

Case Report

Canine non-epitheliotropic CD4-positive cutaneous T-cell lymphoma: a case report

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Abstract

A 5-year-old, spayed female French Bulldog presented with multiple papules on the skin of the scapular area. Histopathological examination of punch biopsy samples revealed dense infiltration of small lymphoid cells in the superficial dermis and in areas surrounding hair follicles. Immunohistochemical analysis indicated that these cells were positive for CD3, CD4, and TCR $\alpha\beta$, but negative for CD1c, CD8 α , CD8 β , CD11c, CD20, CD45RA, CD90, MHC-II, and TCR $\gamma\delta$. In addition, CD45 was highly expressed, and the proliferation fraction was very low. Molecular clonality of T-cell receptor G chains yielded a clonal result. The skin lesions were surgically excised because they had progressed to the lateral front leg. Postoperative clinical course was favorable, and recurrence was not observed until the dog died in a traffic accident, approximately 1 year later.

Keywords: CD4 positive cells, cutaneous T-cell lymphoma, dog.

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Introduction

Canine cutaneous lymphoma is a relatively rare disease and is histologically classified into epitheliotropic and non-epitheliotropic forms (Gross *et al.* 2005; Miller *et al.* 2013). In dogs, primary non-epitheliotropic cutaneous lymphoma is less common than the epitheliotropic form (Gross *et al.* 2005; Miller *et al.* 2013).

Histopathology of the epitheliotropic form features primary epitheliotropism of atypical lymphocytes with Pautrier's microabscesses (Moore *et al.* 1994). Epitheliotropic lymphoma mainly consists of CD3-positive T-cells, which usually express CD8 (Moore *et al.* 1994; Fournel-Fleury *et al.* 2002; Miller *et al.* 2013). While the non-epitheliotropic form consists of heterogeneous groups of both T-cell and B-cell origin (Miller *et al.* 2013), which are either CD8-positive (7/16 cases) or CD4-negative/CD8-negative (8/16 cases) (Moore *et al.* 1998).

Herein, we report an unusual case of CD4-positive, non-epitheliotropic lymphoma confirmed via immunohistochemical analysis, together with detailed dermatological features, including clinical follow-up.

Case report

A 5-year-old, spayed female French Bulldog presented with pruritus with scattered crusty papules and epidermal collarettes on the trunk, mainly the abdominal region. Clinically pyoderma due to hypothyroidism was suspected. Cytological evaluation of direct smears, bacterial culture and serum total T4 level confirmed the diagnosis. Clinical symptoms completely resolved with oral minocycline (Minomycin, Sawai Pharmaceutical Co., Ltd., Osaka, Japan) at 10 mg/kg twice a day and oral levothyroxine (Thyradin-S, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) at 5 μ g/kg twice a day. The dog had

been disease-free along with continuous levothyroxine administration, but multiple non-pruritic papules, which had slightly diffuse erythema without scale and crust appeared on the shoulder area 5 months later (Fig. 1). Newly developed papules were morphologically different from previous one, while the relationship between the previous and latest events was uncertain. Physical examination did not show enlargement of the peripheral lymph nodes, as well as no abnormalities of body temperature were found, heart rate and respiration. Infectious and non-infectious granuloma, cutaneous lymphoma and histiocytic tumour were considered as possible diagnoses on the basis of clinical findings. Based on the results of drug susceptibility testing, minocycline was administered to rule out *Staphylococcus* infection. Dermatophytosis was excluded via microscopic examination and fungal culture. The lesions enlarged gradually, where atypical lymphocytes were observed in a direct smear of a lesion. In addition, haematological testing, including complete blood cell counts, serum biochemical analysis, and serum T4 level, as well as radiography and ultrasonography for the thoracic and abdominal cavities were conducted, but no abnormalities were identified.

