doi: 10.17221/126/2015-VETMED

Extranodal marginal zone B-cell lymphomas of the bilateral third eyelids in a dog

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ABSTRACT: Extranodal marginal zone B-cell type lymphoma of mucosa-associated lymphoid tissue (MALT) in dogs has similar properties to human low-grade B-cell lymphoma. Both are characterised by a relatively low mitotic rate and a slow manifestation of clinical signs. Primary MALT lymphoma of the third eyelid in canines is very rare. In this case report we describe bilateral MALT lymphoma in a 21-month-old miniature poodle. Histological analysis indicated that the masses were mainly composed of lymphoid cells and lymphoepithelial lesions, a typical feature of MALT lymphoma. Immunohistochemical analysis revealed that the neoplastic cells were positive for CD79α, but negative for CD3. We believe that this case report will facilitate the diagnosis, treatment, and prognosis of canine MALT lymphoma of the third eyelid.

Keywords: immunohistochemistry; nuclear atypia; lymphoid cell; polycyctic lesion; ocular adnexal tumor; canine

Neoplasia of the third eyelid is rare in dogs, and when it does develop, it is usually malignant (Aquino 2007). The tumour may originate from the conjunctival or glandular tissue. Generally, it develops in older dogs, and surgical resection is the typical recommended treatment (Aquino 2007; Labelle and Labelle 2013). The most common form is adenocarcinoma (Aquino 2007), but there have also been reports of squamous cell carcinoma, hemangioma, hemangiosarcoma, mastocytoma, mast cell tumour, papilloma, and melanoma (Hallstrom 1970; Peiffer et al. 1978; Lavach and Snyder 1984; Schaffer et al. 1994; Liapis and Genovese 2004).

The third eyelid is important for production of immunoglobulins in the tear film (Alexandre-Pires et al. 2008) and, therefore, it harbours an abundance of immune cells. On rare occasions, a primary tumour can develop within these cells (Ota-Kuroki et al. 2014). Perlmann et al. (2009) reported a case of an extramedullary plasmacytoma in a 7-year-old American cocker spaniel. Hong et al. (2011) reported a case of a mucosa-associated lymphoid tissue (MALT) lymphoma also in a 4-year-old Cocker spaniel. In both cases, the reported prognosis after surgical treatment was excellent.

Herein, we report a bilateral MALT lymphoma of the third eyelids in a 21-month-old Miniature poodle.

Case description

A 21-month-old neutered male Miniature poodle (weight: 4.5 kg) visited the animal hospital for a dental consultation and scaling. During the physical examination, multiple whitish, round nodules 2–5 mm in size were incidentally found in both third eyelids (Figure 1). There were no other specific findings from the physical examination, blood tests, abdominal ultrasonography, and thoracic and abdominal radiography. Basic ophthalmologic examinations including menace response, palpebral reflex and pupillary light reflex were normal, and there were no distinctive lesions on the cornea or conjunctiva. Although there were no clinical symptoms associated with these lesions, both masses

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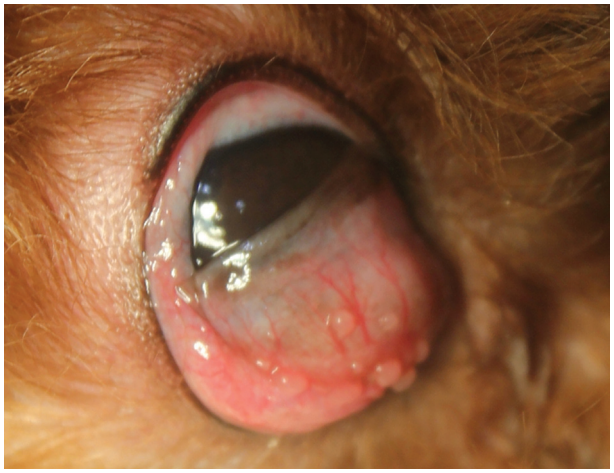

Figure 1. Multiple whitish nodules on the right third eyelid

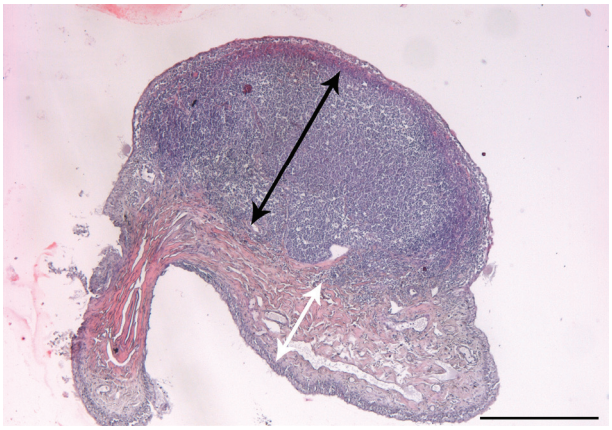

Figure 2. Low magnification image of a third eyelid mass. The mass mainly consists of lymphoid cells (black arrow) and infiltrates into the adjacent collagen stroma (white arrow). Haematoxylin and eosin staining, bar = 500 μm

