Section 6. Medical science

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IMMUNOHISTOCHEMICAL CHARACTERISTICS AND THEIR EFFECT ON THE CLINICAL COURSE AND PROGNOSIS OF DIFFUSED B-LARGE CELL LYMPHOMA WITH EXTRANODAL LESIONS

Abstract. The study included 81 patients with diffuse large B-cell lymphoma with extranodal lesions, who were under examination and treatment from 2015 to 2021 inclusive. We observed 81 patients with 10 types of DLBCL. The most frequently identified primary mediastinal form (19.8%), intravascular form (18.5%), lymphomatoid granulomatosis, which in the usual histological examination is confused with Hodgkin's lymphoma (16%) and with the predominance of T – cells. Less common were plasmablastic, ALC positive, with IRF4 rearrangement, and primary DLBCL of serous cavities.

Keywords: IHC; diffuse B-large cell lymphoma; extranodal lesion.

Introduction

The modern classification of the World Health Organization WHO in 2017 includes more than 40 subtypes of lymphomas, thereby defining various approaches in the diagnosis, treatment and development of new promising therapeutic areas [1]. In the new WHO classification of 2017, DLBCL without additional specification (not otherwised specified – NOS) is divided into various morphological subtypes that do not have prognostic significance: centroblastic, immunoblastic, anaplastic and other rare subtypes [2; 3].

In the field of NHL studies, the focus is on the prognostic value of various morphological variants of the disease, with diffuse large B-cell lymphoma being defined as the most unfavorable [4; 5].

All of the above confirms the extreme relevance of the problem of diagnosis and treatment of primary diffuse large B-cell lymphoma with extranodal lesions and is an unconditional basis for special research in this area. Due to the fact that a relatively small number of works have been done on this topic, there are still a lot of questions in determining the prognosis in patients with diffuse large B-cell lymphoma and the correct management of patients, and the role of immunotherapy in the complex treatment of primary

diffuse B-large cell lymphoma is also relevant. lymphomas with extranodal lesions [6; 7; 8].

Materials and methods

The study included 81 patients with diffuse large B-cell lymphoma with extranodal lesions, who were examined and treated from 2015 to 2021.

For a six-year follow-up in patients with this form of the disease, 81 patients under our supervision, despite the same treatment, five-year survival was 60.5%. In 32 (39.5%) patients during treatment, a lethal outcome was noted due to the progression of the pathological process. To study the causes of an unfavorable outcome of the disease, we conducted this study, so that in the future, taking into account and influencing the factors that affect the final result, it will improve the long-term results of treatment.

In our work, in case of suspected lymphoma, to establish the diagnosis of the disease, we performed morphological studies of the tumor tissue. After identifying the non-Hodgkin nature of the tumor, morphological studies were performed with immunophenotyping.

The purpose of immunophenotyping at the first stage was to determine the B- and T-cell origin of the tumor, as well as the degree of violation of cell differentiation and the similarity of the tumor tissue to a certain anatomical zone of a normal lymph node. This research method is the most highly informative part of the complex diagnosis of non-Hodgkin's lymphoma (NHL).

All patients had a morphologically verified diagnosis – diffuse large B-cell lymphoma. B-cell lymphoma is subdivided into progenitor B-cell lymphomas and mature B-cell lymphomas, which include:

- small cell lymphocytic lymphoma;
- lymphoma from cells of the marginal zone of the spleen;
- extranodal mucosal-associated B-cell lymphoma (MALT-lymphoma);
- nodal B-cell lymphoma from cells of the marginal zone;
- follicular lymphoma;
- mantle cell lymphoma.

B-large cell diffuse lymphoma: (mediastinal or thymic and intravascular large B-cell lymphoma). Mediastinal and intravascular large B-cell lymphoma is one of the morphological subvariants of diffuse large B-cell lymphomaThe mediastinal is localized in the anterior mediastinum and originates from the thymus, and the physiological representatives of lymphoma cells are thymus B cells. Intravascular large B-cell lymphoma is extremely rare. It is characterized by an aggressive course and has an unfavorable prognosis. It affects the skin, lungs, kidneys, adrenal glands, central nervous system.

In this paper, we will consider the diagnosis and treatment, only B-cell diffuse lymphoma.

Diffuse large B-cell lymphoma is always established only on the basis of a morphoimmunohistochemical study of tumor tissue obtained by biopsy or surgical material. Cytological examination of the material obtained by puncture or smear imprints of lymphatic and other tumor nodes is not sufficient to establish this diagnosis. In all cases, histological and IHC examination of incisin or excision material was performed.

In an excisional biopsy, the earliest lymph node that appears is taken and completely removed. When removing the assembly, it must not be mechanically damaged. It is undesirable to use inguinal lymph nodes for histological examination if there are other groups of lymph nodes involved in the process. Histological and immunohistochemical examination of the tumor is carried out.

Only with a deep location of the tumor node, tissue material was obtained using core-biopsy – biopsy. The morphological substrate of DLBCL is usually represented by immunoblasts, centroblasts, cells with multilobed nuclei, polymorphic (anaplastic) nuclear cells. Based on this, three variants of DLBCL are distinguished: immunoblastic, centroblastic, anaplastic (or polymorphic).

