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CASE REPORT

B-CELL MARGINAL ZONE LYMPHOMA IN A PAROTID GLAND LYMPHO-EPITHELIAL CYST OCCURRING IN A PATIENT DIAGNOSED WITH CLONAL B-CELL LYMPHOCYTOSIS: A CASE REPORT

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ABSTRACT: A patient with a history of CD5-negative and CD23-negative clonal B-cell lymphocytosis (CBL) presented at the Department of Maxillofacial surgery of Luigi Vanvitelli University of Naples for a nodule in the deeper left parotid region, discovered accidentally during a follow-up for the lymphocytosis. Ultrasounds and magnetic resonance imaging showed multiple nodules, the biggest of about 1.5 centimeters, partly solid and partly cystic. US-guided fine needle aspiration cytology (FNAC) and flow cytometry (FC) were executed. FC evaluation showed a CD19+, CD10-, CD23-, CD5- cellular population with restriction for kappa chain. Final diagnosis of a monoclonal B-cell lymphoproliferative disorder was rendered, and the patient underwent to partial left parotidectomy. Histological examination showed lymphoid nodules with some cysts in the context. The lymphoid cell population resulted positive for BCL-2 and partially positive for CD43. The immunohistochemical determination for CD23 showed the presence of irregular-shaped residual networks of follicular dendritic cells, and images of follicular "colonization" by small, BCL-2- positive and CD2-negative lymphoid cells. Proliferation index (Ki67) resulted about 10%. The fluorescence in-situ hybridization (FISH) did not show IGH rearrangement. Finally, polymerase chain reaction (PCR) was performed for clonality assessment and showed the clonal IG heavy V-D-J (IGH) gene rearrangement. A final diagnosis of an extranodal non-Hodgkin B-cell marginal zone lymphoma (MZL) occurring in the context of a lympho-epithelial cyst was rendered.

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Impact statement: Exceptionally rare case of an extra-nodal B-cell marginal zone lymphoma arising in the context of a lympho-epithelial cyst of the parotid gland, in a patient affected by clonal peripheral lymphocytosis.

Key words: *lympho-epithelial cyst; clonal lymphocytosis; marginal zone lymphoma; B-cell lymphoma, case report.*

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INTRODUCTION

Lymphoepithelial cysts (LECs) are uncommon cystic lesions located in the lateral upper neck, most commonly occurring in the context of the parotid gland or a lymph-node, in HIV-positive patients (1). Histologically, LECs consist of multilocular cysts lined by glandular or squamous epithelium, with the presence of florid lymphoid tissue with follicular hyperplasia in the thickening of the wall. Although the etiology is unknown, LECs are believed to derive from the degeneration of tissue remnants of branchial clefts (1). At least some cases located in the salivary gland may be due to cystic dilatation of glandular ducts secondary to dense inflammation (2). Clonal B-cell lymphocytosis (CBL) is defined as the presence of <5 x 10^9/L monoclonal B-cells in the peripheral blood of patients, without any sign of lymphoma. Based on the immunophenotype of the clonal cells, and particularly the expression of CD5 and CD23, CBL comprises three sub-types: chronic lymphocytic leukemia (CLL)-like (75% of cases), atypical CLL-like and non-CLL type (CD5-negative). CLL-like and atypical CLL-like types of CBL are actually considered as precursors of CLL, while non-CLL type MBL seems to be related to splenic marginal zone lymphoma (MZL) (3). Based on the concentration of clonal cells in the blood, a low-count (<0.5 x 10^9/L) and a high count (>0.5 x 10^9/L) type are defined (4). Primary salivary gland lymphomas are unusual, accounting for 2.5% to 4.5% of salivary gland tumors. More than 90% are non-Hodgkin lymphomas (NHLs) and extranodal marginal zone lymphoma (EMZL) is the most common histotype. The parotid gland is the most common location. Salivary gland NHLs are divided into two groups: the former develops in patients affected by HIV infection or Sjogren's syndrome, while the latter occurs in otherwise healthy gland. Here we report an exceptionally rare case of parotid gland EMZL occurring in the context of a LEC in a patient affected by CBL.