were resected for histological examination to determine whether they were simple hyperplasias or tumours, as well as to determine an appropriate treatment plan. Under general anaesthesia, the bulbar surfaces of the third eyelids, including the entire lesions, were resected using a microsurgical instrument, and the remaining regions were induced to heal with second intension. The resected masses were fixed in 10% neutralised buffered formalin (BBC Biochemical, USA) and sent to Konkuk University for diagnosis.

Each neoplastic sample was processed using standard methods and embedded in paraffin. The 4-μm-thick sections were stained with haematoxylin (Sigma-Aldrich, USA) and eosin (H&E; BBC Biochemical, USA). For immunohistochemistry, the sections were deparaffinised using a standard method, and endogenous peroxidase activity was quenched by incubation with 3% hydrogen peroxide (Daejung, Kyungkido, Korea) for 30 min. To liberate the cross-linked epitopes for immunohistochemical reactions, the sections were washed with PBS and then exposed to a combination of 0.1M, pH 6.0 citric acid (Sigma-Aldrich, USA) and trisodium citrate (Sigma-Aldrich, USA) for 20 min for heat-induced antigen retrieval. The sections were then incubated in blocking solution (10% normal goat serum, Vector Lab, USA) for 30 min. Subsequently, the sections were labelled with mouse monoclonal anti-CD79α (1 : 100, Santa Cruz Biotechnology, USA) and mouse monoclonal anti-CD3 (1 : 100, Abcam, Cambridge, UK) primary antibodies for 1 h at room temperature. The antibody-labelled sections were then incubated with an avidin-biotin-peroxidase complex (ABC) solution using an ABC kit (Invitrogen, Carlsbad, USA). The Vector SG kit (Vector laboratories, USA) was used for visualisation. Sections were then counterstained with nuclear fast red solutions.

Microscopic analysis showed that the polycystic lesions in the third eyelids had similar characteristics overall. The masses were mainly composed of lymphoid cells (Figure 2). The tumour cells also showed massive infiltrative growth to the adjacent connective stroma (Figure 2). The conjunctival epithelium and glands had been invaded by tumour cells (Figures 3A and 3B).

At higher magnification, the tumour cells appeared to be lymphocytes with a high N : C ratio and nuclear atypia (Figures 4A and 4C). We also found that neoplastic cells were admixed with scant cytoplasmic lymphocytes and small amount of cytoplasmic and large nuclei lymphocytes (Figure 4A). In immunohistochemical staining, the tumour cells stained positive for CD79α and negative for CD3 (Figures 4B and 4D). On the basis of these histological findings, a final diagnosis of malignant lymphoma of B-cell origin of the third eyelid was made.

DISCUSSION AND CONCLUSIONS

The canine third eyelid not only physically protects the eye, but, among other roles, also contributes to the aqueous portion of the preocular

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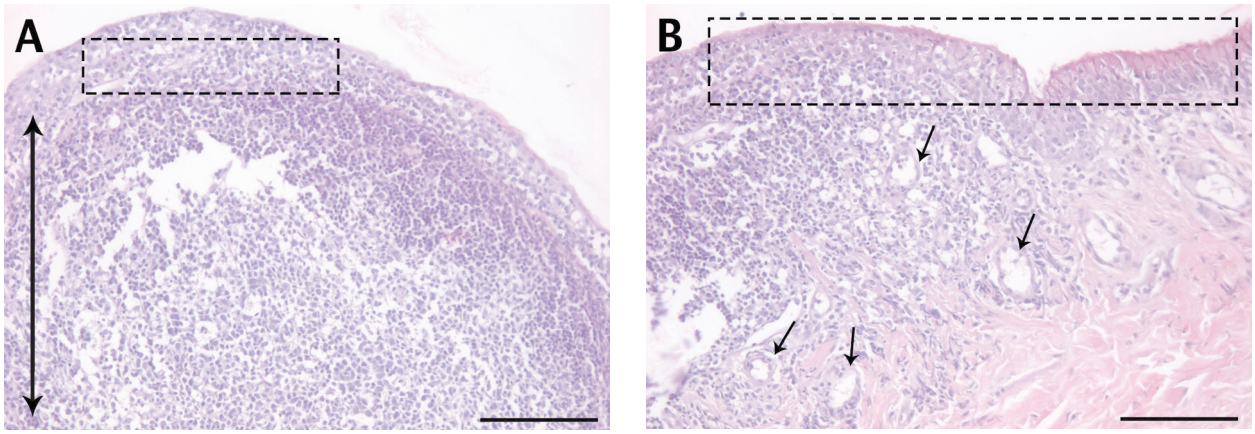

