

## Non-Hodgkin lymphomas of Waldeyer's ring

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### Abstract

Lymphomas represent malignant lymphoproliferative diseases and they are generally classified as Hodgkin's (HL) or non-Hodgkin malignant lymphomas (NHML). Head and neck lymphomas represent one of the most common sites of extranodal lymphomas, second after the gastrointestinal tract. Waldeyer's ring structures include the palatine tonsils, the nasopharyngeal lymphatic tissue, and the lingual tonsil. We investigated 38 patients with malignant lymphoma with ages ranging from 21 to 95 years, all localized in the Waldeyer's ring. Good knowledge of the clinical characteristics of these lymphomas and the methods to establish the differential diagnosis are essential for a correct therapy of the disease.

**Keywords:** lymphoma, Waldeyer's ring, non-Hodgkin.

### Introduction

Lymphomas represent malignant lymphoproliferative diseases and they are generally classified as Hodgkin's (HL) or non-Hodgkin malignant lymphomas (NHML). In the last two decades, there was an increase in the number of non-Hodgkin malignant lymphomas in general and especially in the number of extranodal lymphomas. Head and neck lymphomas represent one of the most common sites of extranodal lymphomas, second after the gastrointestinal tract [1].

Extranodal lymphomas can occur in almost all organs. However, the most common extranodal locations can be grouped: skin, stomach, brain, small intestine, and, although still controversial, Waldeyer's ring [2].

About 2.5% of these lymphomas have oral and perioral location, most of them appearing in Waldeyer's ring. Waldeyer's ring structures include the palatine tonsils, the nasopharyngeal lymphatic tissue, and the lingual tonsil. The most common type of lymphoma with this location is the diffuse large B-cell lymphoma (DLBCL), which represents about 30% of all lymphomas. Although considered as a single entity, DLBCL includes different subtypes with clinical and pathological differences. In fact, their morphology, immunohistochemistry and genetics are different, recently being described two types, some from germinal centers, others from activated germinal centers [3].

### Materials and Methods

Our study was both retrospective and prospective and involved patients with NHML that were diagnosed and treated in the Department of Ear, Nose and Throat (ENT) of the Emergency County Hospital of Craiova, Romania, between 2002 and 2012. We evaluated clinical and laboratory parameters collected from the medical records of the patients.

Tissue specimens were processed and embedded in paraffin. Sections were cut at 5-μm and mounted on poly-L-Lysine coated glass slides. The slides were dried overnight and then routine dewaxing and dehydration were carried out. The sections were routinely stained using Hematoxylin and Eosin stain in order to study the basic morphology.

The histological diagnosis was carried out according to the *Revised European American Classification of Lymphoid Neoplasms/World Health Organization classification (REAL/WHO)*.

Immunohistochemistry was performed on histological sections of formalin-fixed paraffin-embedded tissue samples. The sections were deparaffinized, hydrated, subjected to antigen retrieval for 30 seconds at 120°C, and 10 seconds at 90°C, with 10 mM sodium citrate (pH 6). Peroxide blocking was performed with 3% hydrogen peroxide in methanol at room temperature for 15 minutes, followed by PBS for 20 minutes. Sections were then washed with Tris-containing buffer and incubated for 90 minutes with the primary antibody at

room temperature. Sections were washed and incubated with Biotinylated Link Universal and Streptavidin AP for 30 minutes (LSAB+, Dako) and development with 3,3'-diaminobenzidine chromogen for 5 minutes, rinsed in distilled water, counterstained with Hematoxylin (Dako), and mounted on glass slides prior to evaluation under the microscope.

Antibody dilutions varied during the interval of the study and were used according to the datasheet. Clones and dilutions used are shown in Table 1.

**Table 1 – Antibodies used for the immunohistochemical study**

Antibody	Clone	Manufacturer	Dilution
CD20	MS4A1	Dako	1:100
CD5	4C7	Novocastra	1:50
Bcl-2	L1	Dako	1:50
Cyclin D1	CCND1	Dako	1:100
Ki67	MK167	Dako	1:100
CD79a	JCB117	Dako	1:30
Bcl-6	Bcl6	Novocastra	1:20
CD23	B12	Novocastra	1:100
CD10	56C6	Novocastra	1:100
CLA	DF1485	Novocastra	1:50
Vimentin	V9	Dako	1:100
Cytokeratins	MNF116	Dako	1:50

## Results

A total of 38 patients with malignant lymphoma were included in the study with ages ranging from 21 to 95 years and a mean age of 64 years. The male to female ratio was 0.8/1. Twelve patients experienced night sweats, fatigue, weight loss, fever (B symptoms); dysphagia and dysphonia were identified in 28 cases.