CASE PRESENTATION

A 57 years-old woman presented at the Department of the maxillofacial surgery of Luigi Vanvitelli University of Naples due to the occurrence of a nodule in the deeper left parotid region in the previous weeks. The patient had a history of CD5-negative and CD23-negative CBL in the last three years. The nodule was unapparent at the clinical examination and was discovered during the instrumental clinical follow-up for the lymphocytosis. Ultrasound (US) and magnetic resonance imaging (MR) showed multiple nodules, the largest of about 1.5 centimeters, partly solid and partly cystic in appearance (**Figure 1**). A US-guided fine needle aspiration cytology (FNAC) was executed after informed consent of the patient and according to Institutional Review Board. The patient was consequently submitted to partial left parotidectomy.

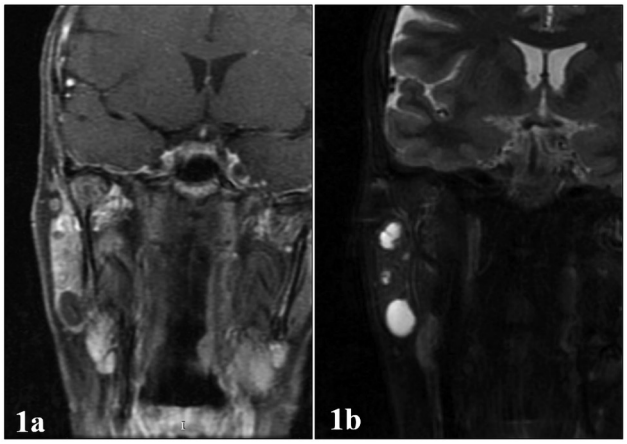

Figure 1. *Magnetic resonance (MR) imaging. (**a**) T1-weighted MR showing multiple nodules in the left parotid; (**b**) the same nodules appearing as cystic in T2- weighted MR images.*

MATERIALS AND METHODS

Cytological preparations

Two passes were performed on the larger solid nodule and one more pass on one of the cystic areas. FNAC material of the solid area appeared dense and whitish; one smear was air-fixed and Diff-Quik stained for rapid on-site evaluation (ROSE). Other smears were alcohol-fixed and Papanicolaou-stained.

Flow cytometry

Therefore, the second pass performed on this area, was suspended in PBS (Phosphate Buffered Suspension) for flow cytometry (FC) evaluation. The fluid material taken from the cystic area was turbid. The material was splitted into two tubes; cytocentrifugates (cytospins) fixed in alcohol were set up by a tube, for FC evaluation. Following lysis of erythrocytes using FACS Lysing Solution (BD

Biosciences, USA), the cells were washed twice in phosphate buffered saline (PBS) (pH 7.2) and stained with the fluorescence-conjugated monoclonal antibody (5ul for each antibody) for 30 min at +4 °C. The cells were then washed twice with PBS and resuspended in the same buffer. The antibodies used were: CD45 APC-H7, CD5 PER-CP CY5.5, CD10 APC, CD3 APC, CD4 V450, CD8 FITC, CD19 PE-CY7, CD22 PE, sIgK FITC, and sIgL PE. The BD ten-color FACS canto II (BD Bioscience, USA) was used. FC calibration and compensation for the overlap between the different fluorochrome spectra were done automatically using FC Beads 8-Color Kit for BD Onflow system. Lymphocyte populations were selected based on CD45 expression versus SSC gating. At least 100,000 events were collected. Data were analyzed using FACS DIVA version 8 software.

Surgical sample and immunohistochemical methods

Surgical sample was formalin-fixed and paraffin-embedded. Immunohistochemistry was performed on Ventana Ultra equipment (Roch Tissue Diagnostic) according to manufacturer's instruction (5).

Fluorescence in-situ hybridization (FISH)

FISH was carried out on three 4-µm-thick sections cut from each formalin-fixed paraffin-embedded (FFPE) sample using the BOND FISH kit (Leica Biosystems, Newcastle Upon Tyne, UK) on the automated BOND system (Leica Biosystems) according to the manufacturer's instructions. This kit consists of a formamide mixture to reduce nonspecific hybridization of nucleic acid probes. IGH fusion detection was performed using specific break-apart probe for each gene: ZytoLight SPEC IGH Dual Color Break Apart Probe (PL67) (ZytoVision, Bremerhaven, Germany). Slides were counterstained with 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) in antifade solution and examined using an automated CytoVision platform (Leica Biosystems). FISH interpretation was performed with the automated fluorescence microscope Leica DM5500 B (Leica Biosystems) using the filter ET-D/O/G for double Spectrum Green plus Spectrum Orange. FISH signals were counted in at least 100 nonoverlapping intact nuclei.

Clonality detection

BIOMED-2 multiplex PCRs and GeneScan analysis were performed according to standard procedures (6).