Figure 3. Microscopic images with conjunctival and glandular epithelium. (**A**) The lymphoepithelial lesion of the conjunctival epithelium was invaded (dashed line box) by tumour cells (arrow) as seen here. (**B**) There was also invasion of tumour cells, not only in the conjunctival epithelium (dashed line box) but also in the glands at the stroma (arrow). Haematoxylin and eosin staining, bar = 100 μm

film and removes foreign bodies from the anterior surface of the eye (Alexandre-Pires et al. 2008). Furthermore, the third eyelid is one of the most important immunological structures among the various adnexa of the eye (Hong et al. 2011). Plasma cells and lymphocytes are part of the normal composition of cells of the lacrimal gland interstitium (Alexandre-Pires et al. 2008). One of the main functions of the immunological cells in the third eyelid is to secrete immunoglobulin A (IgA) as part of tears (Alexandre-Pires et al. 2008). Histologically, B-lymphocytes form lymphoid follicles (nodules) and when activated by antigens they contain germinal centres; T-lymphocytes are mainly distributed in the surrounding area (Hendrix 2007; Alexandre-Pires et al. 2008). Even though ocular involvement is relatively common in cases of canine multicentric lymphoma, primary plasmacytoma or indolent lymphoma of the ocular adnexa have been reported only very rarely (Valli et al. 2006; Olbertz et al. 2013).

There are numerous reports of ocular adnexal lymphoid proliferation in human medicine, and the pathological characteristics of this condition are well known. However, there are few reported cases in veterinary medicine and little is known about pathological characteristics or prognosis for this condition in animals (Coupland et al. 2002; Olbertz et al. 2013; Olszewski and Castillo 2013). Extranodal marginal zone B-cell type lymphoma of MALT in dogs has similar properties to human low-grade B-cell lymphoma, and this indolent lymphoma is known to arise on a base of follicular lymphoid hyperplasia (Valli et al. 2006). Features of this type of tumour include relatively low mitotic rate and slow manifestation of clinical signs (Coupland et al. 2002). Therefore, in many cases it is difficult, on the basis of clinical signs, to distinguish between lymphoid hyperplasia and an actual tumour, or cytology from FNA and imprinting. For a definitive diagnosis, it is necessary to perform histopathological examination in order to assess the mitotic figures, presence of apoptotic bodies, arrangement of neoplastic cells and eventual lymphoepithelial lesions (Hong et al. 2011).

Cytomorphologically, extranodal marginal zone B-cell lymphoma results in expansion of a heterogeneous cell population consisting of centrocyte-like, monocytoid, and plasmacytoid tumour cells with occasional blasts in the marginal zone surrounding reactive follicles (Coupland et al. 2002; Hong et al. 2011). However, it is possible to confuse this heterogenicity with reactive lymphoid hyperplasia and other low-grade B-cell lymphomas (Coupland et al. 2002).

The presence of lymphoepithelial lesions is another factor used to distinguish tumours from lymphoid hyperplasias (Coupland et al. 2002). MALT lymphomas feature lymphoepithelial lesions composed mainly of B-cells (Coupland et al. 2002; Valli 2007; Hong et al. 2011). Extranodal marginal zone B-cell lymphoma can be regarded as a follicular colonisation due to secondary infiltration of neoplastic marginal zone B-cells (Coupland et al. 2002). If it arises in regions with epithelial cells, such as the conjunctiva or lacrimal glands, lymphoepithelial lesions form in the surrounding structures

