Updated requirements for the identification of morphological types of non-Hodgkin's lymphomas

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Abstract

This manuscript describes the updated requirements for diagnosis of morphological types of non-Hodgkin's lymphomas, which are reflected in the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues (2001). It is mentioned that the diagnosis of the tumor of lymphoid tissue should be based on histological and immunohistochemical assessments of the bioptic specimen. General principles of the immunohistochemical studies in the diagnosis of non-Hodgkin's lymphomas consist in the usage of panel of antibodies, which is composed according to the diagnostic hypothesis, resulted from the routine histological examination. The immunohistochemical assessment of the tumor of lymphoid tissue constitutes the selective modality in cases of differential diagnosis of histologically resembling types of non-Hodgkin's lymphomas.

Key words: non-Hodgkin's lymphomas, morphological types, histological examination, immunohistochemical assessment.

Современные требования идентификации морфологических вариантов неходжкинских лимфом

В этой работе изложены современные требования к диагностике морфологических вариантов неходжкинских лимфом, отраженных в Классификации опухолей кроветворной и лимфатической системы, предложенной ВОЗ в 2001 году. Подчеркивается, что диагноз опухолей лимфатической системы должен основываться на гистологических и иммуногистохимических исследованиях биоптата. Общие принципы иммуногистохимических исследований в диагностике неходжкинских лимфом заключаются в использовании в каждом случае панели антител, составленной в соответствии с гипотезой диагноза установленного в результате рутинного гистологического исследования. Иммуногистохимическое исследование опухолей лимфатической ткани является методом выбора в случаях дифференциальной диагностики гистологически похожих вариантов неходжкинских лимфом.

Ключевые слова: неходжкинские лимфомы, морфологические варианты, гистологическое исследование, иммуногистохимическое исследование.

Remarkable progress has been made in the research of non-Hodgkin's lymphomas (NHLs) over the last 15-20 years. Multiple types and subtypes of these disorders were acknowledged and reflected in the updated classifications by the NHL – REAL in 1994 [4] and WHO in 2001 [3], which require the specialist to identify the precise type of NHL by using morphological, immunohistochemical and cytogenetic assays.

The REAL classification [4], as well as the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues [3] which followed were based on the advances in the study of the lymphocyte differentiation mechanisms [6]. It was established that lymphoid cells have particular development stages. The surface antigens on the membrane and cytoplasm allow for the identification of the developmental stage of the lymphoid cell.

The cell surface antigens involved in differentiation are called „clusters of differentiation" (CD) and may be depicted by using specific monoclonal antibodies. The profile of antigens is different for T- and B-lymphocytes, and the antigens change during the different stages of differentiation of the lymphocytes.

The differentiation stages of the lymphocytes were well systematized by S.A.Lugovskaia et al. [10]. It is known that lymphocytes develop from bone marrow stem cells, where antigen independent B-lymphocyte differentiation stage takes place. The early stage of differentiation of B-lymphocytes is called pro-B-lymphocyte. At this stage, the CD19, which is a common marker in all B-lymphocytes, appears on the cell membrane. The emergence of CD19 occurs in cells with expressed HLA-DR molecules, often in combination with CD38, CD34, and TdT. In the next stage of differentiation, CD10 appears on the lymphocyte membrane. This stage is called pre-B-lymphocyte. Subsequently, CD20 molecule emerges on the membrane and the cell obtains the immunophenotype of the pre-B-lymphocyte. This stage corresponds to the stage of mature B-lymphocyte. The antigen independent process of differentiation of B-lymphocytes ends with the expression of IgD that coexists with IgM. The presence of IgM + IgD, CD19, and CD20 on the membrane allows for the classification of a cell as a mature B-lymphocyte (naïve). From the moment when the B-cell has formed the receptor complex, it is able to react to antigens.

The mature B-lymphocytes leave the bone marrow, pass into the circulation and are led to peripheral lymphatic organs (lymph nodes, spleen etc.), where, upon meeting the antigens, they complete the antigen-dependent stage of differentiation.

In these organs they fulfill their functions. The bone marrow is the fundamental source for the renewal of B-lymphocytes.

In the lymphatic nodes, the mature B-lymphocytes get into the primary follicles, the follicles without a germinal center. The secondary follicles differ from the primary ones by the presence of the germinal center.

