

Clinical and histological aspects with therapeutic implications in head and neck lymphomas

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Abstract

Malignant lymphoma (ML) is one of the major issues in modern medical practice, with an increasing incidence in recent years, which makes it, together with leukemia, the most frequent form of neoplasia affecting young people. The onset can occur both inside and outside the lymph nodes, with a quarter of the lymphomas with extranodal onset being located in the head and neck. The purpose of the paper is to conduct a retrospective study over a period of six years on patients diagnosed and admitted to the clinic with malignant lymphomas located in the head and neck, discussing their different histological variations. It emphasizes the importance of the histopathological examination and, in particular, of the immunohistochemical tests, in determining the histological subtype of the lymphoma, as the immunohistochemical and cytogenetic data of the malignant cell play a major role in the evolution and prognosis of patients. The study leads to the conclusion that, in spite of the advancements of the immunological, cytogenetic and molecular techniques, the diagnosis and histological determination of malignant lymphomas continue to be a challenge to clinicians and anatomical pathologists. Of particular importance in the efforts made for the accurate diagnosis and proper treatment of the ENT (ear, nose and throat) malignant lymphomas is the interdisciplinary collaboration between the ENT specialist, the hematologist, the anatomical pathologist, the oncologist and the nutritionist.

Keywords: malignant lymphoma, immunohistochemistry, interdisciplinary management.

Introduction

Malignant lymphomas (MLs), defined as neoplasias of the B- and T-lymphoid cells, are one of the topics of major interest in modern medicine. Unlike Hodgkin's lymphoma, whose incidence has lately declined, non-Hodgkin's lymphomas (NHLs) have seen an increase by at least 5% over the last few years, becoming, together with leukemia, the most frequent form of neoplasia affecting young population [1].

Maybe more than in any hematological–oncological conditions, the pathogenic diagnosis, accurate nosological determination and treatment aspects related to MLs have seen a particularly dynamic evolution over the last decades. Despite the improvements in the molecular biology and genetics techniques, as well as in those of genetic multiplication, which would have been hard to imagine just a few years ago, the histological diagnosis of ML, especially of the NHL ones, is still one of the greatest challenges facing medical practice [2, 3].

MLs are the second most frequent causes of head and neck neoplasias. They can occur in the head and neck lymph nodes, as well as extranodally, being the second most frequent after the primary gastrointestinal lymphomas. They include lymphoproliferations occurring in Waldeyer's tonsillar ring, in the nasal cavity and in the paranasal sinuses, in the larynx, pharynx, in the thyroid

or in the salivary glands. In this area, the mucosa-associated lymphoid tissue (MALT) is already recognized as very rich and as liable to represent the starting point for various lymphoproliferations. This tissue grows in response to frequent inflammations in the area and the Epstein–Barr virus, HTLV-1 (human T-leukemia virus 1) and HHV8 (human herpes virus 8) play a major role [4–9].

The onset of a ML outside the lymph nodes is increasingly frequent and currently over 30% of the ML are diagnosed as having an extranodal onset and particular pathogenic, clinical and therapeutic characteristics. This is why this group of lymphoproliferations has received special attention over the last few years [10].

Most of the extranodal MLs are NHLs. In the medical literature, the head and neck ML frequently include ENT lymphomas. The NHL of the upper aerodigestive tract fall into three major categories, depending on frequency: (1) oral cavity, Waldeyer's tonsillar ring and pharynx lymphomas; (2) lymphomas of paranasal sinuses and of the nasal cavity; (3) larynx and trachea lymphomas, B-cell NHL being the largest category [11].

In addition to these sites, lymphomas occurring in the salivary glands or in the structures of the internal ear were also described. All these lymphomas can be frequently associated with lateral cervical nodal masses, which make it difficult to distinguish, in point of etiological diagnosis of the

neoplasia, between a carcinoma with lateral cervical metastasis and a lymphatic malignancy with extranodal and nodal onset [12].

