Lymphomas of the maxillofacial region – cytological, histological and immunohistochemical aspects

A. IACOB \(^{(1)}\), A. ORMENIȘAN \(^{(1)}\), T. MEZEI \(^{(2)}\), A. ZAZGYVA \(^{(3)}\)*, A. SIN \(^{(3)}\), M. TILINCA \(^{(3)}\)

\(^{(1)}\) Department of Oral and Maxillofacial Surgery, University of Medicine and Pharmacy of Târgu Mureș, Târgu Mureș, Romania
\(^{(2)}\) Department of Pathology, University of Medicine and Pharmacy of Târgu Mureș, Târgu Mureș, Romania
\(^{(3)}\) Department of Cell and Molecular Biology, University of Medicine and Pharmacy of Târgu Mureș, Târgu Mureș, Romania

*Corresponding author

A. Zazgyva
Department of Cell and Molecular Biology, University of Medicine and Pharmacy of Târgu Mureș, Târgu Mureș, Romania, Phone: +40-745-612-397
e-mail: zazgyvaa@yahoo.com,

Keywords. Hodgkin and non-Hodgkin lymphoma, fine-needle aspiration cytology, histology, immunohistochemistry.

Summary

Lymphomas represent the second most frequent malignancy of the head and neck area, after carcinomas. Cytological examination of the material obtained by fine-needle aspiration might be an efficient tool in the preoperative investigation of these lesions. In a prospective study conducted in the Oral and Maxillofacial Surgery Department of the Târgu Mureș Emergency County Hospital that included a consecutive series of 118 patients with tumours/ tumour-like masses located in the maxillofacial area, we identified 9 cases of different types of lymphoma. This paper presents these cases and their cytological, histopathological and immunohistochemical aspects.

Lymphomas of the cervicofacial region were difficult to diagnose only by cytology because of the inability to classify these entities solely based on their cytological appearance. Although a significant number of lymphomas can be sub-classified based on cytological evaluations, fine-needle aspiration cytology could not replace biopsy in all cases, as histopathology and immunohistochemical tests were necessary for a correct diagnosis.

Lymphomas constitute a heterogeneous group of malignancies, being the third most common malignant disorder of the maxillofacial area, after carcinomas and their metastases. These lymphoid malignancies vary in their morphology, clinical and evolutive features, response to treatment, and prognosis. Although lymphomas are the most common hematologic malignancy of the head and neck area, in most cases the disorder is not limited to this anatomical territory, and it is actually a disseminated tumour process, affecting multiple locations in the body (Dunleavy, 2015).

The two main categories are represented by Hodgkin's lymphomas and non-Hodgkin lymphomas. The majority of Hodgkin’s lymphomas have a nodal location, mostly involving the lateral cervical lymph nodes, while non-Hodgkin’s lymphomas are more common than Hodgkin’s lymphomas, and have an extra-nodal location in about 40% of cases. The subtype of lymphomas most commonly found in the maxillofacial area is...
diffuse large B cell lymphomas (Dunleavy, 2015; Walter et al., 2015).

Due to the complexity and multitude of histopathological subtypes, the classification of lymphomas is still a challenge. The World Health Organization’s current classification of tumours of the hematopoietic and lymphoid tissues emphasizes the importance of cellular morphology in addition to immunophenotypic and genotypic characteristics (Sabattini et al., 2010; Schwock et al., 2012).

Clinically, lymphomas may show signs and symptoms of malignant lesions of various sites in the maxillofacial area (oral cavity, oropharynx, paranasal sinuses, salivary glands, lymph nodes, or cervicofacial teguments), thus often making differential diagnosis difficult (Walter et al., 2015). Diagnosis and classification of lymphomas is classically based on biopsy and histopathological examination. But recently there is a trend towards using cytological samples obtained using fine-needle aspiration for the diagnosis of most cases of lymphoma, without the need for regular biopsy.

Given the complexity of lymphoma types, we considered it necessary to present a series of cases identified in a prospective study of tumours and tumour-like lesions of the maxillofacial area. This paper reports the cytological, histological and immunohistochemical aspects found in these cases, highlighting the results of specific immunohistochemical assays for each case.

Materials and methods

Between May 2012 and February 201, a prospective study was conducted in the Oral and Maxillofacial Surgery Department of the Târgu Mureș Emergency County Clinical Hospital. This was aimed to assess the efficacy of fine-needle aspiration cytology in diagnosing tumours or tumour-like lesions of the maxillofacial area. This paper reports the cytological, histological and immunohistochemical aspects found in these cases, highlighting the results of specific immunohistochemical assays for each case.

