

UNIVERSITY OF MEDICINE AND PHARMACY CRAIOVA

**PRINCIPLES AND DIAGNOSIS METHODS IN MALIGNANT
LYMPHOMAS WITH E.N.T LOCALISATION**

DOCTORAL THESIS SUMMARY

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SUMMARY

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Key words : NonHodgkin malign lymphoma, Hodgkin disease, histopatology, Large B cell difuse lymphoma (DLBCL), Waldeyer, lymph node, imunohistochemy, antibodies CD79a, CD20, CD23, CD5, CD10, mantal zone lymphoma,Bcl2, D1 Cycline, B Granzime, Ki67, CD4+, CD24+, Vimentin.

1. Introduction, work motivation .

Lymphomas are malignancies of the immune system cells located in various tissues , which are distinguished from leukemia by the lack of the clinical onset. Thomas Hodgkin is credited with first recognizing them , starting from the observation that lymphadenopathy may be secondary to the primary disease and no lymph node manifestation of an infection or cancer .

Initially the diagnosis was just clinical then, in 1900 came the first elements of histopathology. In the 70s there were developed immunopathology techniques , which allowed differentiation of different subtypes of lymphoma based on immunopathological characters . The '80s brought breakthroughs in molecular genetics , which led to a better understanding of the genetic mechanisms involved in the pathogenesis of lymphomas .

With the development of medical imaging techniques such as high performance computerized tomography, magnetic resonance imaging , early diagnosis was much easier . The incidence of lymphomas has experienced significant growth in recent years, in part due to modern methods of diagnosis.

In ENT neoplasia, lymphoma is a particularly important entity , insidious through the poverty of the early signs . Interdisciplinary collaboration is particularly important in the early diagnosis of lymphoma , which discovered in time , as we see from a properly driven therapies has great chances of entry into complete remission . This possibility of positive evolution and the recent discoveries of molecular genetics led me to choose this work , hoping that through it we can help at all in understanding and diagnosing this formidable disease that is the lymphoma .

ENT specialists are often involved in the diagnosis of lymphoma. A quarter of all lymphomas occur in lymph node belonging to the head and neck . Lymphoma is considered the second type of primitive malignant lesion that occurs in the head and neck. Some studies consider that lymphoma is the most common cause of cervical lymphadenopathy rather than metastatic disease. It is important that non -Hodgkin's lymphoma incidence has increased steadily in recent decades.

Malignant non Hodgkin's lymphomas are more common than Hodgkin's disease . These lymphomas have a variety of subtypes. One of the most common subtypes are found in adults is diffuse B-cell lymphoma , showing a high degree of aggressiveness .

Extranodal lymphomas have a predilection for certain locations such as head and neck and the digestive system .Follicular lymphoma is also frequent , but its performance is less aggressive , as MALT lymphoma (mucosa - Associated lymphoid tumor) .

2. The objectives of this study were to synthesize the methods of diagnosis and staging of malignant lymphoma , an important emphasis is placed on the study of different forms of lymphoma's immunohistochemistry .

I proposed conducting a retrospective study on a group of 79 patients admitted during 2000-2011 , with the following main objectives:

- Evaluation of patients using a diagnostic algorithm including clinical parameters , biological imaging and histopathology and immunohistochemistry of biopsy fragments
- Highlighting features of the lot in terms of histopathologic and immunohistochemical investigations used in the diagnosis process.
- Using the Research immunohistochemical markers in nodal lesions (in selected cases) to highlight significant changes and their correlation with clinical and morphological aspects ;
- Identification of prognostic factors based on clinical and immunohistochemical characteristics .

3. Materials and methods

We reviewed the cases of non Hodgkin's lymphoma of the head and neck diagnosed Otolaryngology Clinic of the Emergency County Hospital Craiova between 2000 to 2011 . All cases studied showed ENT localization . Previously patients were informed and gave their consent to the use of data in this study.

From observation sheets were recorded the following information : age , sex, presentation , clinical symptoms , staging , treatment and evolution. Study lot consisted of 79 tumors , which were investigated using morphological analysis (macroscopic and microscopic) molecular biology methods and statistical analysis and Biomathematics .

In the morphological investigation routine ,the tissue samples were fixed in 10% formalin , included in paraffin , sectioned at 3-5 microns thick microtome and were stained using standard staining technique (hematoxilin- eosin and Giemsa) , applied to all cases studied, which allowed the grouping and classification of lesions based on histology and morphological assessment.

Fixing aimed to preventing alteration of cellular structures and tissue removed from the body by preventing autolysis and cell and tissue preparation for a proper coloring . Determining a duration 36-48 hours at laboratory temperature (20-25 ° C) in a volume of fixative which exceed 10-20 times the volume of the part to be fixed . After fixing the pieces were washed with running

water for about 30 minutes .

These fragments were processed in the usual histological technique resulting paraffin wax blocks containing fragments studied. Inclusion of the wax was the impregnation and fixing parts in paraffin oil.