RESULTS

Cytological features and flow cytometry results

The Diff-Quik stained smears showed a lymphoid population in a serum and proteinaceous background. Subsequently cytological evaluation of all smears (Diff-Quik and Papanicolaou smears) corresponding to the solid nodular area showed a dense cellularity constituted by a relatively monomorphic lymphoid cell population. The cellular population was predominantly composed of small cells with a monocytoid appearance. The nuclei were round with dispersed chromatin; sometimes a thin rhyme of cytoplasm was visible. In the cytospins corresponding to the cystic area, the cell

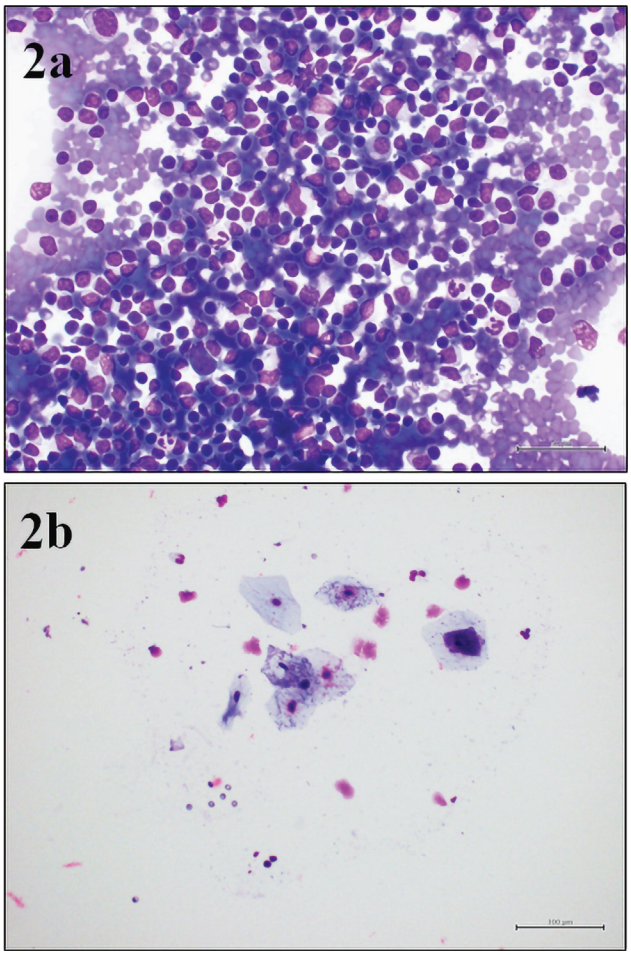

Figure 2. *Fine Needle Aspiration Cytology features. (**a**) Air-fixed and Diff Quik-stained smear showing a relatively polymorphic and constituted by small lymphocytes, plasmacytoid cells e some large elements and histiocytes (Diff-Quik staining, x 20 magnification); (**b**) alcohol-fixed and Papanicolaou-stained smear showing some dispersed squamous cells, referable to the lining epithelium of the cyst (Papanicolaou staining, x 10 magnification).*

population was more polymorphic and dispersed in a proteinaceous fluid background. This last cellular population consisted of small lymphocytes, monocytoid and plasmacytoid cells, plasma cells, few large, transformed lymphocytes and histiocytes (**Figure 2**). The immunophenotype of both lymph nodes by flow cytometric analysis was an abnormal B-cell phenotype: CD19+CD20+CD5-CD22+CD10- sIgK+. The percentage of abnormal B cell population was 84% for lymph node derived from parotid and 37% for lymph node derived from cervical region (**Figure 3**). Cytological diagnosis of a monoclonal B-cell lymphoproliferative disorder was rendered.

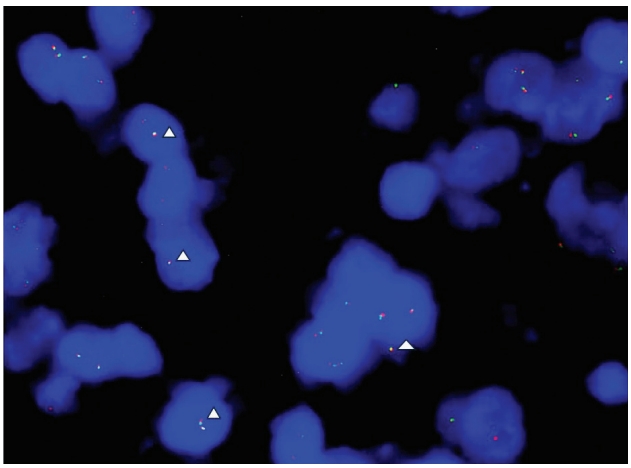

Figure 3. *Flow cytometry showing a B-cell lymphoid population with restriction for the kappa light chain. Kappa:lambda ratio was 84.*

