Immunophenotyping for Diagnosis of Oral Lesions: Is It an Important Tool?

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Authors’ contributions

This work was carried out in collaboration among all authors. Author ARC designed the study and wrote the first draft of the manuscript. Authors BFD, MLL, CCC, DSCV and JAGDM participated in the acquisition, analysis and interpretation of data and managed the literature searches. Authors MCSS and LJG participated in the initial conception and design of the study, analysis and interpretation of data, writing of the intellectual content of the manuscript and critical analysis of important intellectual content. All authors read and approved the final version of the manuscript.

ABSTRACT

**Aims:** To report two cases of onco-hematologic diseases diagnosed by oral biopsy and subsequent flow cytometry immunophenotyping.

**Presentation of Case: Case 1:** A 36-year-old woman, HIV +, referred to the Hospital Dentistry Center presenting an extensive, ulcerated and painless lesion in the left maxilla involving teeth #24, #25, #26 and #27. A fine needle aspiration and incisional biopsy were performed and one sample
was sent to anatomopathological analysis and other to immunophenotyping. Immunophenotyping revealed 31.2% of aberrant plasma cells with phenotype suggestive of plasmablastic lymphoma.

**Case 2**: 62-year-old male patient attended Hospital Emergency presenting extra and intraoral swelling on the left side of maxilla. Fine needle aspiration and incisional biopsy were performed, followed by pathological analysis and immunophenotyping. Immunophenotyping revealed 40.7% of large mature B cells with phenotype suggestive of diffuse large B-cell lymphoma. Both patients presented histopathological and immunophenotyping results leading to the same diagnosis. After definitive diagnosis, both patients underwent antineoplastic treatment through cycle of chemotherapy.

**Conclusion**: Immunophenotyping is a well-established method for the diagnosis of oncohematologic diseases and has been shown to be effective for the rapid diagnosis of oral tumors.

**Keywords**: Flow cytometry; lymphoma; diagnosis; differential.

1. INTRODUCTION

Head and neck cancer includes tumors located in larynx, throat, lips, mouth, nose and salivary glands. Together, these tumors are the ninth most common malignancy in which approximately 90% of the cases are squamous cell carcinomas arising from the epithelial mucosa [1]. Lymphomas account for 3% of all malignant tumors and 2.2% of the head and neck cancers, being overcome only by epithelial malignancies [2].

Lymphomas represent a remarkably diverse group of neoplastic disorders of lymphocytes, being classified depending on the cell of origin from which they arise. The common lymphoid precursor cells give rise to numerous lymphoid subsets that differ both in lineage and degree of functional maturation. In this sense, the origin of the malignancy could be of different subtypes resulting from different disease characteristics and physiological processes [3]. The World Health Organization (WHO) established the current lymphoma classification and revision criteria, and defined a characteristic cell phenotype for all lymphoma entities [4]. In fact, for some lymphoma subtypes this phenotype is very well-established and unique, while for others some degree of phenotypic overlap exists. For this reason, it is very important to bring together information on clinical features, immunohistochemistry/immunocytochemistry (IHC/ICC), flow cytometry (FC) and genetics.

The clinical diagnostic approach to oral lymphomas may be extremely difficult for different reasons. Firstly, the professional experience is an important step for diagnostic accuracy; secondly, there are no specific radiological findings for this group; and thirdly, the lesions have often been described as rapidly growing exophytic masses, which may infiltrate surrounding tissues and exhibit a range of clinical presentations, such as ulceration and necrosis on the surface [5,6].

The gold standard diagnosis for oral lymphomas involves a surgical procedure for biopsy sampling, with the sample being sent to histopathological study followed by IHC analysis [7]. However, in many cases, acquisition of a complete excision or an adequate sized biopsy is difficult. In addition, the final diagnosis can take weeks. Thus, the main objective of this study was to highlight the use of Fine-Needle Aspiration (FNA) cytology combined with flow cytometry as an important tool for diagnosis and sub-classification of oral cavity lymphomas.

