e - ISSN - 2349 - 8005

INTERNATIONAL JOURNAL OF ADVANCES IN CASE REPORTS

Journal homepage: www.mcmed.us/journal/ijacr

# DIFFERENTIAL DIAGNOSIS PITFALLS IN OCULAR ADNEXAL LYMPHOMA WITH ABERRANT PHENOTYPE AND ASSOCIATION WITH CHLAMYDOPHILA PSITTACI: CASE REPORT

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**Article Info**

*Received 15/08/2015*
*Revised 27/08/2015*
*Accepted 02/09/2015*

**Key words:** Flow cytometry, Ocular adnexal lymphomas, MALT lymphoma, Follicular lymphoma, *Chlamydophila psittaci.*

**ABSTRACT**

Ocular adnexal lymphomas (OAL) are a heterogeneous group of malignancies, making up approximately 1-2% of non-Hodgkin lymphomas. The most common subtype of primary OAL is MALT lymphoma, followed by follicular lymphoma. Small B-cell lymphomas share many similarities, especially among indolent lymphomas, in which aberrant and overlapping phenotypes often make defining the diagnosis difficult. In addition, since 2004, *Chlamydophila psittaci* (Cp) has been associated with ocular adnexal MALT-lymphoma. The aim of this study is to report a case of ocular adnexal lymphoma with Cp infection association, which illustrates a complex differential diagnosis. The biopsy of the eye lesion was morphologically and phenotypically analyzed. Eye swab and peripheral blood (PB) samples were analyzed by immunofluorescence and PCR in order to detect the presence of Cp. Histopathological and immunohistochemical analysis of the biopsy of the eye lesion revealed an atypical lymphocytic infiltrate CD20 and lambda positive. Multiparametric flow cytometry showed 54% of mature B lymphocytes with a kappa/lambda ratio equal to 0.46. Lambda B lymphocytes showed greater size, expression of CD19, bright CD20, and CD10. Cp DNA was detected in eye swab and PB samples by PCR. The elected treatment regimen was doxycycline along with radiotherapy sessions. The compilation of clinical information added to the results of histopathologic, phenotypic, and molecular exams indicated the diagnosis of ocular adnexal MALT-lymphoma with aberrant expression of CD10.

## INTRODUCTION

Ocular adnexal lymphomas (OAL) are a heterogeneous group of malignancies, making up approximately 1% to 2% of non-Hodgkin lymphomas (NHLs) and 8% of extranodal lymphomas [1]. The most common subtype of primary OAL is extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type, followed by follicular lymphoma (FL) [1]. MALT lymphoma is an indolent neoplasm which comprises 7% to 8% of all B-cell lymphomas. Most cases occur in adults with a median age of 61 years and a slight female preponderance [2,3]. At diagnosis, most patients have clinical-stage IE disease (extranodal disease limited to the site of origin), but in up to 10% to 20% the regional lymph nodes are involved (stage IIE) and a further 5% to 10% have bone marrow involvement [4]. The gastrointestinal tract is the most common site of origin of MALT lymphoma, comprising 50% of all cases. Additional common sites include salivary gland, lung, head

and neck, ocular adnexa, skin, thyroid, and breast [2,5]. MALT lymphomas are frequently associated with chronic infections. For instance, a lot of attention is given to the well-known correlation between the infection by *Helicobacter pylori* and gastric MALT lymphoma [4,5]. In addition, since 2004, *Chlamydophila psittaci* (Cp) has been associated with ocular adnexal MALT-lymphoma (OAML) [6]. FL is also an indolent-course lymphoma which commonly involves lymph nodes and bone marrow, but less commonly extranodal sites. Median age at diagnosis and gender predominance are very similar to MALT lymphoma. Interestingly, ocular adnexal FL (OAFL) with association to Cp infection has already been reported [7]. Moreover, small B-cell lymphomas share many similarities, especially among indolent lymphomas such as FL and marginal zone lymphomas, in which aberrant and overlapping phenotypes often make defining the diagnosis difficult [8]. Therefore, the present study aims to report a case of OAL with Cp infection association which illustrates a complex differential diagnosis.

