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APPROACHES TO IMMUNODIAGNOSIS OF VIRUS-ASSOCIATED NON-HODGKINIAN LYMPHOS

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SUMMARY

The aim of the study was to study the immunodiagnostic basic cellular values of the immune system that characterize adaptive immunity in patients with non-Hodgkin's lymphomas. Due to the relevance of the presence of infection of patients with the Epstein-barr virus, patients were divided into groups of infected patients and without infection. In this regard, the main markers of adaptive immunity were studied against the background of infection and without infection. The analysis showed that pronounced changes in the state of cellular adaptive immunity were detected in a significant suppression of CD3 + T-lymphocytes, CD3 + CD4 + T-helpers / inducers and an immunoregulatory index against the background of an increase in T-cytotoxic lymphocytes, natural killer cells and B-lymphocytes. It is important to note that markers of lymphocyte activation turned out to be elevated, the study of which is important in immunodiagnostics for lymphomas. These markers characterize the prognosis of relapse of the disease or vice versa, their more favorable course. Moreover, immunological markers of lymphocytes were most indicative in the presence of Epstein-Barr virus infection. There is also a pronounced imbalance of humoral immunity values. The circulating immune complexes and immunoglobulins were increased, while the highest increase in small CIC was observed in groups of patients before chemotherapy for infected EBV.

KEYWORDS: immunodiagnostics, adaptive immunity, immunocompetent cells, non-Hodgkin lymphoma, viruses.

Non-Hodgkin lymphomas are a rather heterogeneous group of tumors of the blood system, and are characterized by various clinical course, localization, morphological, as well as immunological features of the course of the disease.[3,5,9,12] It is important to note that in recent years, the incidence of non-Hodgkin lymphomas has increased significantly.[4,7,10]

In recent years of the development of oncohematology, new data have appeared on immunophenotypic new markers that have contributed to a deeper understanding of the immunopathogenetic mechanisms of the development, course, and prognosis of NHL. It is known that the use of immunodiagnostic methods has influenced the immunophenotyping of lymphocytes, which in turn is an understanding of immunopathogenesis in this disease, and the creation of immunodiagnostic panels necessary for immunodiagnostics.[5,8]

As is known, the diagnosis of NHL is traditionally associated with difficulties dictated by similar cytological and histological characteristics of the tumor, in particular, the group. Only an integrated approach that takes into account the morphological features and uses an expanded diagnostic immunological panel of antibodies aimed at identifying both mature T and B lymphocytes and immature cells makes it possible to correctly approach the immunodiagnosis of lymphomas.[3,10] The problem of improving treatment, which directly depends on the state of the immune system, the presence and degree of secondary immunodeficiency, and the individual development of the disease, remains urgent.

The key role of the Epstein-Barr virus (EBV) in the development of certain lymphomas and lymphoproliferative diseases is currently recognized.[2,7,9] In recent years, EBV-induced lymphomas have increasingly become diagnosed in patients without severe immunodeficiency. Deepening knowledge of the biology of these lymphomas and clarifying the role of EBV in their pathogenesis will lead to improved therapy and the development of new cellular therapies.

The aim of the study was to study the basic cellular and humoral values of immunity in patients with non-Hodgkin's lymphomas infected and not infected with the Epstein-Barr virus.

MATERIALS AND RESEARCH METHODS

The study involved 48 patients with non-Hodgkin lymphomas, which were divided into 2 groups infected with the Epstein-Barr virus and uninfected.

The diagnosis of patients was established on the basis of clinical, laboratory and instrumental data, as well as morphological. The non-Hodgkin's lymphomas in infected patients turned out to be zones of defeat, group 1 patients, cervical and axillary lymph nodes (more than 50%), followed by mediastinal, supraclavicular, retroperitoneal and inguinal (25-45%), lesions of the iliac l / nodes were least, spleen, Valdeyer (20% or less). In group 2, the lesions of the lymph nodes were characterized by lesions of the cervical - more than 70%, while the remaining lesions were much less common, these are 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. Thus, it can be judged that herpetic viral infection can change the picture of lymph node damage in patients with lymphomas compared with the data of uninfected patients.