2. PRESENTATION OF CASE

2.1 Case Report 1

A 36-year-old woman attended the Hospital Dentistry Center complaining of painless and unilateral swelling with a 3-month evolution history. She had a past history of bleeding on the left side of her maxilla, with extra oral examination showing a small swelling on the anterior maxillary labial sulcus. The skin over the swelling had a smooth surface and was normal in color. The consistency of the involved area was firm, presenting no pain nor tenderness or local temperature rise. Regional submandibular lymph nodes were palpable on the same side. Intraorally, there was a growing mass in the left palatal region involving teeth #24, #25, #26 and #27 as well as in the maxillary buccal vestibules (Fig. 1). The consistency of the swelling on palpation was firm. Mobility was detected in the involved teeth and occlusion was unaltered. The overlying surface had a reddish and ulcerated
mucosa on the left palate and a smooth erythematous mucosa on the buccal vestibules.

Based on the history and clinical examination, a few types of oral diseases were considered in the differential diagnosis as: Oral Squamous Cell Carcinoma; Maxillary Sinus Carcinoma; Osteosarcoma; Carcinoma ex-pleomorphic adenoma; Adenocarcinoma; Wegener's Granulomatosis; Plasma cell neoplasms; Carcinoma metastatic.

The lesion was evaluated by means of computed tomography (CT). A large soft tissue density lesion was observed in the left maxilla, causing erosion of the inferior wall of the maxillary sinus with total obliteration. The right maxillary teeth were also involved.

Routine blood investigations were carried out. Significant laboratory findings showed hemoglobin level of 9.9 g/dl (normal range 12-16 g/dl), white blood cell count of 2,950/mm³, platelet count of 215,000/mm³, serum lactate dehydrogenase of 148 U/L (normal range: 81-234 U/L) and HIV infection. In addition, CD4 count was 21 cells/µL, CD4/CD8 ratio was 0.15 and the plasma viral load was 1,452,451 copies/mL.

After informed consent was obtained from the patient, incisional biopsy of intraoral mass and FNA were performed under local anesthesia. The immunophenotypic analysis of FNA detected 31.2% of plasma cells (i.e. CD38++, CD138+, CD45++) with aberrant phenotype (i.e. lack of expression of CD19) and negative expression of CD56, CD30 and CD20 (Fig. 2). Both surface and cytoplasmic Igκ and Igλ were negative. This result suggested the diagnosis of plasmablastic lymphoma (PBL). Sample viability was 93%, with 32,952 of viable cells being analyzed in a lymphoid screening tube (LST) [8]. Immunophenotyping and histopathological results lead to the same diagnosis.

Cytomorphology of oral mucosal biopsy showed presence of large cells with atypical and eccentric nucleus, irregular chromatin and evident nucleoli. In addition, histopathological examination of hematoxylin and eosiin-stained section showed diffuse proliferation of large cells with atypical and hyper-chrome nuclei in a connective tissue stroma with lymphocytes in different maturity stages, which is compatible with undifferentiated malignant cell tumor. IHC/ICC analysis of both biopsy and FNA showed that tumor cells were positive for CD138 and CD79a, but negative for CD20 and CD56. In addition, Ki-67 was positive in 80% of the neoplastic cells. Taken together, one can conclude that these results indicate the final diagnosis of plasmablastic neoplasm.

After diagnosis, the patient was referred to the Hematology Hospital Service for aspiration of bone marrow (BM), cytological analysis of cerebrospinal fluid (CSF) and chest X-Ray. No plasmablastic cells were detected in the CSF analysis, with bone marrow biopsy and x-ray revealing normal results. Both CSF and BM were analyzed by FC, with negative results.

As a result of the HIV diagnosis, the patient started triple antiretroviral therapy (Lopinavir/Ritonavir, Tenofovir plus Lamivudine) during her hospitalization, but had low adherence to treatment at home. The patient received five cycles of EPOCH chemotherapy, and during follow-up she had been in complete tumor remission for 2 years, when died of unknown cause.

