LECTURE

HISTOPATHOLOGIC DIAGNOSIS OF MALIGNANT LYMPHOMAS BASED ON CLASSIFICATION OF WORLD HEALTH ORGANIZATION

*J. Audouin, A. Le Tourneau, T. Molina, S. Camilleri-Brolt, J. Diebold**
Service Central d'Anatomie et de Cytologie Pathologiques, Hqtel Dieu, 75181 Paris Cedex 04, France

ГИСТОПАТОЛОГИЧЕСКАЯ ДИАГНОСТИКА ЗЛОКАЧЕСТВЕННЫХ ЛИМФОМ НА ОСНОВЕ КЛАССИФИКАЦИИ ВСЕМИРНОЙ ОРГАНИЗАЦИИ ЗДРАВООХРАНЕНИЯ

*Ж. Одуэн, А. Ле Турно, Т. Молина, С. Камийри-Брое, Ж. Диболь**
Отдел патологической анатомии и цитологии госпиталя Отель Дье, Париж, Франция

The principles of the diagnosis of malignant lymphomas in accordance with new WHO classification have been considered. The histopathologic criteria provide for 80–95% reproducibility in the diagnosis of the different lymphoma entities. Immunohistochemistry with a large panel of monoclonal antibodies to differentiate lymphomas of B-, T- and NK-cell origin should be done step by step when necessary. The criteria used to delineate lymphomas originating from lymphoid precursor cells and mature (peripheral) lymphocytes have been treated in details. Small B-cell lymphomas, B-CLL, mantle cell lymphoma, marginal zone lymphoma, follicular lymphoma, lymphoplasmacytic lymphoma, large B-cell lymphomas, Burkitt's lymphoma, T-cell lymphoma, Hodgkin's lymphoma have been characterized on the basis of their histomorphology and immunophenotyping.
***Key Words*: lymphoma, diagnosis, classification.**

Рассматривается принцип диагностики злокачественных лимфом в соответствии с новой классификацией ВОЗ. Обычные гистопатологические критерии обеспечивают 80–95% воспроизводимость диагностики отдельных нозологических форм. Иммуногистохимические методы с использованием широкой панели моноклональных антител для распознавания опухолей В-, Т- и ЕК-клеточного происхождения применяют выборочно. Детально описаны критерии, позволяющие выделять опухоли, возникающие из лимфоидных клеток-предшественников и зрелых (периферических) лимфоцитов различного происхождения. Приведена гистоморфологическая, иммунофенотипическая характеристика В-клеточных лимфом из малых лимфоцитов, В-ХЛЛ, лимфомы из клеток мантийной и маргинальной зоны, фолликулярной и лимфоплазмоцитарной лимфомы, В-крупноклеточной лимфомы и лимфомы Беркитта, Т-клеточных лимфом, лимфомы Ходжкина.
***Ключевые слова*: лимфомы, диагностика, классификация.**

In the majority of the cases, the diagnosis of the different lymphoma entities can be done on conventional staining [1]. The histopathologic criteria are now well defined in the WHO classification [2]. The interreproducibility between pathologists and the intrareproducibility are around 80 to 95%. There are only two requirements. First, the pathologist has to learn the criteria and to be trained. Second, the histologic technique should be of good quality: sections 4 μm in thickness, optimal fixation and dehydratation, good HE, and if possible a Giemsa stain of excellent quality. Cytology on the imprints is of good help and should be performed.

Immunohistochemistry with a large panel of antibodies should not be done systematically, but used step by step, according to the hypothesis based on histopathology. CD20 and CD3 are the recommended antigens which should be demonstrated first to assess the B– or T–cell origin of the lymphoma. In case of large cell lymphoma, CD45 is useful for confirming the leukocyte origin of the neoplastic cells.