Histological and immunohistochemical features

Macroscopically, the surgical sample measured 3x2.5x1.5 centimetres. The cut section revealed some solid nodular areas and some cystic cavities, for a total diameter of 1.7 centimetres. Histological examination showed lymphoid nodules with some cysts in the context, lined by multi-layered squamous epithelium. Diffuse lymphocytic exocytosis was observed through the cystic wall. At the periphery of the sample, some lobules of salivary gland were present (**Figure 4**). The lymphoid cell population resulted BCL-2-positive and partially positive for CD43; negative for CD5, CD23, Cyclin D1, CD10, BCL-6. CD138 showed some plasma cells. CD23 showed the presence of irregular-shaped residual networks of follicular dendritic cells, and images of follicular "colo-

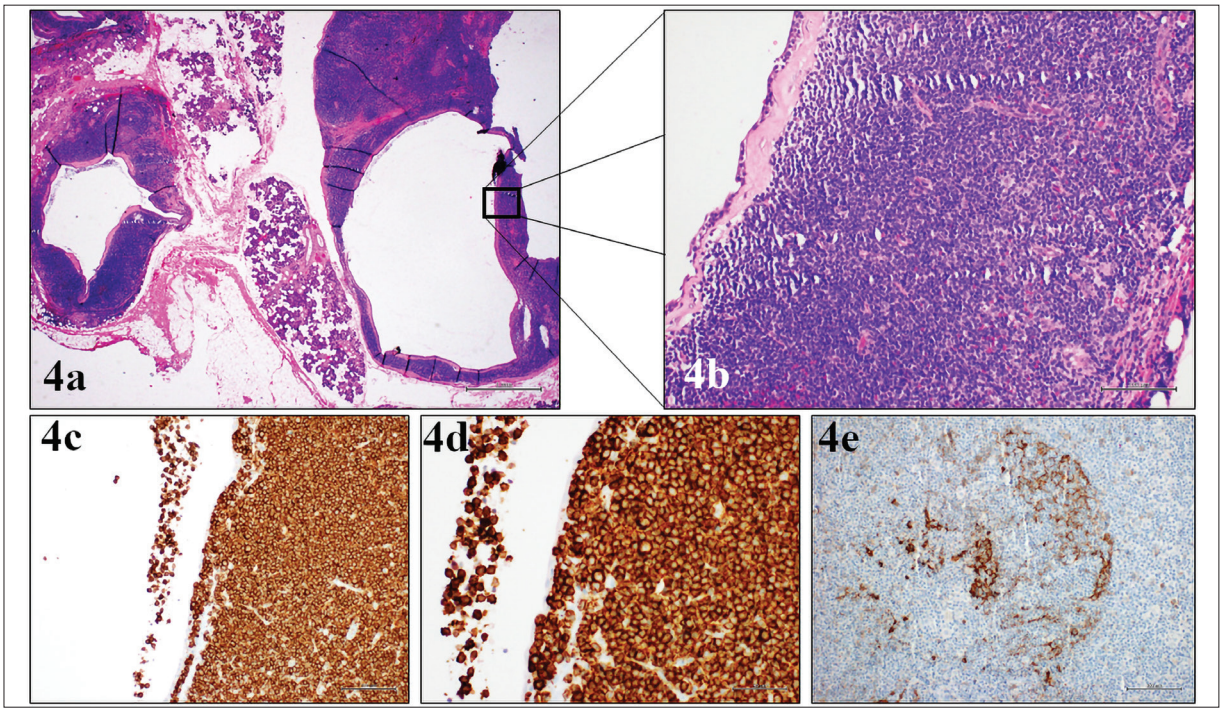

Figure 4. *Histological and immunohistochemical features. (**a**) Histological slide showing the presence of a lympho-epithelial cyst in the context of salivary glandular parenchyma. The cyst is lined by thin squamous epithelium, and a dense lymphoid population fills the wall of the cyst (H&E, x 4 magnification); (**b**) the lymphoid population in the wall of the cyst is constituted mainly by monomorphic small mature lymphocytes with the presence of some medium-sized elements (H&E, x 10 magnification); (**c**) immunohistochemistry demonstrating the expression of CD20 and in almost all the lymphoid cells. In these fields, the lymphoid cells penetrate in the thickening of the epithelium (CD20-immunostaining, x 10 magnification); (**d**) immunohistochemistry demonstrating the expression of BCL-2 in the same cellular population (BCL-2-immunostaining, x 20 magnification); (**e**) CD21 immunohistochemistry highlighting a deconstructed network of follicular dendritic cells in a residual germinal center (CD21-immunostaining, x 20 magnification).*