The DLBCL phenotype is determined by the expression of CD20, CD79a, PAX 5 (monomorphic intense nuclear expression), CD45. Some cells express CD30 with polymorphic morphology. The expression of CD10, BCL – 6, BCL – 2, MUM.1, Ki – 67, CD – 5. When CD – 5 was expressed, IHC with the cyclin D1 antibody was performed to exclude the polymorphocellular blastoid variant of lymphomas from the mantle layer.

Considering the importance of morphological characteristics, the morphological study protocol contained the following:

a) The protocol indicated the macroscopic characteristics of the material to be studied, the number of finished blocks and micropreparations.

b) Histological description of the tumor. In this case, after determining the lymphoma, it is necessary to indicate the type of tumor growth (diffuse, nodular, follicular, etc.), its cellular composition (large or small cells, polymorphic, anaplastic, blast, multinuclear forms, description of the morphology of the nuclei), the presence of reactive and residual components.

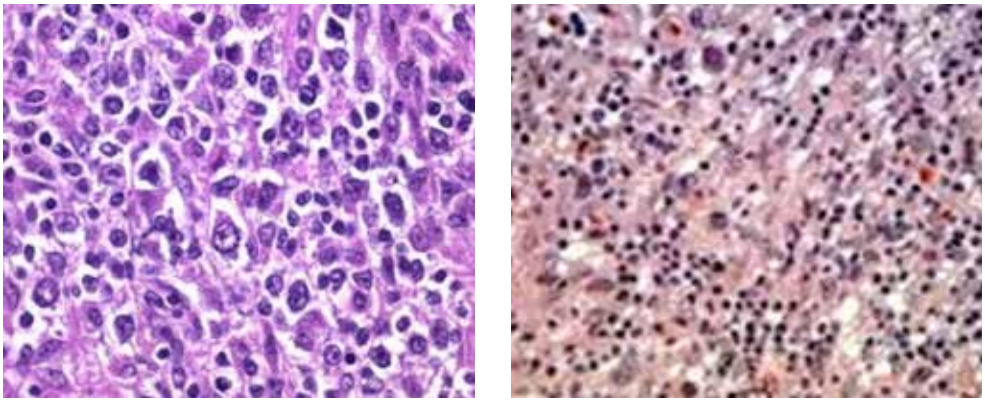
C. In the IHC study, the conclusion is given indicating the antibodies used, their type and staining method. The description is also subject to specific characteristic staining reactions of nuclei, cytoplasm, membranes, etc.

Conclusion in accordance with the international classification.

Research results

As is known, in our study we selected only patients with diffuse B-large cell lymphomas. This lymphoma is characterized by increased aggressiveness.

In diffuse large B-cell lymphoma, tumor cells are found throughout the lymphatic tissue. Histological examination revealed large B-lymphoid cells with pronounced atypia and polymorphism in the lymph nodes, with a large nucleus, several times larger than the size of the nucleus of a small lymphocyte. Malignant cells are mostly diffuse, rarely scattered among mature B-lymphocytes, at times against the background of a T-cell environment, or create concentrated foci.



a) b)

Figure 1. Large B-cell lymphoma. a) Centroblast variant of DLBCL; b) Mixed-cell variant of diffuse B-large cell lymphoma

As a result of a standard histological examination, in 49 (60.5%) patients, a centroblastic (Fig. 1 a) morphological variant of diffuse extranodal B – large cell lymphoma was established, and in 32 (39.5%) cases, a mixed-cell variant of the tumor (Fig. 1 b).

All patients were diagnosed with diffuse large B-cell lymphoma. There are different types of diffuse large B-cell lymphoma, which can only be correctly identified using IHC studies.

Table 1. – Results of IHC study of patients with diffuse B-cell lymphoma with extranodal lesions

Type B-large cell lymphoma	number of cases
Primary mediastinal DLBCL.	16 (19,8%)
Primary DLBCL of the CNS.	5 (6,2%)
Intravascular DLBCL.	15 (18,5%)
Lymphomatoid granulomatosis.	13 (16,0%)
Первичная ДВККЛ серозных полостей.	5 (6,2%)
DLBCL with T-cell predominance.	11 (13,6%)
ALC positive DLBCL..	3 (3,7%)
Plasmablast DLBCL.	2 (2,5%)
HHV8 positive DLBCL, NOS.	7 (8,6%)
Large cell lymphoma with IRF4 rearrangement	4 (4,9%)

Without fail, all patients included in our study underwent an IHC study to determine the tumor subtype (Table 1).

We observed 81 patients with 10 types of DLBCL. The most frequently identified primary mediastinal form (19.8%), intravascular form (18.5%), Lymphomatoid granulomatosis, which in the usual histological examination is confused with Hodgkin's lymphoma (16%) and with the predominance of T – cells. Less common were plasmablastic, ALC positive, with IRF4 rearrangement, and primary DLBCL of serous cavities.