All cases were localized in the Waldeyer's ring. Otolaryngology examination revealed unilateral tonsillar swelling in 21 cases (Figure 1) and bilateral swelling in seven cases. The second most common localization was the rhinopharynx with eight cases (Figure 2), and only two cases in the base of the tongue. A thorough examination of the head and neck was performed (CT, MRI to determine the extent of involvement) and simultaneous nodal involvement was found in 12 cases.

The following histological types were identified (Table 2):

**Table 2 – Histological types of lymphoma**

NHML	No. of cases
Follicular	4
Mantle zone	2
Burkitt-like	3
DLBCL	29
Total	38

There was no difference in the histological features between extranodal tumor and nodal involvement.

Follicular lymphoma was diagnosed in four cases. Patients were women aged between 79 and 95 years. ENT examination found a unilateral enlargement of a tonsil, tumor ulceration being present in all cases. Histological findings included a nodular proliferation with formation of different sized pseudo-follicles with decreased or absent mantle zone, different from the

reactive follicles with germinal centers, numerous centroblasts and centrocytes, tingible macrophages and mantle zone. The interfollicular area was reduced to absent in most cases, with sometimes the presence of diffuse areas with centrocytes. The tumor cells identified were a monotonous population of small cells with irregular nuclei (centrocytes) with a few large cells (centroblasts) with grooved nuclei with one or more basophilic nucleoli, with vesicular chromatin.

The histological grading was established using the WHO classification (2009). The majority of the cases (three cases) were stage I, while the 4<sup>th</sup> was stage II.

From the immunohistochemical point of view, tumor cells expressed CD20 in all cases and other B-cell antigens (CD79a) in the majority of cases (Figure 3). Bcl-2 was positive in 31 cases and Bcl-6 in 24 cases. CD23 was positive focally in the nodular structures. CD5 was negative in all cases.

Mantle zone lymphoma is a B-cell neoplasm composed of small and medium sized cells and was diagnosed in two cases in a man and a woman aged 61 and 81 years respectively. There was an enlargement of the tonsils with presence of ulcerated areas, while the microscopic pattern showed diffuse monomorphic lymphoid proliferation and vaguely nodular pattern. Tumor cells were small and medium sized lymphocytes with nuclei with irregular contour (Figure 4).

Immunohistochemically, the cells are positive for CD20, CD5, and cyclin D1 (Figures 5 and 6).

Burkitt-like lymphoma was diagnosed in three cases and lymph node invasion was present in all cases. Patients were aged between 21 and 53 years, two men and a woman. HIV infection was confirmed in one case. Histologically, there was diffuse infiltration with atypical lymphocytes with numerous mitoses with starry sky appearance due to tingible macrophages. Tumor cells were positive to CD20, CD79a, CD10 and Bcl-6 and negative to CD5 and Bcl-2.

DLBCL was diagnosed in 29 cases, out of which 15 were females. The age range was 45–95 years, with an average of 62 years. Several morphological variants of DLBCL have been described: centroblastic, immunoblastic, plasmablastic, T-cell/histiocyte-rich, and anaplastic B-cell lymphoma (lymphoma, usually kinase-positive anaplastic). While pathological evaluation can distinguish between these alternatives, their prognostic significance remains controversial. In our study, the following subtypes were identified: centroblastic in 12 cases, immunoblastic in 15 cases and anaplastic type in one case. Histological features revealed round cells with hyperchromic irregular nuclei and scanty to moderate amount of cytoplasm. Tumor cells were similar to centroblasts and immunoblasts with round nuclei with nucleoli attached to the nuclear membrane (centroblasts) or central nucleoli (immunoblasts) (Figure 7).

Nine of the cases studied presented necrosis and ulceration and were all developed in a tonsil.

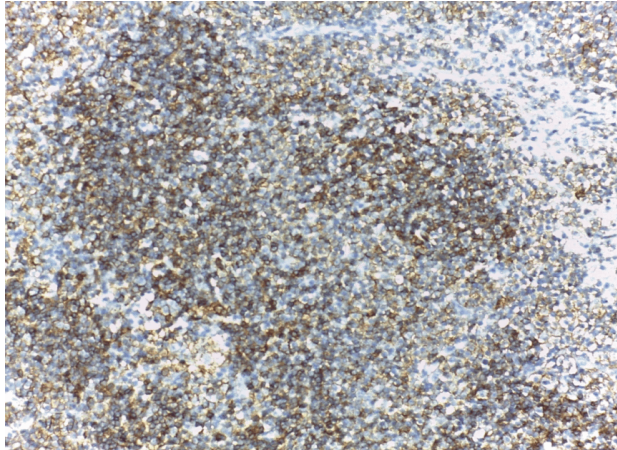
From the immunohistochemical point of view, there was positivity for CLA, CD79a and CD20, and negativity for cytokeratins, vimentin. In all cases, we noted the presence of large B-cells with a high proliferation index (Figure 8).