nization" by small, BCL-2-positive and CD2-negative lymphoid cells. Ki67 resulted about 10%.

FISH AND PCR RESULTS

IGH gene was not rearranged at FISH analysis (**Figure 5**); B cell clonality was assessed through PCR (data not shown).

Final diagnosis

A final diagnosis of an extranodal non-Hodgkin B-cell MZL occurring in the context of a lympho-epithelial cyst was rendered. The 2 years clinical and instrumental follow-up was uneventful.

DISCUSSION

To our knowledge, malignant lymphoma in the context of a cervical LEC has not been reported previously. Rare cases of primary EMZL of parotid gland may acquire a multicystic appearance due to the dilatation of the glandular ducts (2). These cases are characterized by the presence of the lymphomatous population in the glandular parenchyma, while in the case we present the lymphoid population was located substantially out of the gland and was therefore referable to a real LEC. Diagnosing EMZL within the context of an LEC poses significant challenges. Indeed, EMZL often lacks specific immunohistochemical and molecular markers. Microscopically, EMZL may be constituted by a heterogeneous lymphoid population including mature lymphocytes, lympho-plasmacytoid cell, plasma cells, centrocyte-like cells, or may be constituted by a relatively monotonous monocytoid cells (3). A critical histological feature of EMZL is the lymphoepithelial lesion (LEL), characterized by the presence of lymphocytes within the epithelium. Although LELs may be observed in non-neoplastic dysimmune T-cell diseases, such as Sjogren syndrome and celiac disease, they are significantly associated with B-cell lymphoma (5). Nevertheless, reactive B-cell lymphoid proliferation in some specific locations, such as Valdeyer's ring tonsils and LECs, may present LELs without this implying a greater risk of malignancy. Indeed, LELs may be observed in LECs, in both HIV-positive and HIV-negative patients (2). Another histological clue of EMZL is follicular colonization, which consists in the partial