Aim

The aim of this paper is to present the particular aspects concerning head and neck malignant lymphoma diagnosis, emphasizing the importance of an adequate multidisciplinary therapeutic management.

Materials and Methods

The conducted study was a retrospective one, from January 2008 through December 2013, covering 242 patients admitted to “Prof. Dr. Dorin Hociotă” Institute for Phonoaudiology and Functional ENT Surgery, Bucharest,

Romania, and diagnosed with head and neck ML.

The information considered in the study was obtained from: patient observation charts, results of the pathological anatomy tests on the patients who underwent surgery, immunohistochemical (IHC) test reports, photos of the microscope slides held by the Department of Pathological Anatomy.

IHC staining was carried out on paraffin-embedded sections using MaxPolymer Novolink (Leica, UK), in accordance with the manufacturer’s instructions. The panel included many monoclonal antibodies; for each case, the selection of antibodies was made in accordance with pathological diagnostic suspicion after carefully examination of standard Hematoxylin–Eosin (HE)-stained slides (Table 1).

Table 1 – Most important antibody used for IHC diagnosis

Pathological suspicion	Antibody	Specificity (regarding pathological suspicion)	Clone	Dilution	Source
Large B-cell NHL	CD20	Pan B-cells marker	L26	1:400	Dako, Glostrup, Denmark
	CD30	Activated lymphocytes	Ber H2	1:20	Dako, Glostrup, Denmark
	CD10	Centrofollicular cells	56C6	1:100	Novocastra, Leica, UK
	BCL6	Centrofollicular B-cells	LN22	1:100	Novocastra, Leica, UK
	MUM-1/IF-R4	Activated B-cells	EAU32	1:100	Novocastra, Leica, UK
	Ki67	Proliferation index	MIB-1	1:50	Dako, Glostrup, Denmark
	CD5	Pan T-cell marker; 5% of DLBCL are CD5 positive	4C7	1:50	Novocastra, Leica, UK
Small B-cell NHL (nodular and diffuse)	CD20	Pan B-cells marker	L26	1:400	Dako, Glostrup, Denmark
	CD5	Pan T-cells (positive in some B-cell NHL)	4C7	1:50	Novocastra, Leica, UK
	CD23	Follicular dendritic cells (positive in some B-cell NHL)	1B12	1:50	Novocastra, Leica, UK
	Cyclin D1	Mantle-cell NHL	SP4	1:100	Cell Marque, USA
	CD10	Centrofollicular B-cells	56C6	1:100	Novocastra, Leica, UK
	BCL6	Centrofollicular B-cells	LN22	1:100	Novocastra, Leica, UK
	Ki67	Proliferation index	MIB-1	1:50	Dako, Glostrup, Denmark
Plasmacytoma/myeloma	BCL2	Bcl2 oncogene protein	124	1:50	Dako, Glostrup, Denmark
	CD20	Pan B-cells marker	L26	1:400	Dako, Glostrup, Denmark
	CD138	Plasma cells	MI15	Prediluted	Novocastra, Leica, UK
	Kappa	Kappa light chain	CH15	1:200	Novocastra, Leica, UK
	Lambda	Lambda light chain	SHL53	1:200	Novocastra, Leica, UK
T/NK-cell NHL	CD3	Pan T-cells marker	LN10	1:500	Novocastra, Leica, UK
	CD56	NK-cells	123C3	1:50	Dako, Glostrup, Denmark
	CD4	Helper T-cells	1F6	1:20	Novocastra, Leica, UK
	CD8	Suppressor/cytotoxic T-cells	1A5	1:20	Novocastra, Leica, UK
	Granzyme B	Activated cytotoxic cells	11F1	1:40	Novocastra, Leica, UK
	CD30	Activated cells			Dako, Glostrup, Denmark
	CD25	Activated cells	4C9	1:100	Novocastra, Leica, UK
	CD20	Pan B-cells marker	L26	1:400	Dako, Glostrup, Denmark

NHL: Non-Hodgkin’s lymphoma; DLBCL: Diffuse large B-cell lymphoma.