Immunohistochemical reactions were performed on 4 micron sections included in paraffin blocks obtained , which was spread on glass slides pre-treated with polylysine or an electrical charge .

The detection and visualization was used EnVision kit , Dako , Glostrup , Denmark , a method of immunostaining two stages , based on the conjugation of a polymer with secondary antibody HRP . For this study were users from following antibodies : CD79a , CD20 , CD23 , DC5 , CD10 , Vimentin , Bcl12 , Bcl16 , cyclin D1 , Ki67 , GranzinaB , cytokeratins .

4.Results and Discussion

During 2000-2011 , in the ENT Clinic of Craiova , out of the 26 613 patients admitted , 2165 were patients diagnosed with various forms of cancer and the ENT Patients diagnosed with lymphoma were thus 0.29 % of total cases and 3.64 % of all cancer patients , an incidence much higher compared to the incidence in the general population , mainly due to a thorough investigation of the case admitted to the ENT clinic.Although we can not discuss screening tests themselves, absolutely all patients who raised the suspicion of the existence of malignancies of any kind in ENT benefited from the advantages of high performance imaging explorations (Doppler ultrasound , CT, MRI substance contrast), which led to decrease false negative cases .

4.1. Depending on the gender distribution we found a male : female of 0.92 , which is consistent with the data from literature . We can not say that there is a statistically significant difference between the sexes.

4.2 Age of patients included in the study ranged between 28 and 92 years , with an average of 63.08 years , without identifying significant differences statistically between the average age for 5 men and women. In literature it is described as average age of identification of this pathology decade 6 (67 years old) or decade 7 (71 years old) . In our study , 41.77 % of cases were aged below 60 years.

NHL were identified less frequently in the first decade of age , increasing to 5-8 decades , which represented the maximum incidence.

4.3 Head and neck represents the second region in the frequency location extranodal lymphomas after the gastrointestinal tract .

About 2.5 % of malignant lymphomas arising in Waldeyer's ring (for example tonsil , the nasopharynx , the tongue) . NHL topographic distribution showed the highest frequency in the

Waldeyer ring (48% cases) , in accordance with the literature (Kemp , 2008) , followed by laterocervical lymph nodes (34%). Other locations had lower frequencies : sinonasal (9%) , larynx and oral cavity and hypopharynx 4% (1%).

4.4 From the point of view of the accompanying systemic symptoms , they were absent in 31 patients and was characterized by night sweats, fever , weight loss of more than 10 % of body weight in the remaining 38 patients.

Symptoms most commonly described by patients in the study was the presence of persistent painless lymphadenopathy (84.81 %). Symptoms of type B is rarely described in association with NHL and was identified in 48.01 % of cases.

4.5 In terms of histological subtypes , we identified a significant share of B lymphomas (97,46 %). Of type B lymphoma , the most common was diffuse large B cell lymphoma (63%) , followed by follicular lymphoma (11%), diffuse lymphocytic (6%), MALT lymphoma and Burkitt (5%) , lymphoma of the mantle (4%) , plasmacytoma (3%) .

The most common type of lymphoma in the head and neck met in our study was diffuse large B cell lymphoma (DLBCL) - 48 cases. The number of cases with this pathology often was located in the Waldeyer ring (29 cases) , followed by those located in the lymph nodes (17 cases). Other locations were found in a small number of cases: 4 cases at sinonasal and only 1 case with parotid location .

4.6 Using standard procedures for staging (Ann Arbor) , we identified 12 patients in stage I, 12 in stage II , 15 stage III and 11 stage IV.

4.7 The most frequent was the centroblastic (37 cases) , showing similar neoplastic cells reactive centroblasts and germinative centers with reduced amphophile cytoplasm and vesicular nuclei and nucleoli .The Immunoblastic variant , found in 11 cases was characterized by the predominance of similar cells – immunoblastic cells with a single prominent nucleolus and abundant basophilic cytoplasm .Anaplastic variant was found in 2 cases and was characterized by the presence of large cells with marked pleomorphism .

4.8 In terms of neoplastic cells expressed CD20 immunohistochemistry in all cases Cd79a in 46 cases and BCL2 , Ki 67 in 13 cases .

4.8 In terms of location we have identified the presence of similar proportions to the Waldeyer ring (4 cases) and lymph nodes (3 cases) . Other sites have been found in a few cases with 4 cases at sinonasal and only 1 case with parotid location .

4.9 In terms of MALT -type lymphoma , they were found in 4 cases with localization in equal proportions in the Waldeyer ring (1 case) , larynx (1 case) , hypopharyngeal (1 case) and parotid gland (1 case) .

A particularly important aspect was that MALT lymphoma localized to the parotid gland was

identified in a young woman patient under observation for Sjogren's syndrome .