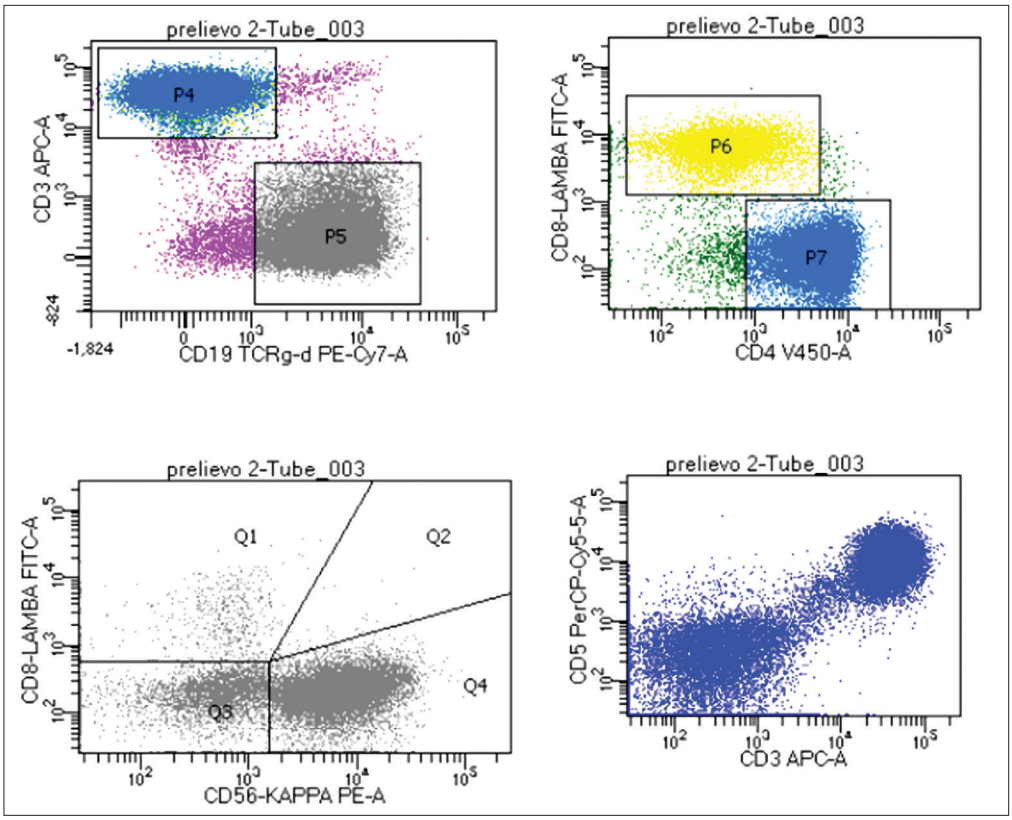

Figure 5. *FISH analysis. 1. Normal IGH gene. The FISH DNA probe is a mixture of a Texas Red-labeled DNA probe (IGH-UPSTREAM) covering centromeric to the IGH breakpoint cluster region, and a fluorescein-labeled DNA probe (IGH-Dowstream) covering telomeric to the IGH breakpoint cluster region. Normal fusion signals (white triangle) are clearly evident.*

loose of network of follicular dendritic cells. In this setting, immunohistochemical tests for CD21 and CD23 highlight the residual follicular dendritic cells and therefore play an important role in the diagnostic evaluation. The histological diagnosis of EMZL in the context of a LEC cannot be rendered without molecular evaluations, proving the clonal nature of the proliferation. This is even more true in case of cytological sample: the correct management of the sample is mandatory for the diagnosis (6). The ROSE allows to exclude rapidly the diagnostic possibility of epithelial or mesenchymal tumors and to recognize a lympho-proliferative disease. Further cytological passes allow to sample sufficient material for the FC evaluation and cell-block realization, to obtain a complete immunophenotype definition including clonal evaluation (7). CBL is a laboratory finding of a clonal mature lymphoid population in the peripheral blood (<5 x 10^9/L). In particular, CD5-negative CBL is characterized by CD19-positive and CD5-negative small lymphocytes which do not express any other distinctive FC markers, being potentially related to B-cells of marginal-zone lymphomas (8). It is a rare condition with an incidence that increase with the age, reaching 50%-75% among the 90-years-old persons (3), with poorly known clinical significance and heterogeneous molecular alterations including NOTCH2, MYD88, TNFAIP3 and CD79B mutations (9). It is actually known that patients affected by CBL have a 4-fold increased risk to develop a B-cell lymphoma in comparison to the general population (10, 11). In a large series by Xochelli *et al*., 17 out of 102 (16.7%) subjects affected by CD5-negative CBL developed a lymphoma after a median follow-up of 5 years, including 15 splenic MZLs, 1 gastric EMZL and 1 cutaneous diffuse large B-cell lymphoma (12, 13). Interestingly, progressive and non-progressive cases seemed to have different genetic background, the former showing 7q deletion, the latter showing a complex karyotype and exclusively the translocation t (2;7)(p11; q22).

CONCLUSIONS

At the best of our knowledge, this is the first reported case of an EMZL developing in the context of cervical LEC in a patient affected by CD5-negative CBM.

The diagnostic evaluation of a LEC has to include the possibility of a concurrent EMZL, mainly in case of high risk biological and clinical conditions like CBL, to avoid underestimation or misdiagnosis of a hematological pathology of more serious clinical significance. The proper management of the cytological sample and the application of clonal tests in both cytological and histological samples are mandatory for a correct diagnosis.

COMPLIANCE WITH ETHICAL STANDARDS

Fundings

There were no institutional or private fundings for this article.

Conflict of interests

The Authors have declared no conflict of interests.

Availability of data and materials

The data underlying this article are available in the article.

Authors' contributions

All Authors attest that they meet the current ICMJE criteria for Authorship. GT participated to the diagnosis, collected the data, and wrote the manuscript. IC and AR performed cytological diagnosis and collected data. GC performed clinical diagnosis and reviewed the paper. VT performed the flow cytometry. PM and LL collected the data. RF participated to the diagnosis, reviewed the manuscript and supervised the work. All Authors have read and approved the final version of the manuscript.

Ethical approval

Human studies and subjects

Consent to publish the case report was not obtained. This report does not contain any personal information that could lead to the identification of the patient.

Animal studies

N/A.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.

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