Table 1 shows the distribution of the 9 cases of lymphoma, based on their diagnosis and reaction to immunohistochemical stains. There were 3 cases of diffuse large B-cell lymphoma, of which 2 w parotid glands, and one in the lateral cervical area (figure 1). Their tumour cells were positive to CD 20, bcl-2, and bcl-6, demonstrating that the malignancy originated from B-cells. The remaining T cells showed a positive reaction to CD3 and CD5. Cytokeratin was positive in the remaining glandular acini and ducts, while tumour cells were negative for other immunohistochemical stains: CD10, CD23, and cyclin-D1 (and in one case, CD30). There were cytology slides inadequate for interpretation, and patients with no histological examination available for the same lesion. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureș (no. 30/26.06.2012). The procedure was explained to all patients and a written consent was obtained from all include patients.

Fine-needle aspiration was performed without radiological guidance by a maxillofacial surgeon, using a 23 G needle and a 10ml or 20ml syringe unattached to an aspiration syringe holder. After a minimum of two needle passes, the aspirated material was spread onto 2-4 slides and immediately fixed by immersion in 95% ethyl alcohol. The slides were stained using the Papanicolaou stain, and evaluated in the Pathology Department of the same Hospital. Biopsy specimens obtained after the surgical treatment of patients were analysed in the same Pathology Department, and special stains and immunohistochemical evaluations were also performed when needed.

The study included 118 patients with tumour lesions in the maxillofacial area. Based on the histopathological assessment’s results, a number of 9 cases of lymphoma were identified.
was also one case with rare positive cells to cyclin-D1 and MUM1.

![Image](image_url)

**Fig. 1.** Lymphoblastic lymphoma (Papanicolaou stain, 40x): the smear is very cellular and is composed of relatively uniform atypical lymphoid cells, a mitotic figure is also seen. Scattered small mature lymphocytes are also observed.

From the total of 9 cases, we identified 2 cases of Hodgkin’s lymphoma with a lateral cervical location. In these cases, Reed-Sternberg cells had a positive stain for CD30 and MUM1. The cells’ staining for CD15 was inconclusive, but in one case the cells were negative for CD20 and CD3, while in yet another case, most lymphocytes were positive for LCA, CD20, and partially to CD3, showing negative reactions to Pan Cytokeratin staining (figure 2).
**Fig. 2.** Hodgkin’s lymphoma (Papanicolaou stain, 20x): the smear is highly cellular and predominantly composed of a polymorphic lymphoid cell population, resembling reactive lymphoid reaction; however a distinctive cellular population is readily observed, composed of large cells, frequently polylobulated (Reed-Sternberg cells), suggestive of the disease.

**Table 1.** The distribution of lymphoma cases, based on diagnosis and reaction to immunohistochemical stains.

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>No. of cases</th>
<th>Positive immunohistochemistry results</th>
<th>Negative immunohistochemistry results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>3</td>
<td>CD20, CD3</td>
<td>Pan Cytokeratin, CD10, CD23</td>
</tr>
<tr>
<td>Hodgkin’s lymphoma</td>
<td>2</td>
<td>Reed-Sternberg cells + to CD30 and MUM1</td>
<td>CD20, CD3/ Pan Cytokeratin</td>
</tr>
<tr>
<td>Grade 2 B-cell follicular lymphoma</td>
<td>1</td>
<td>CD20, CD10, CD3, bcl-2</td>
<td>Bcl-6, CD5, CD23, p53, cyclin-D1</td>
</tr>
<tr>
<td>Grade 1 follicular lymphoma</td>
<td>1</td>
<td>CD20, CD10, bcl-2, bcl-6</td>
<td>Cyclin-D1, CD5, p53</td>
</tr>
<tr>
<td>Marginal zone lymphoma</td>
<td>1</td>
<td>CD20</td>
<td>Bcl-6, cyclin-D, Pan Cytokeratin</td>
</tr>
<tr>
<td>Plasma cell myeloma</td>
<td>1</td>
<td>CD138, kappa chain, CD56</td>
<td>Lambda chain</td>
</tr>
</tbody>
</table>
We found one case of grade 2 B-cell follicular lymphoma, located in the left lateral cervical area. Cells of the tumour lymph node were CD20 positive, while CD3 was positive for T lymphocytes located in between tumour lymph nodes. For CD10 positive reactions were found in the remaining germinal centres, and stained tumour lymph nodes; bcl-2 was positive in cells from these nodes. Other stains showed negative reactions: bcl-6, CD5, CD23, p53 and cyclin-D1, while a positive reaction for Ki67 was found in 30-40% of cells.