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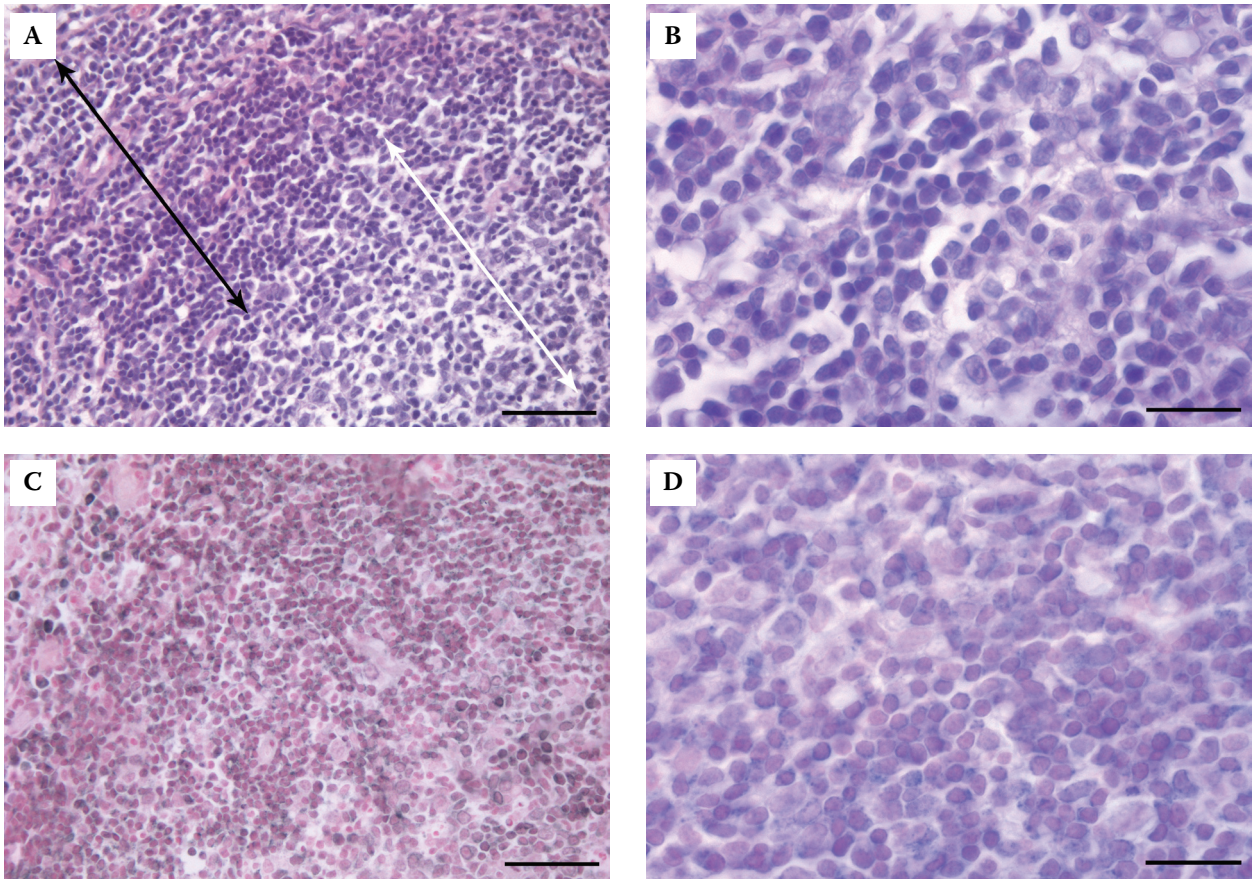

Figure 4. High magnification images and immunohistochemical images of a third eyelid mass. (**A**) Two distinctive phenotypes in the neoplastic lesion. Neoplastic lymphocytes consisted of small lymphocytes with scant cytoplasm (black arrow) and larger immature cells (white arrow). (**B**) The observed tumour cells had high N : C ratios and marked nuclear atypia. (**C**) Immunohistochemical staining showing tumour cells that stained positive to CD79α. (**D**) High magnification image of CD79α-positive cells showing dark-grey colour. (**A** and **B** = haematoxylin and eosin staining, **C** and **D** = immunohistochemical staining, **A** and **C**: bar = 50 μm, **B** and **D**: bar = 20 μm)

(Coupland et al. 2002). Therefore, this lesion can provide additional diagnostic information allowing discrimination between reactive lymphoid hyperplasia and extranodal marginal zone B-cell lymphoma (Coupland et al. 2002). In this case, we also observed cell infiltration in the conjunctival epithelium and the stromal vessels.

In cases of a tumour in the third eyelid, surgical resection is recommended as the treatment of choice (Lackner 2001; Aquino 2007; Labelle and Labelle 2013). In the case report of Hong et al. (2011), only one of the bilateral MALT lymphomas was resected for biopsy, and the other side was left for later follow-up. One year after surgery, there was no recurrence of the tumour and the tumour on the other side was reported to be of similar size to when it was first discovered. The third eyelid plasmacytoma case reported by Perlmann et al. (2009), also exhibited no recurrence one year after resection of the tumour only, as they managed to complete the resection while preserving the third eyelid. Mucosa-associated lymphoid tissue lymphoma is known to generally develop only locally where it first developed and to not spread to other sites (Coupland et al. 2002). The absence of recurrence six months after the surgery was also confirmed in this case.

Based on clinical conditions and histopathological observations, a final diagnosis of bilateral extranodal marginal zone B-cell lymphoma of the third eyelid was made. As very few reports of primarily periocular lymphoma in canines are available, we believe that this case report will provide insight that will facilitate the determination of guidelines in diagnosis, treatment, and prognosis for this kind of tumour.

doi: 10.17221/126/2015-VETMED

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Received: May 12, 2015
Accepted after corrections: January 6, 2017