The cell morphology in the primary follicle corresponds to a small lymphocyte, most of the cells having no signs of activation. The lymphocytes express CD19, CD20, CD22, CD24, and CD37 on their membrane; hence they have the phenotype of peripheral B-cells. The activating antigens CD23, CD5, CD10, and CD38 usually are lacking. Once the CD5-CD23-B-lymphocytes are activated, they migrate to the follicle, whose structure modifies following an accelerated proliferation and the germinal center appears, as well as the so-called mantle zone. A part of the cells migrate and form the marginal zone, which surrounds the follicles, remaining there as memory B-cells.

The process of antigen-dependent maturation and differentiation of B-cells occurs in the germinal center. There, the B-cells loose the CD23 and transform into centroblasts that actively proliferate. On the centroblasts, CD77, CD10, CD19, CD20, and CD38 are characteristically expressed. In the germinal center, a portion of centroblasts is differentiated into centrocytes – small cells with cleaved nuclei.

Subsequently, plasmocyte memory B-cells are formed from centrocytes. The mature plasmocytes produce immunoglobulins, which ensure the humoral function of the body. These cells loose the majority of B-cellular receptors, keeping only CD38.

The cells in the marginal zone of the follicles in the lymph nodes are few in comparison to the spleen and mucosal lymphatic tissue – the so-called monocitoid B-cells. Their name reflects the morphological resemblance with monocitoid elements.

The immunophenotype of these cells corresponds to the activated B-cells in the terminal differentiation stages (CD19, CD20, CD22, CD37, CD40). CD21, CD23, and CD24 are not usually expressed, a feature that makes them different from the cells of the germinal center and mantle zone of the follicle. This cell type more closely resembles the lymphocytes in the marginal zone of the spleen, differing from them, however, by the more permanent expression of CD20, CD39, and CD38.

The natural killers (NK-cells) represent the lymphocyte fraction that lacks the markers of T- and B-cells. Their phenotype is CD3, CD16+, and CD56+. They are largely contained in the liver and spleen, and are found in small numbers in the lymph nodes, bone marrow, lungs, and the lymphatic follicles of the small bowel. Morphologically, they correspond to large granulated lymphocytes. Their fundamental function is contact cytolysis of viruses and intensively proliferating cells.

This way, the differentiated lymphocytes remain morphologically mature cells in lymph nodes, spleen, bowel submucosa, and other organs until they meet the antigens. When an antigen acts, a new lymphocyte multiplication and differentiation cycle begins, with the morphological stages of pro-lymphocyte, blast cell, and immunoblast. A.I.Vorobiov and A.M.Kremenetskaia [8] mention that of all the hematopoietic cells, the lymphocytes are the only able to transform into blast several times. B-lymphocytes pass through this stage at least 3 times: a) at the level of stem cells; b) at the stage of primary immune response; c) at the stage of secondary immune response when the memory cell transforms into immunoblast, and finally becomes a plasmocyte.

Tumors may develop at all these levels of cell differentiation. The blast cells may belong to any of these three levels of differentiation and differ from one another by the immunophenotype. We cannot exclude that these cells differ morphologically as well, however the specific morphological features have not yet been established.

There is a continuous research in the stages (levels) of differentiation of the lymphocytes. As a consequence, cellular lines have been stratified (B-, T- and NK cells). Each of these cell lines consists of antigen-dependent and antigen-independent lymphocytes (correspondingly „immunologically mature" and „immunologically young" lymphocytes).

It has been mentioned many times that each tumor cell has its analogue – the normal cell which, upon malignization, forms the morphological substratum of the respective NHL type. This leads to meticulous studies of the lymphocyte transformation in peripheral organs and the identification of lymphoid cell at different development stages.

It is important that the morphological study includes the identification of the NHL type. The diagnosis of NHL is not difficult to determine. Nonetheless, the morphological assessment is not always sufficient, because it does not allow for the differentiation of a specific NHL type. The data of immunogenesis, which reflect the process of transformation of lymphoid cell from immature state to the immunologically differentiated stage, are necessary. Without exaggeration we could say that the study of NHL types is based on the principle of morphoimmunological comparison (13). Based on this principle, all NHLs are divided into tumors formed of predecessor cells (including tumors of thymus cells – the central organ of the immune system for T-lymphocytes) and tumors consisting of cells having the phenotype of the lymphoid elements of the peripheral organs of the immune system. The last ones form the majority of NHLs.

