL in comparison with patients without viral load: in group I, axillary, mediastinal, supraclavicular, retroperitoneal, and inguinal l / nodes were transformed malignantly (25-45%). Group I patients were most often infected with HSV herpes simplex virus and Epstein-Barr virus (65-80%). Cytomegalovirus infection (CMV), human papillomavirus (HPV) in 43.4 ± 7.7% of patients, and Varicella-Zoster virus were found in 26.0 ± 6.8% and 8.6 ± 4.4%, respectively. The examination included patients undergoing examination in the chemotherapy department. All patients underwent clinical and laboratory blood tests, which included the study of a general analysis of blood and urine, biochemical and immunological parameters, as well as the blood coagulation system. Patients underwent a comprehensive clinical and instrumental examination aimed at clarifying the prevalence of the tumor process and identifying existing complications.

Immunological studies included the study of the humoral parameters of the immune system of patients. The humoral immunity parameters were determined by studying the main immunoglobulins A, M, G using commercial test systems for enzyme-linked immunosorbent assay production "Human", Germany.

Determination of cellular immunity (CD3 +, CD3 + CD4 +, CD3 + CD8 +, CD16 +, CD20 +), as well as identification of activation markers of lymphocytes (CD25 +, CD38 + and CD95 +) was carried out by flow cytometry using Accuri C6 (USA) using monoclonal antibodies.

When conducting a statistical analysis of the data presented in the work, the results of the study were entered into databases prepared in the Microsoft Excel XP program. Numerical (continuous) values were presented as arithmetic means and mean errors (M ± m). Comparison of quantitative characteristics was carried out using Student's criterion, for continuous variables - paired Student's criterion. $P < 0.05$ was taken as a boundary comparative criterion of statistical significance.

The obtained research results and their discussion. Thanks to the modern achievements of fundamental immunology, molecular biology, new biologically significant indicators have now appeared in the arsenal of researchers, which can help the practitioner, in particular in the diagnosis, treatment and prognosis of diseases, as well as in the choice of immunotropic therapy. In this regard, in recent years, much attention has been paid to immunological markers that can help in identifying an immunodeficiency state, diagnosing, predicting and preventing relapse of the disease, which is a kind of indicator of the nature of the course of the disease, especially in oncological diseases.[8,11]

Despite a significant deepening in the last decade of ideas in the etiology, immunopathogenesis, course and progression of malignant processes, many questions regarding the mechanisms of the development of the pathological process and its course, evaluation of treatment effectiveness, remain open.[10] Moreover, in recent years, many etiopathogenetic factors, such as viruses, which have a pronounced carcinogenic factor, have been understood. So, an important role in the course

and effectiveness of therapy for lymphomas belongs to the cellular and humoral factors of the immune system.[4,7]

At present, great attention is paid to the study of immunological mechanisms in the development and progression of lymphomas. An important issue remains the immunodiagnostics of cellular and humoral immunity. As the literature shows, publications devoted to the study of immunity are sporadic. Basically, articles are devoted to clinical study and the description of the results of treatment methods, while there are no works of a pathogenetic nature. Reliable data can only be obtained by analyzing a sufficiently large amount of clinical material, which makes it possible to study the frequency of occurrence of various NHL variants; comparison of clinical, morphological and immunological data, as well as treatment results for T- and B-cell lymphomas, which opens up the possibility of improving the diagnosis and individualization of chemotherapy programs depending on the immunophenotypic characteristics of tumor cells.[1,7,8,12] Based on the foregoing, we evaluated the humoral factors of the immune system in patients with NHL depending on the virus carrier.