Results

The study of the cases of extranodal and nodal ENT NHL reinforce the idea that they are not one disease, but a group of extremely (clinically, anatomo-pathologically and histologically) heterogeneous and variable diseases.

The most frequent in the studied group were the ML with head and neck nodal onset, with a number of 71 cases representing 29.36% of all patients. Single lymphadenopathies were found in 26.76% of patients and multiple lymphadenopathies in 73.24%. The material for the histopathological examination was surgically obtained,

leading to an accurate diagnosis, while the detailing of the anatomic pathology examination using IHC methods permitted the precise determination and classification of the type of lymphoma according with WHO (*World Health Organization*) Classification.

The studied group included particular clinical situations in which surgery played a greater role in the complex treatment of the ML. We refer to a nodal marginal zone B-cell lymphoma (MZL) that caused a cervical neurovascular, pharyngeal and esophageal compression syndrome, in the purpose of surgery included both diagnosis and surgical decompression (Figure 1, a and b).

An important group of lymphomas with extranodal onset was the MLs of the oropharynx and hypopharynx. In this group, the tonsillar ML was the most frequent, occurring in 71.66% of the cases. Histologically, the most frequent type was the diffuse large B-cell lymphoma (DLBCL) (67.44%). Extremely important for the histological accuracy was to establish the diagnosis not after biopsy of a tonsil fragment, but after tonsillectomy. We encountered a clinical situation in which the result of the initial biopsy indicated a non-differentiated carcinoma, while the subsequent histopathological and IHC examination of the fully excised piece established the final diagnosis as being DLBCL (Figure 2, a and b).

A particular site of the head and neck lymphoma was the nasal cavity and paranasal sinuses, with 12.8% of the total number of treated patients. The clinical situations were diverse, from simple sinusitis to tumors invading the orbit and involving the nasal base. Some of the histological forms were aggressive and highly malignant, tending to rapidly and massively expand to the orbit area and the soft parts.

The surgical ablation of a nose and sinus lymphoma that proved to be a nasal cavity and paranasal sinus plasmacytoma resulted in complete remission with very good long-term results (seven years after the diagnosis) (Figure 3, a and b).

Another case was an aggressive form of nasal cavity T/NK-cell NHL with orbit invasion and involving the nasal base, in which the surgical treatment combined with polychemotherapy proved to be the best solution to eliminate the nose, sinus and eye symptoms, as well as in the local and regional control of the disease (Figure 4, a and b).

Of the head and neck ML, nasal cavity lymphomas represented a major group, ranking second in point of frequency. A number of 70 of the total 242 patients included in the study (28.92%) had this form of lymphoma. Concerning histopathological diagnosis, the nasopharyngeal site raised major debates: IHC proved essential in determining the histological subtype and, in particular, in distinguishing between B-cell lymphomas, undifferentiated carcinomas and poorly differentiated carcinomas (Figure 5, a and b).

Other ML located in other ENT sites included two cases of laryngeal lymphoma, one case of ear lymphoma, four cases of thyroid lymphoma and three cases of salivary gland lymphoma. Most of these cases were diagnosed by taking biopsy samples from the suspicious areas. In the two cases of submandibular gland lymphoma, surgery had a double role, of taking biopsy samples and removing the tumor, and it consisted in the ablation of the submandibular glands (Figure 6).