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*Correspondence. Fax: (33–01) 42348641
E–mail: anapath.hd@htd.ap–hop–paris.fr

Then three possibilities can occur:

1. The lymphomatous cells are CD20 positive and CD3 negative. You are dealing with B–cell lymphoma. The size of the cells (small, medium, large), the pattern of the proliferation, diffuse and/or nodular may be enough for proposing a precise diagnosis. Immunohistochemistry can be useful to confirm the morphologic diagnosis. As an example the immunohistochemical patterns of small B–cell lymphomas are presented in Table 1.

2. The lymphomatous cells are CD3 positive and CD20 negative. The diagnosis of T–cell lymphoma can be proposed. The site of involvement, the size and shape of the cells, the pattern of proliferation are sometimes enough for a precise diagnosis allowing the choice of an appropriate treatment. A more extended immunohistochemistry is sometimes of good help to recognize some entities (for example, NK versus T cytotoxic) but without influence on the therapeutic strategy.

3. The cells are CD20 and CD3 negative. Some B–cell lymphoma may express CD20 faintly (for example, B–CLL) or may be negative (for example, some lymphoma with a plasmacytic differentiation or plasmacytoma). The demonstration of the expression of CD79a is then very useful. This marker is an antigen expressed by B cells from the

primitive stage to the mature plasma cell. For these entities, the plasmacytoid morphology of the cells on imprints and histologic sections allows the diagnosis. Demonstration of an intracytoplasmic production of a monotypic immunoglobulin using antibody against kappa and lambda light chains may be useful. T/NK–cell lymphomas can be negative for CD3. In many cases, the site of proliferation and the morphology allows to recognize an entity and to complete the phenotype. Other T–cell markers (CD2, CD5, CD7), NK markers (CD56, CD57) and demonstration of proteins present in cytotoxic granules (TiA–1, granzyme, etc.) can be useful. Anaplastic large cell lymphomas are often negative for CD3 and CD20. CD30 should be studied systematically particularly in large cell lymphoma. In such a case CD45 may be useful for confirming the leukocyte origin of the neoplastic cells.

DIAGNOSIS OF SMALL B–CELL LYMPHOMAS

In the majority of the cases, histopathologic criteria allow us to recognize precisely the type of lymphoid neoplasias. But in some cases immunohistochemistry using only five antibodies (CD5, CD10, CD23, bcl–2, cyclin–D1) is necessary for a precise diagnosis.

We will now summarize the most important criteria of the different entities.

B–CLL. The infiltration is diffuse, obscuring the normal architecture and is constituted by small lymphocytes with dark round nuclei. Proliferative centers are recognized as pale areas, constituted by medium–sized cells (prolymphocytes) with round nuclei, an open chromatin and a centrally situated nucleolus and by large transformed cells (paraimmunoblasts) with an amphophilic cytoplasm, a round pale nucleus and a large nucleolus. In addition, the lymphoplasmacytoid variant is recognized on Giemsa stain due to basophilic cytoplasm visible on paraffin section or on imprints. Immunohistochemistry discloses cytoplasmic immunoglobulin with a light chain restriction.

Mantle cell lymphoma. The proliferative cells may resemble centrocytes (small/medium cells with cleaved nuclei) or small lymphocyte with round nuclei as in B–CLL. Often the neoplastic cells have an intermediate morphology of the nuclei, slightly irregular. Numerous histiocytes with large pale cytoplasm are dispersed. One of the most important criteria is the absence of proliferative centres. Then the pattern of proliferation is very important. In many cases, the organization is nodular. Silver impregnation is useful for demonstrating these nodules. In the centre of the nodules either typical reactive germinal centres or small remnants of the previous colonized germinal centres can be observed. In other cases, the proliferation is more diffuse resembling B–CLL. The morphology of the nuclei and the absence of proliferative centres allow the diagnosis. In some cases, immunohistochemistry is needed (see Table 1). Demonstration of nuclei positivity for cyclin D1 is one of the most valuable criteria in favor of mantle cell lymphoma.