2.2 Case Report 2

A 62-year-old male attended the emergency of the University Hospital complaining of a mild extraoral swelling in the middle third of the face on the left side with nasal obliteration. The patient reported that the symptoms have been present for four months with progressive worsening. On physical examination, a mild extraoral swelling was diffusely presented in the left maxilla (Fig. 2). Due to the swelling, the left corner of the mouth transposed downwards and the tip of the nose pointed towards the right side. The left nasolabial sulcus was slightly erased. The area was firm and no peripheral submandibular or cervical lymphadenopathy was detected. Intraoral examination showed a well-defined growth in the left hard palate and in the buccal sulcus on the same side. The overlying mucosa showed a mild erythema with no ulceration (Fig. 2).

Based on the clinical findings, the following differential diagnoses were established: Odontogenic cyst; Pleomorphic adenoma, Myoepithelioma; Adenocarcinoma, Cystic adenoid carcinoma; Ameloblastoma, Odontogenic myxoma; Fibro-osseous dysplasia; Central ossification fibroma.

CT showed a soft tissue density lesion in the left maxilla causing obliteration of the maxillary and
Fig. 1. Clinical presentation and FNAC immunophenotypic analysis of Case Report 1

(A) Extensive, ulcerated lesion located in the left maxilla. (B) Lesion in the buccal region of teeth #21 to #27. (C) Total lesion remission after chemotherapy. Teeth #25, #26 and #27 removed due to lack of bone support. (D) FNAC immunophenotypic analysis of Case Report 1. Flow cytometric analysis detected presence of 31.2% of large-sized cells (D-A) with high expression of CD45 (D-B), CD38 and CD138 (D-C) as well as absence of expression of CD19 and CD20 (D-D), CD30 (D-E) and CD56 (D-F), as illustrated by bivariate dot plots. Neoplastic cells are colored in red and normal background cells in grey.

Laboratory findings showed hemoglobin level of 12.2 g/dl (normal range 13-18 g/dl), white blood cell count of 4,450/mm³, platelet count of 170,000/mm³, serum lactate dehydrogenase of 279 U/L (normal range: 85-227 U/L) and negative for HIV 1 and 2 infection.

The patient's informed consent was obtained. An incisional biopsy was performed and a diagnosis of diffuse large B-cell lymphoma (DLBCL) was made by using IHC. To determine a definitive diagnosis, FNA of hard palate was performed. Immunophenotypic analysis of FNA showed presence of 40.7% of large cells with mature B cells phenotype (CD19+, CD20++, CD45++),
Fig. 2. Clinical presentation and FNAC immunophenotypic analysis of Case Report 2
(A and B) The initial aspect of the lesion and repercussions in the face of the patient. (C and D) Total lesion remission after chemotherapy. (E) FNAC immunophenotypic analysis of Case Report 2. Flow cytometric analysis detected the presence of 40.7% of large-sized cells (E-A) with high expression of CD45 (E-B), CD19 (E-C) and CD20 (E-D), light chain restriction (Igκ +) (E-E), as illustrated by bivariate dot plots; and dim expression of CD10 (E-F), shown by histogram. Neoplastic cells are colored in red and normal background cells in grey.

light chain restriction (Igκ +), aberrant expression of CD10 (low intensity) (Fig. 2G) and the following phenotype: IgM+, CD79b+, Bcl-2+low and CD38 negative. Sample viability was 93% of viable cells, with 9,010 viable cells being analyzed in LST. In addition, a sample of posterior buccal sulcus was also obtained by using FNA technique and sent for immunophenotypic analysis. A total of 2.3% of neoplastic cells with the same phenotype was
found, with 90% viability and 5,878 viable cells. Taken together, these results were indicative of DLBCL with germinal center phenotype.

Simultaneously, FNA analysis by using cytomorphology and ICC revealed the presence of large cells with phenotype Bcl-2++, CD10+, CD20++ and a high Ki-67 expression (95%), thus confirming the diagnosis of DLBCL with germinal center phenotype. Immunophenotyping and histopathological results lead to the same diagnosis.