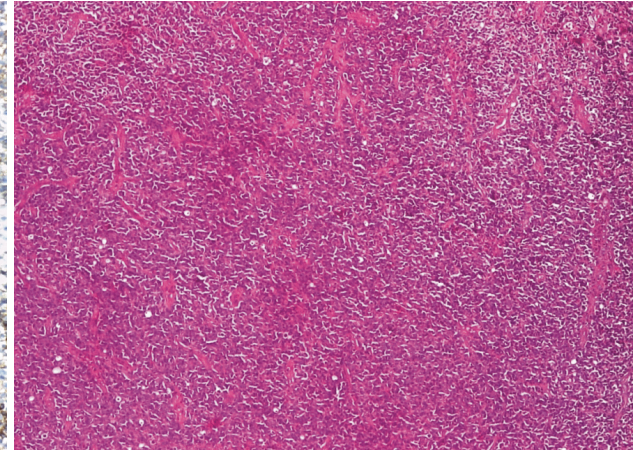
**Figure 1 – Non-Hodgkin lymphoma localized on left palatine tonsil.**



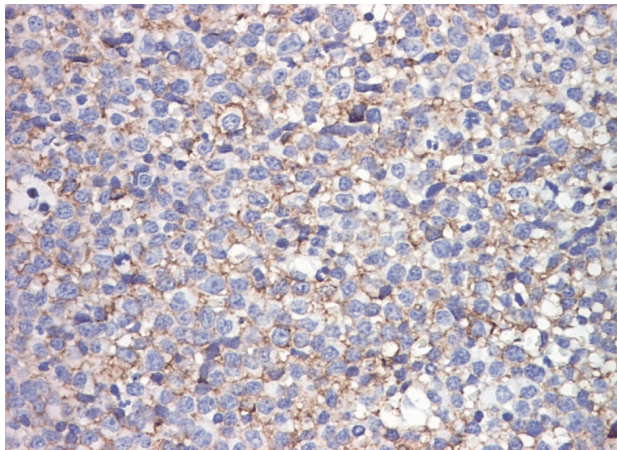
**Figure 2 – Rhinopharyngeal non-Hodgkin lymphoma.**



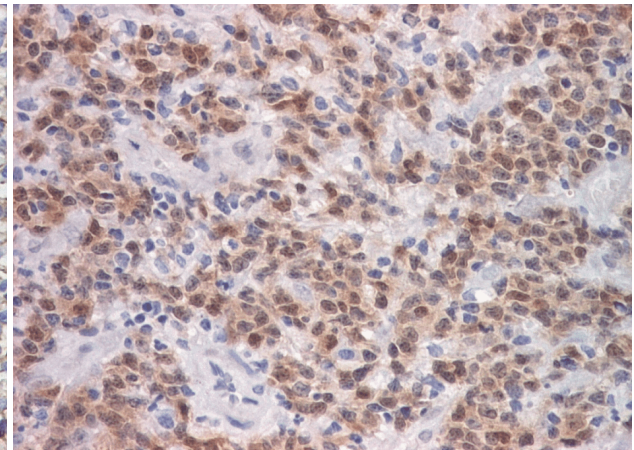
**Figure 3 – CD20-positive follicular lymphoma (immunohistochemical stain,  $\times 100$ ).**



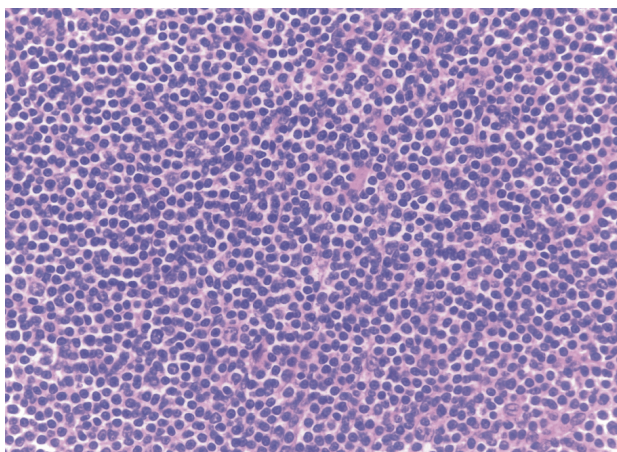
**Figure 4 – Mantle cell lymphoma (HE stain,  $\times 40$ ).**