## CASE REPORT

This report describes the case of a 45-year-old male diagnosed in 2004 with B-cell lymphoma in the University Hospital of the Federal University of São Paulo in the state of São Paulo, Brazil. This diagnosis was obtained through pathological analysis and immunohistochemistry of biopsy of the lower temporal conjunctiva. The patient was treated with radiotherapy (17 sessions, with a total dose of 3060 cGy), which resulted in disappearance of the tumor.

After ten years, in 2014, the patient looked for medical assistance in the University Hospital of the Federal University of Santa Catarina, in the state of Santa Catarina, Brazil. He developed a progressive right ocular adnexa lesion, diplopia, and foreign body sensation in the upper eyelid for around two months (Figure 1A). The biopsy of the new lesion in the right eye was performed and histopathological analysis revealed an atypical lymphocytic infiltrate, which mimicked a reactive follicular hyperplasia. Lymphoid cells had small to medium size, slightly irregular nuclei, moderately condensed chromatin, inconspicuous nucleoli, and a diffuse arrangement with associated lymphoepithelial lesion (Figure 1B). Immunohistochemistry showed positivity for CD20 and lambda (Igλ) on atypical lymphocytes (Figure 1C and 1D) and Ki-67 index of 60% on germinal center cells and 10% on interfollicular cells, whereupon the diagnosis of a low-grade lymphoma was concluded, though the presence of reactive follicles did not exclude the possibility of a follicular hyperplasia. Immunophenotypic analysis of the biopsy demonstrated 54% of mature B lymphocytes, of which 31.6% were kappa (Igκ) and 68.4% were lambda (Igλ), which resulted in a kappa/lambda ratio equal to 0.46. Significantly, lambda B lymphocytes showed greater size, expression of CD19, bright CD20, CD10, CD95, CD22, CD39, CD81, CD23, and CD79b, and no expression of CD5, CD27, CD200, CXCR5, CD43, CD31, LAIR-1, CD11c, sIgM, CD103, CD49d, CD62L, CD38, and cyBcl-2 (Figure 2 and 3).

With these last findings, a differential diagnosis was required of MALT lymphoma with aberrant expression of CD10 or FL without expression of cyBcl-2 and CD38, since both types of lymphoma can affect the ocular adnexal region.

Simultaneously, the research for the presence of Cp was conducted. Initially, the assay using direct immunofluorescence in ocular discharge was negative. However, eye swab and peripheral blood samples were analyzed by PCR and Cp DNA was detected in both samples. In addition, blood tests showed values within the normal range and cranial computed tomography (CT) showed no changes. The lymphoma was defined as stage IE. As previously mentioned, Cp has been extensively associated with the development of OAML and, much more rarely, associated with OAFL. As a whole, the research group believed the results suggested the diagnosis of OAML associated with Cp infection with aberrant expression of CD10. However, the possibility of an OAFL was not excluded. The elected treatment regimen was doxycycline at a dose of 100 mg given twice per day for 14 days, along with 20 radiotherapy sessions. After this period, regression of the lesion was noticed through reduced volume and whitening of the site. In October 2014, a new cranial CT scan showed normal orbits and the presence of a prominent lymph node in the neck, which is under close observation.