Lymphoplasmacytic lymphoma. The diffuse infiltration is constituted by lymphoplasmacytic cells, typical plasma cells and less than 20% of immunoblasts. Reactive follicles can be still present. The demonstration of intracytoplasmic immunoglobulins with a light chain restriction confirms the diagnosis.

Table 1. Immunohistochemical characteristics of small B–cell lymphomas

	CD20	CD79a	CD5	CD10	CD23	bcl–2	cyclin D1
B–CLL	+/–	+	+	–	+	+	–
Mantle cell	+	+	+	–	–	+	+
Lymphoplasmacytic	+/–	+	–	–	–	+	–
Follicular	+	+	–	+	–	+(65%)	–
MALT	+	+	–	–	–	+	–
Nodal marginal zone	+	+	–	–	–	+	–
Splenic marginal zone	+	+	–	–	–	+	–
Hairy cell leukemia	+	+	–	–	–	+	–

Follicular lymphoma. In the majority of the cases, the diagnosis is really easy due to the nodular pattern. In some cases, nodularity is difficult to recognize, particularly when there is a heavy interfollicular infiltration, but the nodular pattern can be demonstrated by silver impregnation. Follicular origin of these nodules is recognized by the morphology of the neoplastic cells and by the presence of follicular dendritic cells. The neoplastic cells are centrocytes, small or medium (with cleaved nuclei) and centroblasts (small and large). Immunohistochemistry is often not useful. Follicular lymphoma should be graded according to the number of large cells (bigger than 2 lymphocytes or even larger) with the morphology of centroblasts or sometimes of immunoblasts. It seems also logical to count large cells with multilobated nuclei and Reed–Sternberg–like large cells but not large centrocytes. The large round cells are counted in the follicles in ten high power fields (x 40). Grade I is defined by the presence of 0 to 5 large cells by high power field, grade II by 6 to 15 large cells and grade III by more than 15 large cells.

Marginal zone lymphoma. These lymphomas, whatever the site of involvement are defined by a diffuse proliferation around and between reactive germinal centres which are progressively colonized. Three types of cells can be recognized. The most prevalent are small or medium–sized lymphoid cells with pale cytoplasm and more or less irregular nucleus called *centrocyte–like*. In addition it is possible to recognize larger cells with a more abundant pale cytoplasm and a larger ovoid nucleus with a more open chromatin, called *monocytoid cells* and typical *plasma cells*. In extranodal MALT lymphomas, the diffuse infiltration is associated with lymphoepithelial lesions. In such extranodal sites, the main differential diagnosis is constituted by mantle cell lymphoma. However, the tumor cells of MALT lymphoma do not express CD5 nor cyclin D1 (see Table 1). In nodal marginal zone, monocytoid B cells are often prominent, presenting as large pale ring around follicles colonized by centrocyte–like cells and often associated with plasma cells. One can distinguish primary nodal lymphoma from secondary involvement by MALT lymphoma mainly on the basis of the clinical data.

In splenic marginal zone, the morphology is so peculiar that the diagnosis is easy. The reactive follicles are surrounded by a large pale ring of monocytoid B–cells associated, within about half of the cases, with a plasma cell component. In almost all the cases, the follicles are more or less homogenous due to colonization by centrocyte–like cells.

Hairy cell leukaemia. This lymphoma today is mainly observed on bone marrow biopsy. There is a diffuse interstitial infiltrate patchy or massive constituted by medium–sized lymphoid cells with an abundant pale cytoplasm surrounding an ovoid or kidney–shaped nucleus.

Systemic myelofibrosis is demonstrated by silver impregnation in the areas infiltrated by leukaemic cells.

DIAGNOSIS OF LARGE B–CELL LYMPHOMAS

The expression of CD20, or sometimes of CD79a and the morphology of the cells allow easily the diagnosis of diffuse large B–cell lymphomas. Definition of the morphologic variants is based on morphologic criteria: centroblastic (monomorphic, polymorphic, multilobated), immunoblastic with or without plasmacytoid differentiation, T–cell/histiocyte rich B–cell lymphoma, anaplastic large B–cell lymphoma. Even the clinical variants are defined by the site of involvement and the pattern: mediastinal large B–cell lymphoma with fibrosis, intravascular large B–cell lymphoma, pleural effusion lymphoma.