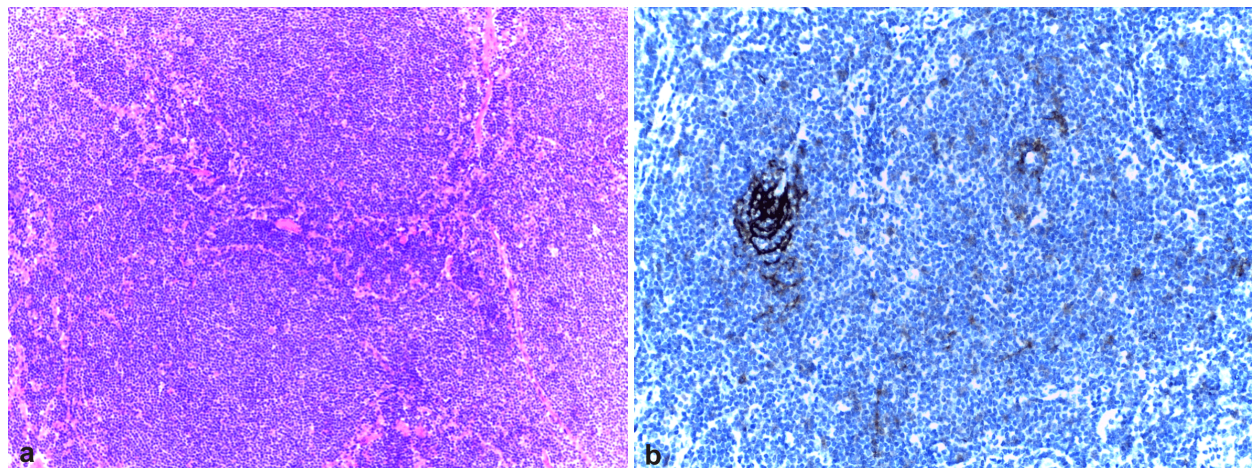


Figure 1 – Lymph node, MZL: (a) HE staining, $\times 100$; (b) CD23 positive in restant FDC (follicular dendritic cell). Immunostaining with CD23 antibody, $\times 100$.

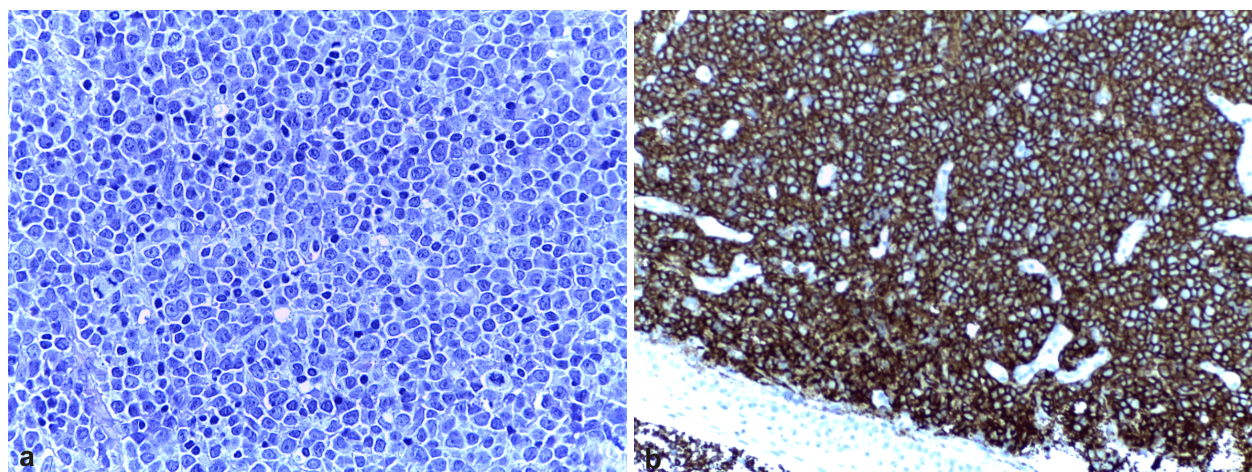


Figure 2 – Tonsil, DLBCL: (a) HE staining, $\times 400$; (b) Tumor proliferation expresses CD20. Immunostaining with CD20 antibody, $\times 200$.