**Figure 1. (A) Right ocular adnexal lesion. (B) Histological appearance of the right ocular adnexal lesion biopsy (H & E 100x). (C) Immunohistochemical expression of CD20 (100x) and (D) immunohistochemical expression of lambda (400x)**

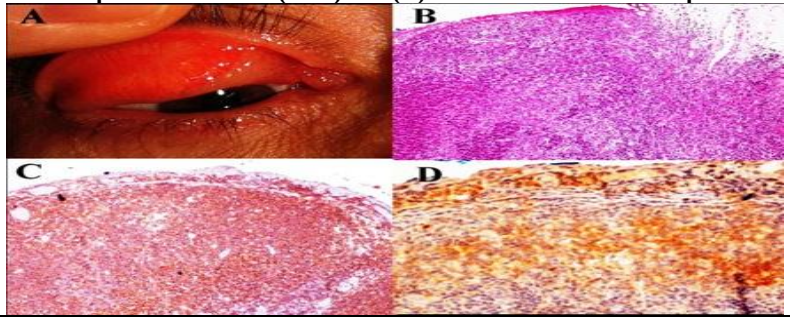


**Figure 2. Immunophenotyping by flow cytometry of the right ocular adnexal lesion biopsy. Plot A shows the sample viability analysis with vital dye 7-AAD. Positive cells are non-viable cells, while negative cells are viable (90%). Plot B shows B-cells identified by gating the CD19+ events, which represent 54% of the total cellularity. Maturity of B-cells is represented in Plot C and Plot G (CD20+, bright CD45+). Plot D shows (gate on CD19+ and CD20+ cells) the existence of two distinct populations considering expression of Ig light chain: colored in red is Igλ type and in blue, Igκ type, which resulted in a κ/λ ratio of 0.46. Interestingly, plot E demonstrates that Igλ B-cells are 1.14 times larger compared to Igκ B-cells. In addition, Igλ B-cells expressed CD20 more brilliantly (Plot C). When CD10 expression was evaluated (Plot F), only Igλ B-cells expressed this marker. Considering CD5 and CD43 expression, both cell populations were negative (Plots G and H). In the background, T-cells are represented in gray.**

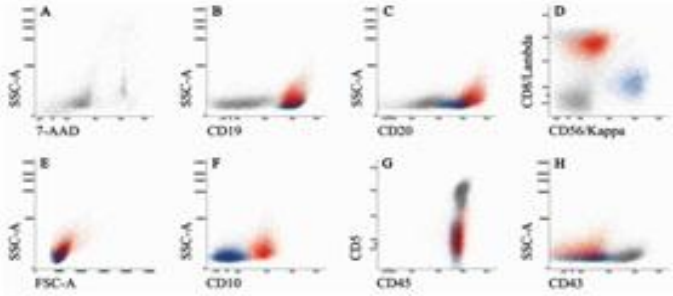
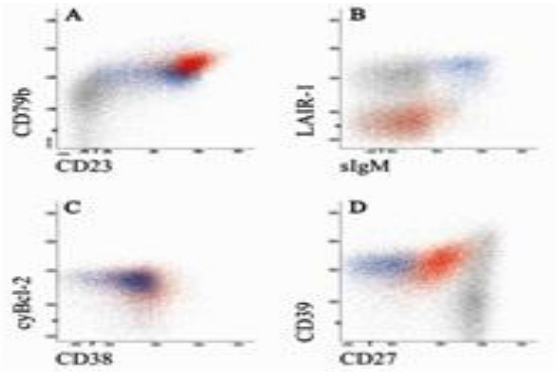



**Figure 3. Immunophenotyping by flow cytometry of the right ocular adnexal lesion biopsy. Igλ CD10+ B-cells are colored in red, and Igκ CD10 negative B-cells, in blue. Again in the background, T-cells are represented in gray. Plot A demonstrates the double-positivity for the markers CD79b and CD23 while plots B and C show double-negativity for LAIR-1, sIgM, cyBcl-2, and CD38, respectively. Plot D shows positivity for CD39 and negativity for CD27 (compared to T-cells).**




## DISCUSSION

More than 95% of all OAL are of B-cell origin, and 80% are low-grade lymphomas. The most common subtype of primary OAL, accounting for 35% to 80% of cases, is MALT lymphoma, followed by FL (~20%), and diffuse large B-cell lymphoma (DLBCL) (~8%). The most common site of origin is the orbit (40%), followed by the conjunctiva (35% to 40%), lacrimal gland (10% to 15%), and eyelid (10%). In addition, bilaterality occurs in 10% to 15% of cases (80% simultaneous and 20% sequential events), and nodal involvement is reported in approximately 5% of patients [1]. Clinical presentation of conjunctival OAL consists of a classic "salmon-red patch" or "salmon-pink patch" appearance with swollen conjunctiva, causing redness and irritation [1,9].