DIAGNOSIS OF BURKITT'S LYMPHOMA AND VARIANTS

The diagnosis is sometimes difficult with some large B–cell lymphomas with high apoptosis and numerous mitosis. This type of lymphoma represents the lymphoma for which the histopathologic diagnosis is the least reproducible. To confirm the diagnosis of true Burkitt's lymphoma, immunohistochemistry (CD10 positive, bcl–2 negative, bcl–6 positive, 100% of Ki–67) as well as cytogenetics are of great importance.

DIAGNOSIS OF T–CELL LYMPHOMA

The expression of CD3 and the morphology allow us to recognize the majority of the cases of T–cell lymphomas which in total represent less than 10% of all the lymphomas in the occidental world. The most frequent are anaplastic large cell lymphoma (ALC), peripheral T–cell, AILD type and peripheral T–cell lymphomas NOS. The ALC express CD30, EMA, ALK–1, sometimes CD3. T–AILD can be confused with some peripheral T–cell lymphomas NOS. The demonstration of an hyperplastic network of follicular dendritic cells outside the germinal centre is regarded as an argument for the diagnosis of the T–cell lymphoma, AILD type.

Mycosis fungoides and Sйzary's syndrome are mainly recognized on morphologic and clinical data. In the different variants of T–cell lymphomas with leukaemic presentation, expression of CD3 or a NK marker associated with the morphology and the clinical presentation allow the diagnosis.

DIAGNOSIS OF PRECURSOR CELL LYMPHOMA

T and B lymphoblastic lymphomas have many clinical and morphological characteristics in common. The morphology allows us to recognize the diagnosis of lymphoblastic lymphoma. This diagnosis is confirmed by nuclear TdT expression and by high percentage of Ki–67–positive cells. Immunohistochemistry on paraffin sections is needed for the demonstration of the B– or T–cell origin, often with a larger panel of antibodies: CD20, CD10, CD79a, CD3, CD2, CD5, CD1a. But the immunophenotype is mostly demonstrated by flow cytometry on circulating blood cells.

DIAGNOSIS OF HODGKIN'S LYMPHOMA

The diagnosis of Hodgkin's lymphoma is morphologic. In the majority of the cases, immunohistochemistry is not really useful for the diagnosis. But in difficult cases, immunohistochemistry is necessary for the differential diagnosis between lymphocyte predominance and classic Hodgkin's lymphoma (Table 2), or between classic Hodgkin's lymphoma, nodular sclerosing type, grade 2 and anaplastic large cell lymphoma (Table 3).

Table 2. Immunohistochemistry in Hodgkin's lymphomas

	Nodular lymphocyte predominant	Classic lymphocyte rich
CD30	–	+
CD15	–	+
EMA	+/–	–
CD20	+	+/–
FDC	Network in the nodule in contact with tumor cells	Tumor cells at the periphery of nodules or absence of nodules
CD57	Numerous cells in nodules, rosetting around giant cells	Rare cells
LMP–1	–	+ (40–50%)

Table 3. Immunohistochemistry in classic Hodgkin's and anaplastic large cell lymphomas

	ALC	Classic Hodgkin's nodular sclerosing, grade 2
CD30	+	+
CD15	–	+/–
EMA	+/–	–
CD20	–	+/–
CD3	+/–	–
ALK–1	+	–

CONCLUSION

Immunohistochemistry on paraffin sections allows us to recognize more easily all the different varieties of lymphomas. We learn a lot on morphology from immunohistochemistry. So at present, in many cases, immunohistochemistry is not really necessary, but is more "comfortable". On the other hand, when it is not possible to propose a diagnosis on pure morphologic criteria, then immunohistochemistry is mandatory.

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