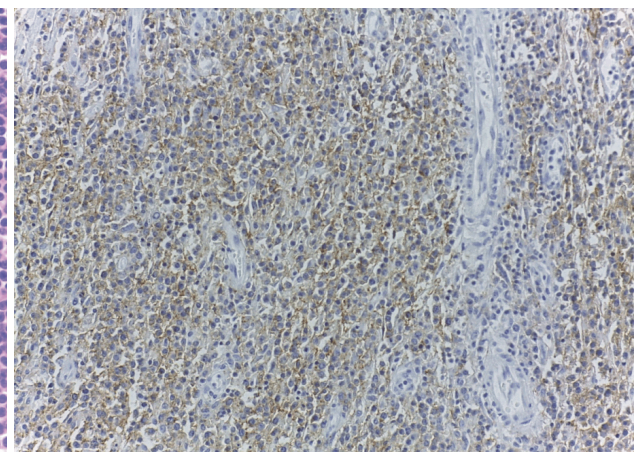
**Figure 5 – CD20-positive mantle cell lymphoma (immunohistochemical stain,  $\times 200$ ).**



**Figure 6 – Cyclin D1-positive mantle cell lymphoma (immunohistochemical stain,  $\times 200$ ).**



**Figure 7 – Diffuse large B-cell lymphoma (HE stain,  $\times 200$ ).**



**Figure 8 – CD20-positive diffuse large B-cell lymphoma (immunohistochemical stain,  $\times 200$ ).**



## Discussion

Non-Hodgkin lymphomas (NHL) arise in about 20–30% of the cases from extranodal sites [4] and the head and neck are some of the most common sites of extranodal non-Hodgkin lymphoma, commonly involving oropharyngeal lymphoid tissue comprising Waldeyer's ring, but also infiltrations of non-lymphoid tissue. The most affected sites are tonsils (55% of oral cases), palate (30% of cases) and genial mucosa (2% of cases) [5].

Waldeyer's ring includes tonsils in the oropharyngeal sidewall (palatine tonsils), rhinopharyngeal tonsils, pharyngeal lymphatic tissue and lingual tonsil [6]. Waldeyer's ring is the most common site of lymphoma of the head and neck, representing over 50% of the cases [7] and extranodal non-Hodgkin lymphoma (NHL) located here account for 10–20% of all cases of NHL [8].

Waldeyer's ring is considered in *Ann Arbor* classification [9] as a lymphatic tissue, together with the thymus, spleen, appendix, and Peyer's patches of the small intestine and therefore involvement of these areas does not constitute an 'E' lesion, mainly because primary extranodal involvement is rare in patients with Hodgkin's disease, for whom this classification was originally designed. However, most clinicians distinguish these sites as special entities with unique pathological and clinical characteristics and report their involvement as an extranodal one.

Our study focused on the lymphomas located in Waldeyer's ring and in agreement with the literature; in the present study, we identified involvement of palatine tonsils more common than the other two locations – 73% of cases. The diagnosis of tonsillar NHL may be challenging because frequently there is clinical suspicion of squamous cell carcinoma, which is the most common malignant tumor at this site.

Most patients in our study were adults (median age in the 60's), and the male to female ratio was 0.8. Most data in the literature describe a unitary or higher ratio, although there are studies that mention a female predominance [10].

The commonest histology in NHL of Waldeyer's ring is DLBCL with natural history similar to primary nodal NHL [11] and more than half of the patients in our study had this diagnosis. Although standard Hematoxylin and Eosin stained sections still provide the basis for all lymphoma diagnosis, most of the cases benefit from ancillary techniques for accurate sub-classification with high sensibility and specificity.

## Conclusions

Malignant lymphomas represent approximately 5% of all malignant neoplasms of the head and neck area and the most frequent extranodal site is Waldeyer's ring.

For the non-Hodgkin lymphoma, the most common manifestation is cervical lymphadenopathy, thus making the otolaryngologists the first physicians to deal with lymphoma. Good knowledge of the clinical characteristics of these lymphomas and the methods to establish the differential diagnosis is essential for a correct therapy of the disease.

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