4.10 Mantle zone lymphoma is a B -cell lymphoma found in our study in 3 cases , localized to the Waldeyer ring (2 cases) and lymph node (1 case) .

The mantle cell lymphoma (3 cases) showed diffuse lymphoid infiltrate with nodular aspect , without the presence of proliferation centers . The tumor cells were monotonous nuclei with varying degrees of indentation and angulation, chromatin condensation and reduced cytoplasm .

4.11 Burkitt lymphoma was found in 3 cases . Morphological appearance of these entities included sized cells with nucleus and nucleolus located paracentral and basophilic cytoplasm arranged in a cohesive monomorphic pattern . We have also identified numerous mitosis . The presence of macrophage tumor cells created a characteristic " starry sky "

4.12 Regarding NK lymphomas / T, our study identified two cases in male patients aged 53 and 57 years. Small cell histological pattern identified with angulated nuclei and granular chromatin and cell medium / large granular chromatin and visible nucleoli . Squamous epithelium infiltrated lymphoid proliferation in the presence of large areas of necrosis and was characterized by patterns of growth and angiodestruction . In these cases, the reactions were carried out in situ hybridization to identify Epstein Barr virus (EBV) . This reaction positivity supported the diagnosis of lymphoma NK / T as peripheral T-cell lymphomas that express CD56 are often EBV negative .

5. Conclusions .

- In the group of patients studied , histopathological analysis of biopsies taken from patients confirmed the clinical diagnosis .

Symptoms most commonly described in patients in the study was the presence of persistent painless lymphadenopathy (84.81 %). Symptoms of type B is rarely described in association with NHL and was identified in 48.01 % of cases.

Clinical reasons for admission to 67 of the patients included in the study were painless and only 7 persistent lymphadenopathy in 12 patients has been described to accelerate tumor growth , accompanied by pain .Accompanying systemic symptoms were absent in 31 patients and the remaining 48 patients were characterized by night sweats, fever , weight loss more than 10 % of body weight .

- The most frequent location was the tonsils and lymph laterocervical and the rarest of the larynx and hypopharynx .

Most common histological types were diffuse large B cell lymphoma (51 cases) , follicular 9 cases, 4 cases MALT , mantle zone lymphoma and Burkitt -like lymphoma by 3 cases plasmacytoma and lymphoma NK / T type nasal in 2 cases.

- The most common type of lymphoma in the head and neck in our study was diffuse large B cell lymphoma (DLBCL) - 51 cases. The number of cases with this pathology often was located in the Waldeyer ring (29 cases. Other locations have been found in a few cases (4 cases at sinonasal and 1 single case with parotid location) .
- Age greater than 60 years and a bad clinical performance status are negative prognostic factors , even associated with low histological types of malignancy .
- Lymphoma localized in the cavum has insidious onset , often the first manifestation is 1 laterocervical lymphadenopathy . Histologically , we meet both B cell shape and cell shape T.
- Every form of non -Hodgkin lymphoma presented various microscopic aspects , for each subtype there is a microscopic pattern that is most frequent . Lymphoid tissue architecture in all cases had been replaced by proliferation of malignant lymphocytes consistent with the microscopic characters of the form of lymphomawhich it belongs.
- Histopathological subtypes of DLBCL were centroblastic (37 cases) , immunoblastic 11 cases , anaplastic (2 Cases) , plasmablastic 1 case .
From the point of view of cancer cells expressed markers for immunohistochemical pan B in nearly all cases (49) ; CD20 in 48 cases in 50 cases Cd79a , aberrant phenotypes were found in two cases, BCL2 was positive in 43 cases , Ki 67 were positive for more than 60 % in 39 cases .
- Diffuse lymphocytic lymphoma was identified in 5 cases and characterized by deletion of the microscopic nodal architecture , with the presence of lymphoid infiltrates in 3 hyperchrome punctual presence of outbreaks of pale , round centers of proliferation . Pan B markers showed immunoreactivity in tumor cells, but CD20 had a lower intensity than Cd79a . We also currently identified for CD5 and CD23 immunostaining and absence from CD20 .8
- Regarding lymphoma of MALT type , they were found in 4 cases with localization in equal proportions in the Waldeyer ring (1 case) , larynx (1 case) , hypopharynx (1 case) and parotid gland (1 case) . A particularly important aspect was that MALT lymphoma localized to the parotid Landei was identified in a patient with Sjogren's syndrome .
- Mantle zone B -cell lymphoma was found in our study in 3 cases , localized to the

ring Waldeyaer (2 cases) and lymph node (1 case) .

- Lymphoma NK / T in our study , was identified in two cases, male patients aged 53 and 57 years. Identified immunophenotype was CD2 positive cytoplasmic CD56 and CD3e positive and negative TCR .
- 17 . Lymphomas of the head and neck is the second in extranodal lymphomas after the digestive system and pathogenesis involved viral risk factors (EBV), primitive and secondary immunodeficiencies and toxic factors (pesticides) .

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