It has been established that the functional usefulness of B-lymphocytes in the immune response is characterized by the production of immunoglobulins.[10] It is known that immunoglobulins play an important function of mediators in the cascade development of the immune response and can partly determine the effectiveness of the final, effector responses of cellular immunity in the inactivation and elimination of antigens.[7,8] It is also known that circulating antibodies are one of the effector factors of immunity, which provides antigen-specific protection.[1,3,4,8] We have analyzed the serum concentrations of the main immunoglobulins IgG, IgA, IgM in NHL. As can be seen from the data presented, the content of the main serum immunoglobulins varied widely. The highest serum IgG content was detected in the group of patients before treatment without virus carrier, and the lowest content was noted in the group of patients with virus carrier, that is, due to a factor that suppresses immunity. The serum IgM content in almost all groups of patients with NHL was within the normative values, and there were no specific deviations between the groups of patients. Thus, the serum IgG content in the group of patients before treatment with virus-bearing was 1374.5 ± 46.4 mg%, in the group of patients without virus-bearing - 1287.9 ± 56.8 mg%, with a standard value of 1148.5 ± 35.6 mg%. Analysis of IgA content revealed the presence of a significant increase in serum IgA in all groups of patients with NHL. Moreover, the highest content was noted in the group of patients with virus carrier. Consequently, the humoral immunity was characterized by an increase in serum concentrations of IgG and IgA in groups of patients with NHL.

One of the most important humoral markers of immunity is the circulating immune complexes (CICs). It has been established that one of the most important biological functions of immunoglobulins is antigen binding and the formation of CICs.[6,10] An important characteristic of the CIC is their size, which can be large and small. The analysis showed that the CIC of large and small sizes in all groups of patients were significantly increased. So, CIC of large sizes were significantly increased before treatment. As for the CIC of small quantities, a significant increased content is also observed. The highest average value of small CIC was revealed in groups of patients before treatment and after treatment without immunotherapy. Apparently, this is due to the lack of necessary detoxification and effects on the functioning of the immune system. Also, a CIC of 4% of small values is observed, the lowest value being close to normal in the group of patients without virus carrier. It is known that CIC3% of large quantities formed with an excess of antibodies, although they are able to bind complement, are large, insoluble, quickly phagocytized and have low pathogenicity.[2,3,5] The greatest pathological potential is possessed by soluble immune complexes of small sizes, which were formed with an excess of antigen.[4,7] A high level of CIC can be caused not only by activation of the immune response, but also by suppression of the mechanisms of their elimination.[2,6,10] The latter, apparently, is associated with a weakening of the function of the cells of the monocyte-macrophage system - cells that absorb and disintegrate the immune complexes. Therefore, activation of the humoral immunity is observed along with severe depression of the cellular immunity.

The main factors of immunity that have been studied and will be presented below include cellular factors of immunity, such as population and subpopulation markers of lymphocytes. It should be noted that the listed immunity parameters are specific factors, but studying them against a specific nosology and comparing the results with the clinical manifestations of the disease are definitely important, specific value, because it is the elements of the immune system that accompany all processes of pathogenesis, the development of the disease, its progression and outcome. It is known that all malignant processes relate to immunodeficiency conditions, accompanied by immunosuppression of any parts of the immune system.[6,9] Therefore, the study of the immunoreactivity status of cancer patients is an important factor necessary to establish the depth of immunodeficiency, to predict the disease and, most importantly, to identify the most radical treatment modalities, including immunotropic therapy in the future. The immunological parameters of patients with NHL were analyzed before the start of complex treatment to identify pathogenetic characteristics of the response. Analysis of the results showed that the average content of leukocytes in peripheral blood in all groups of patients was reduced compared with the value of the control group. Moreover, a significant decrease in the content of leukocytes was detected in groups of patients before treatment compared with data from a group of practically

healthy individuals. The highest leukocyte count was detected in the group of patients and amounted to 6280.9 ± 280.7cl / µl. The study of the relative content of the total pool of lymphocytes between the studied groups of patients showed that the number of lymphocytes was significantly suppressed in the group of patients compared with the value of the control group. It was found that the most significantly low value of lymphocytes was observed in groups of patients before treatment with virus carrier. So, the level of lymphocytes in the group of patients with virus carrier was 29.3 ± 1.2%, while in the control group it was 33.8 ± 1.11%.