In the case of grade 1 follicular lymphoma, the tumour was located in the submentonian region. Cells showed positive immunohistochemical reactions for CD20, CD10, bcl-2, and bcl-6. Tumour cells were negative for cyclin-D1, CD5 and p53. Immunomarking for CD23 revealed an expanded network of follicular dendritic cells. There was a reduced number of centroblasts per high-power field (5/10), and Ki67 was positive in about 15%.

A rare case of marginal zone lymphoma was also identified. The tumour had developed in the right parotid gland, and tumour cells were intensely positive to CD20. The surrounding reactive T lymphocytes were marked by CD3 and CD5. However, CD23 and CD10 were positive in the remaining centres, but negative in tumour cells. There was also a positive reaction to bcl-2 in the remaining germinal centres, with a strong positive reaction to Ki67 in the centres and approximately 10% positive reaction in the tumour cells. Immunohistochemical staining for bcl-6, cyclin-D and Pan Cytokeratin was also negative.

A rare occurrence of plasma cell myeloma was identified in one patient in the maxillary bones. Upon immunohistochemical assessment, tumour cells were positive to CD138, CD56, and kappa chains, and negative to lambda chains.

Cytological examination alone proved to be difficult in lymphomas, where the lesions could only be identified as lymphoproliferative masses in all studied cases. A conclusive diagnosis was obtained only after histopathological and immunohistochemical assessments.

Discussion

When a patient presents with the enlargement of a neck lymph node and has risk factors for a head and neck malignancy, fine-needle aspiration cytology is usually recommended as an initial step in the diagnostic management. This is usually followed by excisional biopsy if the histologic examination does not find squamous cells (Lash).

In recent years fine-needle aspiration cytology has become a first-line investigation in the evaluation of maxillofacial tumours due to its minimally invasive nature, diagnostic efficiency and optimum cost/efficiency ratio. The clinical value of aspiration cytology is not limited to tumours, as it might have a great significance in the assessment of inflammatory lesions as well (Muddegowda et al., 2014). In addition, processing cytological material obtained by fine-needle aspiration biopsy only takes a few hours up to a few days, depending on the special stains and ancillary techniques applied. Thus the result are most often available in a short time, which is an advantage when compared to the histological assessment, where a longer time is needed for getting the results (Kocjan, 2006).

However, in the studied cases, cytological evaluation was difficult in cases subsequently diagnosed as different types of lymphomas. In these situations, cytology only gave a result of lymphoproliferative masses, without final diagnostic details. A definitive diagnosis was only established after using histopathological and immunohistochemical tests.

One of the major limits of cytology is that of the poor architectural details obtained when compared to histopathology (Schwock et al., 2012). The current classification system of lymphomas states that a correct diagnosis is made on clinical examination, immunophenotypic and genotypic details, combined with histomorphologic features (Gong et al., 2002). Diagnosis, classification and sub-classification of lymphomas are classically based on biopsy and histopathological examination. In recent years, the application of ancillary techniques
(e.g., flow cytometry) on cytological material obtained using fine-needle aspiration made it possible to diagnose most types of lymphomas solely based on cytology, without the need for regular biopsy. Moreover, a significant number of lymphomas can be subclassified based only on cytological evaluation (Gong et al., 2002). However, some studies show that fine-needle aspiration cytology cannot replace biopsy in all cases – in these situations histopathology is required for a correct diagnosis (Das et al., 2009; Roseman et al., 2008; Roshal et al., 2011; Skoog et al., 2009).

For example in Hodgkin’s lymphoma, a histologic diagnosis is always required. An excisional biopsy of a lymph node is mandatory because lymph node architecture is very important for correct classification. The usual appearance of smears in Hodgkin’s lymphoma is polymorphous, with mature lymphocytes, follicular centre cells, and neutrophils and eosinophils in variable amounts; epithelioid cells can also be found. Cytological diagnosis is made in the presence of Reed-Sternberg cells, related to equivalent histotypes. Immunocytochemical tests show CD15 and CD30 positivity, but CD20 positivity may be equivocal because other lymphoid cells may also be CD20+ (Roshal et al., 2011).