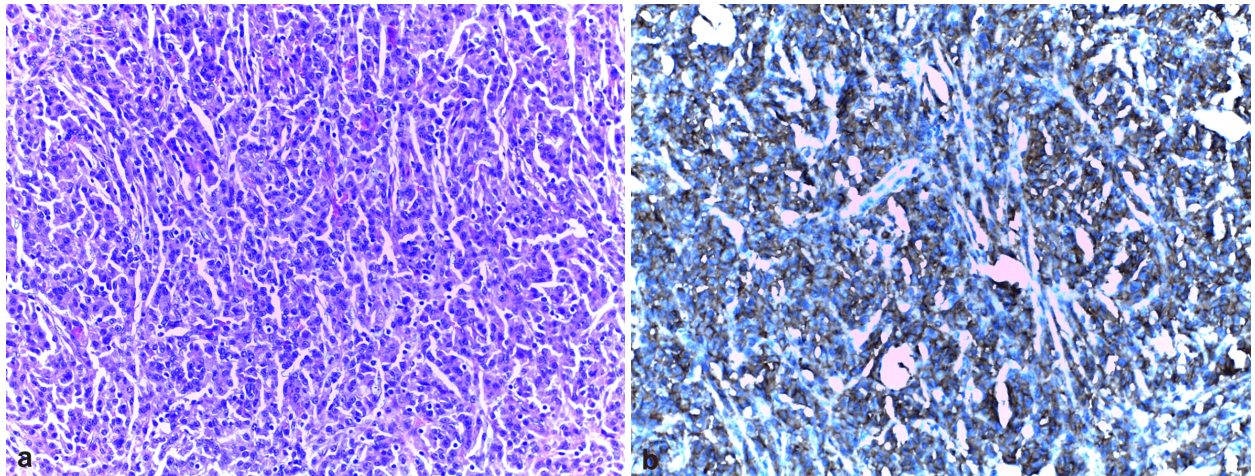


Figure 3 – Nasal cavity, plasmacytoma: (a) HE staining, $\times 200$; (b) CD138 positive in tumor cells. Immunostaining with CD138 antibody, $\times 200$.

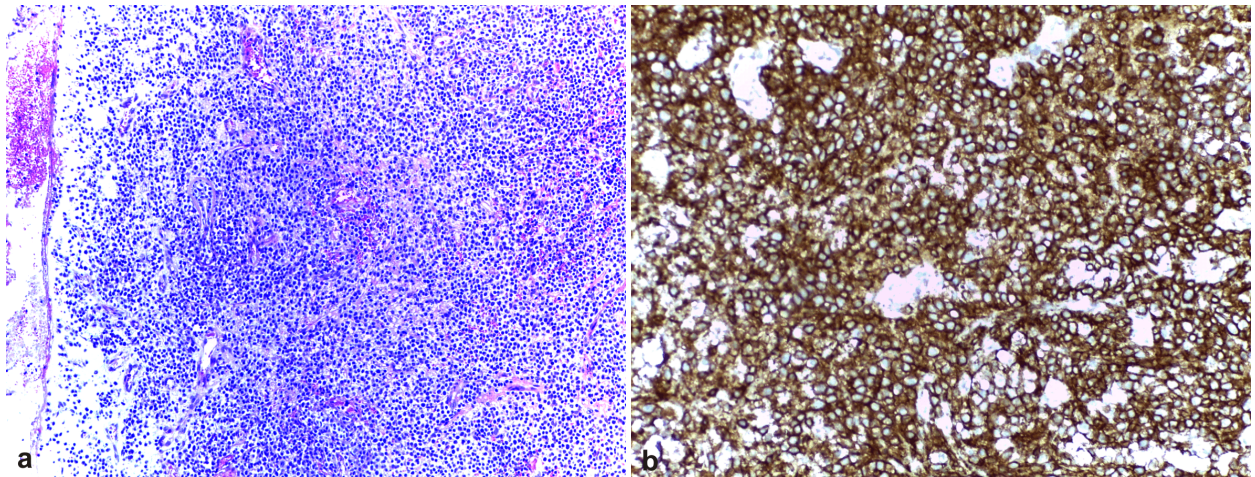


Figure 4 – Nasal cavity, T/NK-cell NHL: (a) Immunostaining, $\times 100$; (b) CD56 positive in malignant cells. Immunostaining with CD56 antibody, $\times 200$.