It was found that phenotypic markers of lymphocytes include such markers as CD3 +, CD3 + CD4 +, CD3 + CD8 +, CD20 +. It is widely shown in the literature that the triggering and regulation of the effectiveness of the immune response is largely determined by the specific antigen of T-lymphocytes. Responsible for this function are antigen-recognizing receptors - TCR. It is known that the surface expression level of CD3 + receptors on the T-lymphocyte membrane reflects its transmissible function and allows the total number of T-lymphocytes to be identified.[5,9] Thus, the analysis of the immunophenotype of CD3 + T-lymphocytes in patients with NHL showed that the presence of a reliable suppression of the expression of CD3 + on T-lymphocytes is observed in all groups of patients compared with data from a practically healthy group ($p < 0.05$). The lowest CD3 + value was observed in the group of patients with virus carriers before chemotherapy. Obviously, a decrease in the total pool of T-lymphocytes (CD3 +) was observed mainly due to the suppression of the expression of CD3 + CD4 +. A study of the expression of CD3 + CD4 + on T-lymphocytes, which are the main regulatory cells of the immune system, showed that the lowest value was noted in groups of patients before treatment ($p < 0.05$). Expression of CD3 + CD4 + was most suppressed in the group of patients with NHL with virus carrier, as indicated in the literature.[3,5,10] The analysis showed that in the group of patients with virus-bearing CD3 + CD4 + it was 21.4 ± 1.3%, while in the groups of patients without virus-bearing it was 25.8 ± 0.8%, and in the group of practically healthy individuals it was 35.7 ± 1.14%.

Cytotoxic CD3 + CD8 + T-lymphocytes play an important role in the pathogenesis of cancer.[4,8] The biological role of this activation is the removal of mutant cells.[4,9] CD3 + CD8 + T-lymphocytes play a major role in the elimination of the virus, which is caused, on the one hand, by their ability to cause the death of infected cells expressing the corresponding peptides presented by MHC class 1 molecules, and, on the other hand, the ability to secrete antitumor factors – cytokines.[7,11]

Analysis of the expression of CD3 + CD8 + on T-lymphocytes revealed a significant increase in all groups of patients compared with the value of the control group. It should be noted that the maximum increase in CD3 + CD8 + was detected in the group of patients before treatment ($p < 0.05$). An analysis of the values of CD3 + CD8 + on T-lymphocytes between the studied groups of patients shows that before treatment, the expression of CD3 + CD8 + was significantly increased and amounted to 37.9 ± 1.72%. The immunoregulatory index (IRI), which is the ratio of the values of CD3 + CD4 + / CD3 + CD8 +, is of significant importance in secondary immunodeficiency states. It is known that in normal IRI in healthy individuals averages 1.62 ± 0.02. Obviously, suppression of the expression of CD3 + CD4 + against the background of increased expression of CD3 + CD8 + leads to a decrease in IRI. According to our data, the lowest decrease in IRI is observed in the group of patients before treatment with virus-bearing compared with the data of patients without virus-bearing ($p < 0.05$). Therefore, pronounced immunosuppression was characteristic of patients with NHL in groups of patients before treatment with virus carrier. Apparently, a decrease in IRI is an important criterion for the depth of the T - cell immunodeficiency state, especially against the background of infection with latent chronic viruses.