Cytological diagnosis of non-Hodgkin’s lymphoma is also difficult. They represent clonal expansion of lymphoid cells in different stages of maturation therefore they show poor relevant nuclear atypia. Furthermore, neoplastic cells might be mixed with different amounts of benign reactive lymphocytes, making diagnosis more difficult. The cytological diagnosis of non-Hodgkin’s lymphoma is determined by a combination of cytological features and phenotypic patterns, with the use of immunocytochemistry or flow cytometry (Sharma et al., 2014).

Differentiated B-cell lymphomas usually have a quite monomorphous cell population, but a final diagnosis may only be made by light chain clonality or IgH gene rearrangement. Still, flow cytometry can assess some phenotypic patterns which define a lymphoid cell population as B-lymphoma and are sub-type specific: CD5/CD19 co-expression, CD10/bcl-2 co-expression or CD10 positivity in all the gated cells, combined with a specific cytological pattern. T-cell lymphomas are more complex. Peripheral T-cells currently express CD2/3/7 and alternatively CD4 or CD8. Loss of one antigen may suggest a clonal proliferation. CD3/CD56 expression is indicative for natural killer cell lymphoma (Sharma et al., 2014).

Some authors reported that low-grade lymphomas account for a significant percentage of false-negative or indeterminate results when using cytology alone. However, they also found that the diagnosis of malignant lymphomas could be made in 82% of cases with a significant contribution occurring through the use of immunophenotyping, which led to a substantial improvement in diagnostic accuracy (Chhieng et al., 2001; Schwock et al., 2012).

There is still significant scepticism regarding the role of cytology for the diagnosis of lymphomas and excisional biopsy is frequently regarded as essential for an accurate initial diagnosis and classification of malignant lymphomas, despite the development of sophisticated methods for ancillary testing (Hehn et al., 2004; Schwock et al., 2012).

The significant impediments of fine-needle aspiration biopsy as a stand-alone approach for malignant lymphoma diagnosis were discussed in a commentary which also stressed the need for interdisciplinary collaboration, multi-parametric testing and on-site specimen adequacy assessment as requirements for optimal results (Schwock et al., 2012; Wakely, 2010).

There are many situations with difficulties in cell assessment, and the morphologic ambiguity is part of the reason why ancillary testing, in particular immunophenotyping studies, are considered mandatory in the diagnosis of lymphoproliferative disorders. Ancillary studies are crucially guided by the initial cytological evaluation, providing the base for a proper interpretation of immunophenotypic and molecular testing results (Schwock et al., 2012).
Excisional biopsy should be considered when cytological diagnosis is ambiguous or the lymphadenopathy persists without a definitive cause – in these cases there might be a suspicion for T-cell lymphoma, Hodgkin’s lymphoma or composite lymphomas (Landgren et al., 2004). Histologic examination may also be necessary due to persistent controversies, such as those related to the grading of follicular lymphomas.

Lymphomas are difficult to recognize on cytology alone due to the fact that tumor cells show overlapping morphology among different entities. Nevertheless cytologists should be well acquainted with the cytologic features of these lesions in order to be able to differentiate it from other lesions, such as metastasis. Sometimes the most important question to be answered when evaluating an enlarged lymph node is whether the lesion is metastatic carcinoma or a lymphoid neoplasm. If a lymphoid neoplasm is suspected, flow cytometry and immunophenotyping are required for definitive diagnosis and surgical removal, and subsequent histological evaluation is usually performed.

Conclusions

Lymphomas constitute a heterogeneous group of malignancies, with a multitude of histopathological subtypes. Cytology does not replace histopathology in the diagnosis of lymphomas of the maxillofacial area, but in selected cases it might narrow the list of possible differential diagnoses. Still, a correct diagnosis is based on immunophenotyping, genotyping, and clinical and histomorphologic assessment.

Since samples obtained by fine-needle aspiration provide excellent material for both cytomorphologic assessment and ancillary testing, the acceptance of cytology as a diagnostic tool for lymphomas has increased considerably.

Clinical data, immunophenotyping and cytogenetics provide the context for cytomorphology, and offer the possibility to establish an accurate diagnosis even in this challenging area.

Acknowledgement

The project was funded by the University of Medicine and Pharmacy of Târgu Mureș within Scientific research grants – Research groups (contract no. 20/2014).

References

Schwöck, J. and W. R. Geddie: Diagnosis of B-cell