MALT lymphoma is an extranodal lymphoma composed of morphologically heterogeneous small B-cells including marginal zone (centrocyte-like) cells, so-called monocytoid B-cells, small lymphocytes, and scattered immunoblasts and centroblast-like cells [10].

Pathognomonic histopathologic features include "follicular colonization" and the formation of "lymphoepithelial lesions" formed by invasion of individual mucosal glands or other epithelial structures by aggregates of lymphoma cells [1,4]. These features are consistent with the morphology found in the present case. Immunophenotypically, MALT lymphomas have a characteristic profile, which usually allows their differentiation from benign lymphoproliferative disorders and other small B-cell lymphomas, such as FL. They are characterized by the expression of pan-B markers, as CD19, CD22, CD79b, and a bright expression of CD20, while CD10, CD23, CD5, and CD27 are frequently negative, and expression of CD43 and CD11c varies [1,2]. The most frequent translocation in OAML, observed in 15% to 40% of patients, is t(11;18), although t(14;18) is also found in a large number of patients [1]. FL usually presents with asymptomatic lymphadenopathy and bone marrow involvement [11].

Although extranodal involvement is relatively uncommon, as stated earlier, cases of OAFL have been reported [12]. Immunophenotypically, FL cells express CD19, CD20, CD22, CD10, and Bcl-6 and are negative for CD5, CD43, and CD23. In general, FL cells overexpress cyBcl-2 protein [10,11]. Nevertheless, cyBcl-2 is expressed by a variable proportion of the neoplastic cells in 85% to 90% of cases of grade-1 and grade-2, but only 50% of grade-3 FL [10]. In about 85% of cases, demonstration of t(14;18) will help in diagnosing FL, but approximately 10% of FL cases do not have this cytogenetic abnormality [8,11].

Considering morphological and phenotypic features, the medium-sized lambda B-cells found in the biopsy were not totally conclusive for OAFL or OAML. The detection of t(14;18) was not performed since is not available in the Institution. Nevertheless, this translocation is not pathognomonic for FL, and, as cited above, can be found in OAML cells. Thus, this exam is usually not used for routine diagnosis and therapeutic decisions concerning OAML. Additionally, the phenotype of lymphoma cells could not be related with that observed in 2004, because ten years earlier immunophenotyping and research for *Cp* were not performed. Nevertheless, the light-chain restriction is not consistent, since the left OAL was kappa- and the right OAL was lambda-type. OAML shares several clinic pathologic features with other MALT lymphomas. In fact, it arises in tissues normally devoid of innate immune system, often develops on a background of preexisting chronic inflammation, and usually shows an indolent clinical course. The presence of a preexisting inflammatory background seems to be pathogenically significant for MALT lymphomas, underlying the possible role of infectious agents and autoimmune reactions [9].

Several infectious agents have been proposed as risk factors for the development of this type of lymphomas, and some of these agents have been considered as targets for new therapeutic strategies [6,13]. The list of infectious agents associated with lymphomas has grown longer with molecular investigations. At the moment, it comprises at least five distinct members: *Helicobacter pylori*, *Campylobacter jejuni*, *Borrelia burgdorferi*, Cp, and hepatitis C virus (HCV), which have been associated with gastric MALT lymphoma, immunoproliferative small intestinal disease, cutaneous MALT lymphoma, OAML, and splenic marginal zone lymphoma, respectively [14-16].