The immunologic diagnosis of NHL is based on the detailed study of the membrane and cytoplasm antigens of the tumor cells to determine the origin of lymphoma (B- or T-cell) and the stage at which normal development ended. Subsequently, the immunophenotype of the tumor cells is compared to the immunophenotype of the normal cell analogue [11, 12].

It was shown that NHLs are B-cellular more frequently (85-90%), with the expression of pan-B-cellular antigens: CD19, CD20, and CD22 in complex with HLA/DR, as a rule. The other B-cellular antigens (CD5, CD10, CD23, and CD38) allow for the identification of B-cellular subtypes. The presence of CD4, CD7, and CD8 is characteristic for T-cell tumors.

The assessment of the immunophenotypical features of different morphological types of NHLs is of major impor-

tance, because they represent a very relevant component in the complex diagnosis of NHL [12].

Usually, NHLs are tumors consisting of peripheral cells of the immune system. Acute lymphoblastic leukemias and a small part of lymphomas (lymphoblastic lymphomas) develop from immunologically young cells or antigen-independent predecessor cells. These cells are found normally in the central organs of immunogenesis – bone marrow and thymus. Predecessor B- and T-cell lymphomas essentially correspond to the extramedullary manifestations of acute lymphoblastic leukemia.

The WHO Classification (2001) [3] pays major attention to the possibilities to improve the diagnosis of lymphomas and leukemias developed from mature lymphoid cells by applying immunologic criteria. This group of lymphoproliferative disorders is predominantly seen in adults. The lymphomas formed of peripheral mature cells are developed from antigen-dependent immunocompetent cells of the peripheral lymphoid organs (lymph nodes, spleen, mucosal lymphoid tissue etc.).

At present, the description of the immunophenotype for almost all types of NHLs consists of the list of the tumor cell markers [3].

Using the list of markers makes it possible to identify the nosologic form when the tumor cells constitute a majority or when not less than 30% of the cell are positive, but is not useful in the cases with a smaller percent of tumor cells. In these cases, the decisive role belongs to the concomitant expression (co-expression) of different markers on the same cells [9].

A.I.Vorobiov et al. [9] consider that the main issue of immunophenotype assay in hematology is the determination of the nosologic variant of the disease to facilitate the optimal therapy for the particular patient, but not to assess the prognostic risks. The authors consider useful the assessment of the tumor immunophenotype on treatment.

Although the identification of subtypes in the case of macrocellular NHLs is not accepted, a worsening of the prognosis in the cases with large numbers of immunoblasts and centroblasts (> 90%) in the tumor tissue has been noted.

The differential diagnosis imposes a rational selection of the components of immunological markers panel, which allows differentiating histologically similar NHLs [1].

The general principles of the immunohistochemical assays in the diagnosis of NHLs presume the use of antibody panel every time correspondingly to the diagnostic hypothesis emerged following the routine histological examination, and not to a specific antibody. It is necessary to consider the combination of positive and negative results with respect to the known data regarding tumor morphology and the immunophenotype of the tumor cells [2].

As it was mentioned in the updated International Classification of Tumors of Hematopoietic and Lymphoid Tissues, the NHL types are classified correspondingly to the normal cellular analogue. Beside this, some types include the structures of the secondary lymphatic organs in their name. Therefore, the list of nosologic types includes such names as follicle center cell lymphoma, mantle cell lymphoma, and marginal zone lymphoma.

The diagnosis of lymphatic tissue tumor should be based on the histological and immunohistochemical assessments of the bioptic specimen. The immunohistochemical assay of the lymphatic tissue tumors is the prefered method in cases of differential diagnosis with tumors with similar histological changes [5].

The identification of the normal equivalent for tumor cells in hemoblastoses represents one of the major advancements of the recent years. It embodies the key for immune diagnosis and opens new research directions aimed at finding alternative ways (noncytotoxic) to control the proliferation and differentiation of tumor cells. The need to determinate the normal equivalent for lymphoma cells is also justified by the fact that the features of tumor growth, progression, proliferative activity, sensitivity of tumor cells to chemotherapy largely depend on the biological traits of these cells, and, primarily, the belonging to a cellular line and the level of differentiation.

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