Epstein-Barr virus infection studies were carried out first by enzyme-linked immunosorbent assay, after obtaining positive results, polymerase chain diagnostics of Epstein-Barr virus DNA was performed. The examination involved patients undergoing examination and treatment in the chemotherapy department. After diagnosis, immunological studies were performed, which included cellular and humoral markers of immunity. Determination of cellular immunity (CD3 +, CD3 + CD4 +, CD3 + CD8 +, CD16 +, CD20 +), as well as identification of activation markers of lymphocytes (CD38 +, D95 +) was carried out by flow cytometry on Accuri C6 (USA) using monoclonal antibodies. Humoral studies were performed by enzyme immunoassay.

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. In recent years, much attention has been paid to immunological markers that can help in the diagnosis and identification of immunodeficiency states, the determination of which is important in predicting and preventing relapse of oncopathology, which is necessary to establish the nature of the course of the disease.[4,7] Moreover, it is known that the Epstein-Barr virus has a pronounced immunotropic effect, which is expressed in the lymphotropy of the virus, and its persistence in immunocompetent cells.[7,12] The main factors of immunity include cellular factors of immunity, such as population and subpopulation markers of lymphocytes. It should be noted that the listed parameters of immunity are specific factors of immunity or are also called components of adaptive immunity, but studying them against a background of 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.

All malignant processes relate to immunodeficiency conditions, accompanied by immunosuppression of any parts of the immune system.[3,5] The study of the immunoreactivity status of cancer patients is an important factor necessary for establishing the depth of immunodeficiency, predicting the disease, and most importantly identifying the most radical treatment modalities, including immunotropic therapy in the future. The immunological parameters of patients with NHL before chemotherapy were analyzed 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, which differed significantly. 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 revealed that a low value of total lymphocytes was observed in the group of patients infected with Epstein-Barr virus. It was found that phenotypic markers of lymphocytes include such markers as CD3 +, CD3 + CD4 +, CD3 + CD8 +, CD20. Thus, the analysis of the immunophenotype of CD3 + T-lymphocytes in patients with NHL showed the presence of reliable suppression of CD3 + T-lymphocytes compared with the data of a practically healthy group (p <0.05). The smallest number of CD3 + was detected in the group of patients infected before chemotherapy. A decrease in the total pool of T-lymphocytes (CD3 +) was observed mainly due to the suppression of the number of CD3 + CD4 + T-helpers / inductors. The study of CD3 + CD4 + T-helpers / inducers, which are the main regulatory cells of the immune system, showed that the lowest value was noted in the group of patients infected with herpes infection (p <0.05). It is known that the CD4 + T-cell response to tumor proteins is an important cellular defense mechanism of the macroorganism, since CD4 + T-helpers stimulate antibody production by B-lymphocytes and activate CD8 + T-lymphocytes specific for tumor cells.[5,7] The analysis showed that in the group of infected patients, CD3 + CD4 + T-helpers / inducers was 21.4 ± 1.1%, while in the groups of uninfected patients it was 26.2 ± 0.74%, and in the group of practically healthy individuals it was 35.7 ± 1.14%. Cytotoxic CD3 + CD8

+ T-lymphocytes are known to play an important role in the pathogenesis of cancer.[8,9] The biological role of this activation is the removal of mutant cells.[6,10] Analysis of the content of CD3 + CD8 + T-cytotoxic lymphocytes revealed a significant increase in all groups of patients compared with the value of the control group. The maximum increase in CD3 + CD8 + T-lymphocytes is observed in the group of infected patients before therapy ($p < 0.05$). 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. Suppression of 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 infected patients before treatment compared with the data of uninfected patients ($p < 0.05$). Therefore, pronounced immunosuppression was characteristic of patients with NHL in groups of patients infected with herpes infection.

The content 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.[5,11] A significant increase in the expression of CD16 + was revealed in all groups of patients infected and uninfected. It was shown that the highest expression of CD16 + is observed in the group of infected patients ($p < 0.05$). So, in the group of patients before treatment, the expression of CD16 + was 26.8 ± 1.1%, in the control group (uninfected) - 23.9 ± 1.12%, in the group of healthy individuals - 16.7 ± 1.4%.