For us, in terms of prognosis, it was more interesting to study the immunophenotype of DLBCL. In this connection, we studied the degree of expression of a number of antigens in correlation with the long-term results of the treatment.

We have studied the expression of pan-B-cell antigens CD20, CD79a, PAX 5 (monomorphic intense nuclear expression), CD45, CD30, BCL-6, Ki-67, BCL-2, MUM.1, GCET1, FOXP1, CD5. To exclude polymorphocellular blastoid variant of lymphoma in the case of CD5 expression from mantle cells, cyclin D1 was studied.

The group of antigens (or markers) referred to as CDs are the cluster of differentiation of human leukocyte antigens.

We want to give a brief description of each marker:

- T1 lymphocyte receptor CD5 is a membrane protein, a receptor, expressed on the outer membrane of T- and B-lymphocytes of subgroup B-1a. Controls the proliferation of T-lymphocytes;
- B – CD10 lymphocytic antigen – a protein, a co-receptor, is located on the surface membrane of B-lymphocytes. The function of this protein has not been established, it is assumed that it is involved

in the activation and proliferation of B-lymphocytes;

- CD30 is a cell membrane protein from the tumor necrosis factor receptor family;
- CD45 (receptor type tyrosine protein phosphatase C) is an enzyme encoded by the PTPRC gene. They are components of cell signaling pathways, regulate cell growth, differentiation, mitotic cycle and malignant transformation;
- CD79 is a membrane protein that forms a complex with the receptor and is involved in signal transduction after the receptor binds to the ligand;
- PAX-5 encodes B-cell activator protein (BSAP); marker of B-lymphoblastic neoplasms. Diffuse large B-cell lymphomas express PAX-5, except in cases with terminal B-cell differentiation. There is a close association of this antigen with CD20 expression;
- Bcl-2 is a regulator of apoptosis, a protein factor located intracellularly. The main task of this gene is to suppress apoptosis and regulate cell death by controlling the permeability of the mitochondrial membrane;
- Bcl, transcription factor 6, proto-oncogene. BCL6 is involved in the formation of germinal centers in lymphoid follicles;
- MUM1 is a regulator of interferon factor 4, also known as a protein that in humans is encoded by the IRF4 gene;
- FOXP1 is involved in the stimulation of the expression of pluripotency genes (OCT4, NANOG, NR5A2 and GDF3), while simultaneously suppressing the expression of genes that promote cell differentiation.

The definition of markers in oncology is used for immunophenotyping. The presence of established molecules may be associated with proper immune functions. Although the presence of one type of marker usually does not allow one to accurately determine the population of a cell (with the exception of a few examples), combinations of markers make it possible to establish it quite distinctly.

We were more interested in the influence of the presence of certain markers on the outcome of the disease. In this connection, in the first stage, we studied the self-sharing distribution of these antigens in the sample of patients we studied (Table 2).

Table 2.– Immunophenotypic pattern of patients with diffuse B-cell lymphoma with extranodal lesions

markers	expression averages	
	number of cases	percent
1	*2*	*3*
CD – 5	22	27,2
CD – 10	46	56,8
CD – 30	31	38,3
CD – 45	67	82,7
CD – 79	49	60,5
PAX-5	33	40,7
Bcl-2	38	46,9
Bcl-6	61	75,3
MUM1	55	67,9

1	*2*	*3*
FOXP1	27	33,3
Ki-67	57	70,4
GCET1,	33	40,7

Since the markers studied by us are traditionally used to diagnose this pathology, as we see from the data table, all the studied markers in DLBCL patients had moderate or high expression. Moderate expression was characteristic of markers CD-5, CD-30, PAX-5, FOXP1, and GCET1. High CD – 10, CD – 45, CD – 79, Bcl-6, MUM1 and Ki-67.

Before the start of treatment, after determining the nosology, the stage of the tumor process and its phenotyping, the patients underwent a set of diagnostic measures aimed at assessing the somatic state. Clinical and laboratory blood parameters were studied for all patients, ECG studies were performed, and, if necessary, consultations of narrow specialists were carried out.

The revealed changes in the body of patients corresponded to changes in the general population, and were of little use for use as a prognostic criterion. Some changes in clinical and biochemical parameters were subject to correction, and corresponded to the indicators of patients characteristic of oncological patients. In view of this fact, we refused to present in detail the identified changes in this work. In this chapter, we present only data that determines the risk affecting the outcome of the disease, the distribution of patients according to the International Prognostic Index IPI (Table 3).

Table 3.– Distribution of patients with DLBCL depending on the prognostic index IPI

Degree of risk	**number of patients**	
	abs.	**percent**
0–1 factor – low risk	8	9.9
2nd factor – low/intermediate risk	17	20.9
3rd factor – high/intermediate risk	25	30.9
4–5 factor – high risk	31	38.3

High and high-intermediate risk, according to the IPI criterion, was detected in 56 patients (69.1%), low risk in only 9.9%, and only 1/5 cases (20.9%) revealed low-intermediate risk.

Conclusions

Thus, the patients included in the study were characterized by a wide range of DLBCL varieties, high expression of the studied markers, and a relatively high prognostic risk according to the IPI criterion.

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