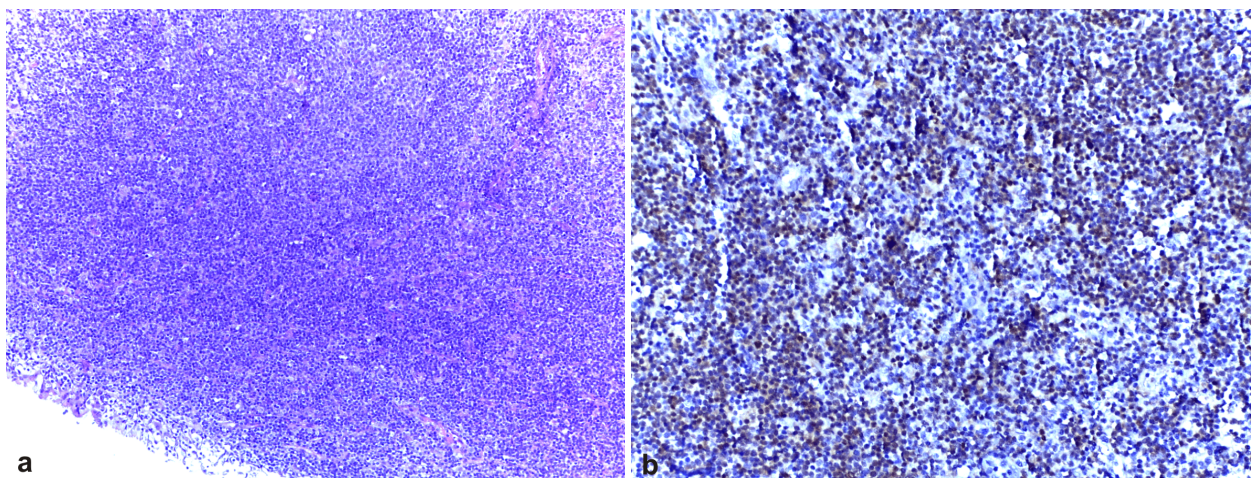


Figure 5 – Cavum, MCL: (a) HE staining, $\times 100$; (b) Tumor is cyclin D1 positive. Immunostaining for cyclin D1, $\times 200$.

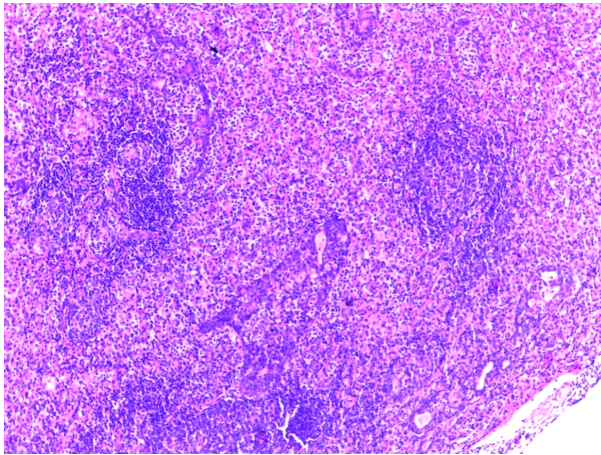


Figure 6 – Submandibular gland, MZL MALT-type, lympho-epithelial lesion. HE staining, $\times 100$.