A skin biopsy using a 6-mm diameter biopsy punch was performed under local anaesthesia using 2% lidocaine. The excised tissue was fixed in 10% formalin solution, and fresh frozen sections were obtained. Histopathological examination revealed proliferated



Fig. 1 Clinical features of the dog. Multiple papules are present on the right scapular area.

small lymphoid cells, which were characterized round nuclei with condensed chromatin and scant cytoplasm on the superficial dermis and the perifollicular areas. The cells exhibited chromatin-rich, oval nuclei with mild anisokaryosis, and mitosis were not prominent (Fig. 2). Immunohistochemical examination using formalin-fixed tissue revealed a positive reaction for CD3. An additional immunohistological analysis of fresh frozen sections was conducted via the avidin-biotin-peroxidase complex method, and the results were positive for CD4 and TCR $\alpha\beta$, but negative for CD1c, CD8 α , CD8 β , CD11c, CD20, CD45RA, CD90, MHC-II and TCR $\gamma\delta$ (Fig. 3). CD45 was highly expressed, and the proliferative fraction was low (Ki-67 labeling <5%). Furthermore, monoclonal reconstitution of T-cell receptor G chains (TRG) was observed. On the basis of these findings, the dog was diagnosed with non-epitheliotropic lymphoma, derived from CD4-positive T-cells. One month following the biopsy, lesions on the shoulder progressed to the lateral side of the front leg. Subsequently, the lesions were widely excised at the owner's request. The postoperative clinical course was favourable, and the lesion did not recur at the original site or on any other part of the body. Unfortunately, the dog died in a traffic accident approximately 1 year later, and a post mortem examination was not performed.

Discussion

To the best of our knowledge, this is the first case report describing canine cutaneous CD4-positive non-epitheliotropic lymphoma with detailed dermatological features, including the clinical course.

The majority of canine non-epitheliotropic lymphomas are observed as nodules or plaques on the face, limbs, neck and trunk (Affolter *et al.* 2009; Moore *et al.* 2013). The lesions in the present case were distributed on the shoulder area and the dorsal side of the front leg, which is consistent with previous reports. However, the lesions were relatively small, locally scattered, and expanded peripherally. Immunophenotyping analysis revealed that the lymphoma cells in the dog expressed CD4 and TCR $\alpha\beta$, but not CD45RA. As mentioned above, non-epitheliotropic T-cell lymphoma is usually either CD8-positive or CD4-negative/CD8-negative (Moore &

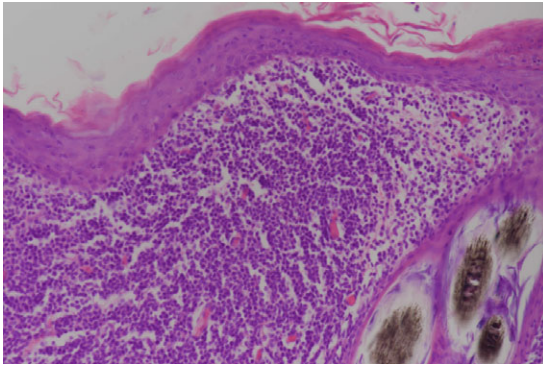


Fig. 2 Histopathological features of the lesion. Small lymphoid cells are proliferative at the superficial dermis and the perifollicular areas.

Olivry 1994; Moore *et al.* 1998). There has been only one reported case of canine non-epitheliotropic CD4-positive T-cell lymphoma, but the clinical features of that case are not available (Moore *et al.* 1998). Interestingly, non-epitheliotropic T-cell lymphoma is generally CD45RA-positive, and epitheliotropic T-cell lymphoma is CD45RA-negative (Moore *et al.* 1998), which is also common in cases of canine indolent T-cell dermal lymphocytic disease (Affolter *et al.* 2009). In contrast to that in cases of pseudolymphoma, T-cell receptor monoclonal

reconstruction was observed in this case (Landa *et al.* 1993). Furthermore, the proliferation fraction was extremely low. These findings suggested that the lymphoma was likely not aggressive.

The dog showed a good clinical outcome, without any medical management, for over 1 year. Prognosis of non-epitheliotropic cutaneous lymphoma is generally guarded, and the median survival period is 1–36 months (Moore *et al.* 2013). There are few reports on the long-term survival and clinical course related to non-epitheliotropic lymphoma, from which at least a part may be described as cutaneous lymphocytosis (Affolter *et al.* 2009).

Surgical excision of solitary cutaneous lymphoma may result in long-term local control (Miller *et al.* 2013; Moore *et al.* 2013). Unfortunately, immunohistochemical analysis of the lesion was not conducted in the previous cases. Clinically an early stage of indolent lymphoma (“cutaneous lymphocytosis”) should be considered in the dog, but CD4-positive, CD45RA-negative, non-epitheliotropic T-cell lymphoma might be categorized as Primary cutaneous CD4⁺ small/medium-sized T-cell lymphoma (CD4⁺ SMTL) in human (Willemze *et al.* 2005). Additional cases are warranted for further confirmation.