Ferreri and colleagues (2004) were the first to report a possible association between Cp and OAML and the benefit of antibiotic treatment has been documented. Cp DNA has been detected in 80% of patients with this lymphoma [6]. Besides that, detection of the DNA of such bacteria in peripheral blood mononuclear cells of 40% of patients with Cp-positive OAML indicates this is often a systemic infection [7]. The prevalence of Cp in OAL ranges from 47% to 80% in Italy, Austria, Germany, and Korea, while the percentages are much lower in other countries [3].

Regarding the antibiotic treatment, Kiesewetter and Raderer (2013) evaluated nine studies published between 2004 and 2012 on antibacterial therapy in patients with OAML including Korean, Italian, Hungarian, US-American, Chilean, Spanish, Swiss, and Austrian study groups. Out of the 116 patients tested within these studies, 66 (56%) showed a positive test result for the presence of Cp, whereas the others were rated negative for this pathogen. A single course of oral doxycycline, a tetracycline derivative, at a dose of 100 mg given twice a day for three weeks was the most popular regimen and was used by most investigators [13]. This antibiotic treatment has been proposed as a valid alternative for OAML patients [9]. Prospective trials with standardized objective response criteria and longer follow-up are necessary to further evaluate the role of antibiotics in the treatment of OAML [1]. Concerning local treatment with radiotherapy, most irradiated patients with localized lymphoma achieve an objective response, which is slow and gradual [9.17]. Relapse rate at 4 years is 20% to 25% and it could be related to low-radiation doses or to the use of lens shielding. Most relapses involve the contralateral orbit (half of relapses) and distant extranodal organs [9]. Stefanovic and Lossos (2009) recommend 30 Gy as the optimal radiation dose for local disease control in patients with OAML [1].

The present case report had a lymphoma relapse involving the contralateral conjunctiva after ten years of local treatment with radiotherapy (3060 cGy). For this reason, and especially after the identification of Cp, a combined treatment approach with doxycycline for two weeks and radiotherapy was applied. Prognosis seems to be favorable, especially since conjunctival OAML is associated with the lowest risk of disseminated disease [1]. Indeed, in the present case, after treatment, the lesion's volume decreased.

Millar and colleagues (1999) reported a case of CD10-positive thyroid marginal zone non-Hodgkin lymphoma. It showed the typical morphological features of a marginal zone lymphoma: nodular low-power architecture which effaced most of the gland, with prominent lymphoepithelial lesions. In that study, immunohistochemistry showed CD10 and LN1 (CDw75) positivity, which is normally associated with follicle center cells only. No evidence of t(14;18) was found by PCR. Those authors concluded that the results suggested the diagnosis of MALT lymphoma with aberrant expression of CD10 [18]. Previously, in 1998, a pulmonary MALT lymphoma case was reported due to aberrant CD5 and CD10 (weak) expression [19]. Additionally, studies have reported rare cases of nodal marginal zone lymphomas with aberrant expression of CD10 since staining for cyBcl-2 was negative [20,21]. Moreover, Ponzoni and colleagues (2008) investigated Cp infection in human lymphomas through a screening on 205 cases of nodal and extranodal lymphomas besides a variety of non-neoplastic lymphoid tissues. In summary, Cp turned out to be present not only

in OAML, but also in an unexpected proportion of nodal and extranodal lymphomas. Concerning the group of OAL with orbit involvement, from 31 patients, 29 were classified as marginal zone type with a frequency of Cp infection of 69%, one case was classified as DLBCL and the other as FL. Importantly, Cp DNA was detected by PCR in both cases [7]. Therefore, although very rare, Cp could be associated with the development of other types of lymphoma, in spite of OAML.

## CONCLUSION

The present study described a case of OAL associated with Cp that highlights diagnostic pitfalls within the group of mature small B-cell lymphomas, especially for FL and marginal zone lymphomas with uncommon phenotype. According to the above, the compilation of clinical information added to the results of histopathologic, immunohistochemical, immunophenotypic, and molecular exams indicated the diagnosis of OAML with aberrant expression of CD10.

**ACKNOWLEDGEMENT: NIL**

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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