Discussion

The histological, clinical and evolutionary heterogeneity of ML raises many challenges concerning diagnostic and treatment. The treatment strategy should be based on an accurate diagnosis and the histopathological and IHC examination of the completely excised piece play a key role in this aspect. There are several techniques of outstanding importance in the analysis of the lymphoproliferative disorders. Immunophenotyping permits distinguishing between the polyclonal reactive and the malignant monoclonal proliferation of B- and T-lymphocytes. It can indicate the cell line, subset and differentiation stage of the malignant lymphocytes. The genotype analysis (demonstration of gene rearrangement) for immunoglobulin IgH and the T-cell receptor allows identifying the cell line and the clonality of B- or T-cell proliferation on a molecular level [13–16].

Histopathologically, there are three major types of lymphomas occurring in the upper aerodigestive tract: MZL MALT-type, DLBCL and the nasal peripheral T/NK-cell lymphoma, with cytotoxic phenotype. DLBCL is the most frequent form of lymphoma. It is most often seen in localized forms of the disorder with extranodal onset [17, 18].

The nasal peripheral T/NK-cell lymphoma is extremely aggressive, with a significant local expansion tendency (especially to the orbit, the palate and the soft parts) and CNS tropism. The B-cell phenotype is most frequently located in the paranasal sinuses, while the T-cell phenotype occurs more often in the nasal cavity. The T-cell proliferation lymphomas frequently cause obstructive lesions and perforation of the nasal septum in their evolution. Frequent association was demonstrated between Epstein-Barr virus infections and the development of nasal cavity and paranasal sinus primary lymphomas. Parts of them are caused by the proliferation of CD56+ phenotype NK-cells [19–22].

The treatment of these ML has seen major changes over time; the advancements in cellular biology in recent years permitted the use of modern treatment techniques to stop the evolution of the neoplastic process in the hope of possibly curing the disease. The main treatment means used in the therapy of ML include traditional chemo-

therapy, radiotherapy, surgery, salvage treatment, interferon treatment, treatment with monoclonal antibodies, bone marrow transplantation and treatment with peptides. Great importance is currently attributed to adapting the treatment protocols for each individual case [23–26].

This paper aims to present some clinical situations that have generated discussion about the difficulty in establishing a correct and complete diagnosis, or in the management of these cases. Thus, there are clinical situations in which surgery indication was dictated by the existence of cervical neurovascular and pharyngo-esophageal compression syndrome, in case of MZL, MALT type.

Another case presented was that of a tonsillar ML, which highlighted accuracy of final diagnosis after tonsillectomy and not using a tonsillar biopsy fragment, to establish an incorrect initial diagnosis of carcinoma. The presented histological subtype was DLBCL.

In case of sino-nasal ML, a clinical form of aggressive lymphoproliferation, the importance of surgical treatment along with hematological conventional treatment in obtaining long-term remission (six years and seven years after diagnosis) is emphasized.

In case of rhinopharyngeal ML, a histological form of small B-cell lymphoma (MCL) that shows frequent diagnostic difficulties of other small B-cell lymphoma and, in cases of pleomorphic variant, with DLBCL.

In the case of ML of the salivary gland, a case of MZL MALT-type of the submaxillary gland is presented, in which surgery had a double role, both diagnostic and ablative in complete removal of the tumor.

Conclusions

Although ML is eminently a blood disease, there are some particular situations where surgery has been shown to have an increased role. Surgical removal of the lymphoma tumors in the head and neck area contributes along with specific hematological treatment to obtain lasting complete remissions and to improve the prognosis of these patients. Considering the efforts made for accurately diagnosing and properly treating a head and neck malignant lymphoma, a complex approach, integrating clinical, morphological and cytogenetic elements and the interdisciplinary collaboration of the ENT surgeon with the anatomic pathologist, the hematologist, the oncologist, the radiotherapist, the nutritionist and the psychotherapist are the most legitimate methods of approaching a patient with a ML.

Conflict of interests

The authors declare that they have no conflict of interests.

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