The study of CD20 + B-lymphocytes is important in the development of humoral immunity, the analysis showed that CD20 + B-lymphocytes were significantly increased in the group of infected patients ($p < 0.05$), which once again proves the lymphotropicity of the virus. The study of the content of B-lymphocytes is an important criterion that allows you to assess the depth of immunodeficiency and determine the next steps in terms of diagnosis and treatment.

It is known that activation markers of lymphocytes began to be studied relatively recently, therefore, scientific papers on the functional activity of activation markers of lymphocytes, in particular in oncological processes, are rarely covered in the literature. From the available literature data it is clear that the study of activation markers of lymphocytes, especially in proliferative processes, is of great scientific and practical importance. analysis of activation markers of lymphocytes allows one to study the processes of activation, proliferation, differentiation and apoptosis of immunocompetent cells and characterizes cell cycles associated with these processes.[4,12] So, lymphocyte markers such as CD38 + and CD95 + have been studied. It is known that CD38 + is an activation marker represented by a transmembrane glycoprotein, which is considered as a multifunctional protein.[7] 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.[3,7] CD38 + is expressed on immature T- and B-lymphocytes, activated T-lymphocytes, plasmocytes.[10] 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 noted in the group of infected patients and amounted to 33.6 ± 0.93%, while normal - 23.4 ± 0.6%. Consequently, the increased expression of CD38 + is apparently associated with the proliferative activity of specific T and B lymphocytes in response to the malignant process and chronic long-persisting virus carriage. According to published data, APO-1 / Fas (CD95 +), the receptor during apoptosis is a reflection of the level of lymphocyte apoptosis.[7] It has been established that an increase in the expression of CD95 + receptors on lymphocytes indicates an excessive and ineffective process of stimulating blood lymphocytes, which indicates an apoptotic pathway for the death of lymphocytes.[11] The analysis showed that significantly increased expression of CD95 + was observed in the group of infected patients. Apparently, excessive apoptosis in combination with activation of the humoral immunity and deep T-cell immunodeficiency contribute to the progression of the oncological process.

It is known that immunoglobulins play an important function of mediators in the cascade development of the immune response and can partially determine the effectiveness of the final, effector responses of cellular immunity in the inactivation and elimination of antigens.[4,9] It is also known that circulating antibodies are one of the effector factors of immunity, which provides antigen-specific protection.[4,6] Serum concentrations of the main immunoglobulins IgG, IgA, IgM in NHL, as well as circulating immune complexes, were analyzed. The content of major serum immunoglobulins varied widely. The highest serum IgG content in the group of infected patients was revealed, and the lowest content was noted in the group of patients uninfected with herpes infection. An increase in serum IgM was observed in the group of infected patients compared with the data of other compared groups. The IgA content was also significantly increased in the group of infected patients compared with the data of the compared groups. Consequently, the humoral immunity was characterized by an increase in serum concentrations of IgG, M, and IgA in groups of infected patients with NHL. One of the most important humoral markers of immunity is the circulating immune complexes (CICs) of various sizes. It has been established that one of the most important biological functions of immunoglobulins is antigen binding and the formation of CICs.[9, 11] 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. But significantly increased in the group of infected patients with herpes infection. Apparently, this is due to the persistent activation of latent viral infection and the lack of antigen neutralization. It is known that CIK3% 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.[8,9] The greatest pathological potential is possessed by soluble immune complexes of small sizes, which were formed with an excess of antigen.[8] 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.[8] Therefore, there is an activation of the humoral immunity coupled with severe depression of the cellular immunity. Thus, in NHL, especially against the background of infection with herpetic EBV infection, a pronounced imbalance of humoral and cellular immunity is observed, which is characterized by an increase in the CIC of large and small, especially in the group of infected patients, increase in basic immunoglobulins, which also logically corresponds to the data obtained on circulating immune complex background of deep T-cell immunodeficiency. Therefore, our data will contribute to the accumulation of knowledge in the field of immunodiagnostics of oncological diseases, which in turn can contribute to the creation of immunodiagnostic protocols for patients with various forms of the disease, and will also serve to assess the state of immunity in the dynamics of therapy and prognosis.

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