Further, the expression of killer cells, which are the third population of lymphocytes providing maintenance of genetic homeostasis, which are phenotypically and functionally significantly different from T and B lymphocytes, was studied.[5,8] Killer lymphocytes belong to the category of the main effectors of natural or innate immunity, which are able to lyse target cells or carry out antibody-dependent cellular cytotoxicity. They perform the functions of the first line of defense before immune T-lymphocytes and specific antibodies arise.[12,13] Killer cells with CD16 + phenotypes were studied. A significant increase in the expression of CD16 + was revealed in all groups of patients. It was shown that the highest expression of CD16 + is observed in the group of patients with virus carrier ($p < 0.05$). So, in the group of patients before treatment, the expression of CD16 + was 27.6 ± 1.1%, in the control group (without virus carrier) - 24.5 ± 1.31%, in the group of practically healthy individuals - 16.7 ± 1.4%. Consequently, the highest expression of CD16 + was observed in groups of patients prior to the start of chemotherapy with virus carrier. A study of the expression of CD20 + on B-lymphocytes, which are the main regulatory cells of the immune system and are important in the development of humoral immunity, showed that the expression of CD20 + was significantly increased in all groups of patients ($p < 0.05$). Despite this, the study of the content of B-lymphocytes is an important criterion to assess the depth of immunodeficiency and determine the next steps in terms of diagnosis and treatment. Next, activation markers of peripheral blood lymphocytes were studied. It is known that the activation markers of lymphocytes began to be studied relatively recently, therefore, small works on the functional activity of activation markers of lymphocytes, in particular in oncological processes, have been reported in the literature.[3,7,8,9] So, we studied lymphocyte markers such as CD38 + and CD95 +. It is known that

CD38 + is an activation marker represented by a transmembrane glycoprotein, which is considered as a multifunctional protein.[9] The enzymatic functions of CD38 + provide its main immunoregulatory role; this is the binding of various agents to this receptor, which enhances the synthesis of cytokines, activation of kinases, and protein phosphorylation.[6,9] CD38 + is expressed on immature T- and B-lymphocytes, activated T-lymphocytes, plasmocytes.[13] Analysis of the expression of CD38 + on lymphocytes revealed a significant increase in this marker in groups of patients before treatment. The highest value of CD38 + was observed in groups of patients before treatment with virus carrier. The expression of CD38 + before treatment was 32.2 ± 1.6%, without virus carrier - 28.4 ± 1.5%, and normal - 23.4 ± 0.6%. Therefore, increased expression of CD38 + was observed before the treatment of patients, which is apparently associated with the proliferative activity of specific T and B lymphocytes in response to the malignant process and chronic virus carriage. Also, increased expression of CD38 + may be associated with activation of CD3 + CD8 +, CD16 + and CD20 +. According to published data, there is information about the role of APO-1 / Fas (CD95 +) receptors in the process of apoptosis, and its degree is a reflection of the level of lymphocyte apoptosis.[3,6] It was established that the increase in the expression of CD95 + receptors on lymphocytes indicates an excessive and ineffective process of stimulation of blood lymphocytes, which indicates an apoptotic pathway for the death of lymphocytes.[10] Binding of CD95 + to the Fas ligand induces apoptosis of cells expressing CD95 +. The analysis showed that in groups of patients increased expression of CD95 + is noted. Moreover, the greatest expression is observed in groups of patients before treatment with virus carrier. Apparently, excessive apoptosis in combination with the activation of the humoral immunity and deep T-cell immunodeficiency contribute to the progression of the disease. Thus, the analysis of the results allowed us to identify pronounced changes in the cellular component of immunity, which are manifested by a suppression of the expression of CD3 +, CD3 + CD4 +, IRI, increased expression of CD3 + CD8 +, CD16 + and CD20 + cells, as well as increased expression of CD38 +, CD95 +. Based on the results obtained, it is seen that with NHL there is a pronounced imbalance of humoral immunity. CIC of large and small sizes are also increased, however, the largest increase in CIC was observed in groups of patients prior to chemotherapy with virus-bearing compared with data from patients without virus-bearing. An increase in major immunoglobulins indicates the presence of humoral imbalance, and an increase in CIC indicates intoxication of the body either due to the decay of the tumor cells themselves and infected cells. An increase in CIC of 4% always indicates the progression of the pathological process and is a marker of the progression or worsening of the clinical course. CIC3% quickly disintegrate in the organism, so they have no pathological potential.

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