No neoplastic cells were detected in the CSF or BM by FC, and BM biopsy revealed normal cellular marrow. Chemotherapeutic induction consisted of intravenous R-CHOP. After six courses of chemotherapy, the oral swelling was greatly decreased. The patient was clinically free of the disease after just 1 cycle. Rigorous long-term follow-up over 4-years is presented with no recurrence.

2.3 Technical Procedure for FNA Analysis by Flow Cytometry

With regard to cytometric and staining procedures for both cases, cell suspension was obtained by FNA and processed within two hours after sample aspiration. First, the total volume of the FNA was concentrated in 1mL. After that, 100 µL of the concentrated sample was incubated with CD45 and 7-AAD in order to detect the presence of leucocytes and evaluate cell viability. Simultaneously, 500 µL of the concentrated sample was washed three times with phosphate-buffered saline (PBS) with 2% fetal bovine serum and resuspended with 200 µL of PBS. From there, 100 µL was used for LST staining [8]. After 30 minutes of incubation with the antibodies, red blood cells were lysed with 1 mL of lysing solution (1:10) (Becton Dickinson, Franklin Lakes, New Jersey, USA) for 15 minutes and then washed. Finally, 200 µL of PBS was added to cell pellet before being immediately acquired on an eight-color three-laser flow cytometer (FACSCanto II, Becton-Dickinson). All events/cells were recorded and the resulting data analyzed with Infinicyt Software 1.7 (Cytognos, Salamanca, Spain). After analysis, further staining procedures were performed according to the result obtained (i.e. antibody panel for plasma cell disorders or for B-cell chronic lymphoproliferative diseases) [8]. Cell fixation and permeabilization kit (Nordic-MUBio, Susteren, the Netherlands) was used for cytoplasmic Igκ and Igλ staining, according to the manufacturer’s recommendations.

3. DISCUSSION

FNA cytology combined with FC is a well-established, useful tool for diagnosis of non-Hodgkin lymphoma in patients with easily access to enlarged lymph nodes [9-14]. Over the past decade, studies have evaluated the importance of FNA combined with FC in oral cavity lesions [5,6,15,16]. Although some studies claim that FNA provides samples with far fewer cells than biopsies [17], in the present study it was observed a sufficient amount of cells for complete flow cytometry analysis and a very high percentage of viable cells in the two cases. As cells obtained by FNA are in suspension, it is not necessary to cut, macerate and filter the sample as it is done for biopsies. Therefore, this procedure is easier and faster, which is in accordance with Cozzolino et al. [6]. In addition, for diagnosis of non-Hodgkin lymphoma, FNA combined with FC showed levels of diagnostic sensitivity and specificity of, respectively, 95% and 85% [10]. Ensani et al. [18] reported 75% sensitivity, 94% specificity, 90% positive prediction and 83% negative prediction when comparing FC to IHC. Furthermore, Maroto et al. [19] found a similar level of detection of B cell lymphomas by using FC and single-round polymerase chain reaction (PCR).

Multi-parametric FC analysis of eight colors or more is important when dealing with samples with small volume or low amount of cells. For instance, in the LST analysis, it is possible to analyze simultaneously cell size and 12 surface markers in order to identify subtypes of leukocytes and lymphocytes, including expressions of Igκ and Igλ light chains for evaluation of B cell clonality. The major advantages of the technique consist of a multi-parametric analysis that may allow a quicker diagnosis and an almost immediate onset of treatment. On the other hand, the main disadvantages include loss of tissue architecture – which sometimes requires later biopsy to elucidate the diagnosis; possible difficulties in aspirating lesions; and an immediate dispatch of the sample to the laboratory or use of cell preservation media for analysis of viable cells [17].

4. CONCLUSION

FNA cytology combined with FC is an important tool for diagnosis of oral cavity lesion, which can also guide the ICC analysis in order to achieve a faster diagnosis and further clinical intervention.
CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this paper and accompanying images.

ETHICAL APPROVAL

The patients agreed to participate in this study and signed an informed consent form approved by the Human Research Ethics Committee of the Federal University of Santa Catarina, Brazil - CEPSH/USFC no. 2.985.964/2018.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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