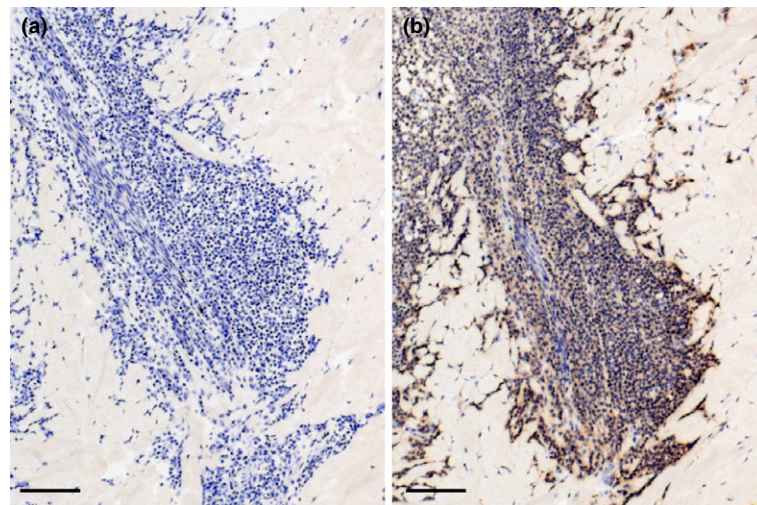


Fig. 3 Immunohistochemical analysis via the avidin-biotin-peroxidase complex method. Dense infiltration of CD4-positive small lymphoid cells is evident at the superficial dermis. Bar = 200 μ m.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethics statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and that no ethical approval was required for this particular case report.

Contributions

HK, PFM and MN wrote the manuscript. YK, KS and PFM performed histopathologic evaluation. All of the authors reviewed, revised and accepted the manuscript.

References

Affolter V.K., Gross T.L. & Moore P.F. (2009) Indolent cutaneous T-cell lymphoma presenting as cutaneous

lymphocytosis in dogs. *Veterinary Dermatology* **20**, 577–585.

Fournel-Fleury C., Ponce F., Felman P., Blavier A., Bonnefont C., Chabanne L. *et al.* (2002) Canine T-cell lymphomas: a morphological, immunological, and clinical study of 46 new cases. *Veterinary Pathology* **39**, 92–109.

Gross T.L., Ihrke P., Walder E., Affolter V. (2005) *Skin Diseases of the Dog and Cat*. 2nd edition, pp 866–893. Blackwell Publishing Co: Oxford.

Landa N.G., Zelickson B.D., Peters M.S., Muller S.A. & Pittelkow M.R. (1993) Lymphoma versus pseudolymphoma of the skin: gene rearrangement study of 21 cases with clinicopathologic correlation. *Journal of the American Academy of Dermatology* **29**, 945–953.

Miller W.H., Griffin C.E., Campbell K.L. (2013) *Muller and Kirk's Small Animal Dermatology*, 7th edition, pp 810–816. W.B. Saunders Co: Philadelphia.

Moore P.F. & Olivry T. (1994) Cutaneous lymphomas in companion animals. *Clinics in Dermatology* **12**, 499–505.

Moore P.F., Olivry T. & Naydan D. (1994) Canine cutaneous epitheliotropic lymphoma (mycosis fungoides) is a proliferative disorder of CD8⁺ T Cells. *American Journal of Pathology* **144**, 421–429.

Moore P.F., Affolter V.K. & Olivry T. (1998) The use of immunological reagents in defining the pathogenesis of canine skin diseases involving proliferation of leukocyte. *Advances in Veterinary Dermatology* **3**, 77–94.

Moore P.F., Affolter V.K. & Keller S.M. (2013) Canine inflamed nonepitheliotropic cutaneous T-cell lymphoma: a diagnostic conundrum. *Veterinary Dermatology* **24**, 204–211.

Willemze R., Jaffe E.S., Burg G., Cerroni L., Berti E., Swerdlow S.H. *et al.* (2005) WHO-EORTC classification for cutaneous lymphomas. *Blood* **105**, 3768–3785.