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CASE REPORT

Cutaneous epitheliotropic T-Cell Lymphoma with Multiple Metastases in a Dog Diagnosed by PCR to Antigen Receptor Rearrangement Analysis

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ABSTRACT

Herein, a case of canine cutaneous epitheliotropic T cell lymphoma (CETL) with multiple organ metastases is described. An 11-year old female Yorkshire terrier presented with chronic generalized dermatitis and respiratory distress. Multiple erosive plaques and nodules in the skin, enlarged submandibular lymph nodes and tachypnea were noted at the initial presentation. Antigen receptor rearrangement (PARR) analysis by PCR revealed clonal rearrangements from the T-cell receptor gamma (TCRγ) in cells that were fine-needle aspirated from the skin and submandibular lymph node. Histopathological findings in the skin also revealed infiltration of neoplastic lymphoid cells with epithelial tropism. Furthermore, clonal rearrangement from the TCRγ was detected by PARR in the lung nodules. Based upon those findings, the present case was considered as CETL with multiple metastases, which has been only rarely reported in canine cases. In this case, diagnosis of CETL and metastases to other organs could be made by PARR analysis alone.

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INTRODUCTION

Canine cutaneous epitheliotropic T-cell lymphoma (CETL) is a rare skin tumor in dogs characterized by infiltration of neoplastic T lymphocytes with a tropism specific to the epidermis and adnexal structures (de Mello Souza *et al*., 2010). Clinical presentations and morphological features are heterogeneous, and three subforms of cutaneous epitheliotropic lymphoma have been recognized in humans and dogs: mycosis fungoides, pagetoid reticulosis and Sėzary syndrome (Ponce *et al*., 2010). Mycosis fungoides (MF) is the most common type of epitheliotropic lymphoma seen in older dogs; T lymphocytes distribute in the epidermis and adnexal structure as well as in the underlying dermis. Pagetoid reticulosis is described as an exfoliative erythroderma with scaling, alopecia, and erosion without forming palpable masses and the lymphocytic infiltration is mainly localized to the epidermis and its adnexa (Moore *et al.,* 1994). Sėzary syndrome is a leukemic phase characterized by the coexistence of cutaneous epitheliotropic lymphoma, lymph node involvement and the presence of circulating Sėzary cells in the peripheral blood (Foster *et al*., 1997). In canine literature, pagetoid reticulosis and Sėzary syndrome have only rarely been reported (Moore *et al.,* 1994). Here, a canine case of CETL with multiple tumor metastases is reported. PCR to antigen receptor rearrangement analysis (PARR) was used for rapid diagnosis of CETL and metastases in this case.

History and clinical examination: An 11-year old female Yorkshire terrier was brought to the Veterinary Medical Teaching Hospital (VMTH) at Chonbuk National University for evaluation of a chronic generalized dermatitis and respiratory distress. The owner initially had noticed skin erythroderma with alopecia over a year prior to the clinic visit. The dog was tentatively diagnosed with allergic dermatitis, which the local veterinarian had treated using corticosteroids, but the clinical symptoms were not responsive to medication. The owner noticed breathing difficulties several days prior to presentation at VMTH.

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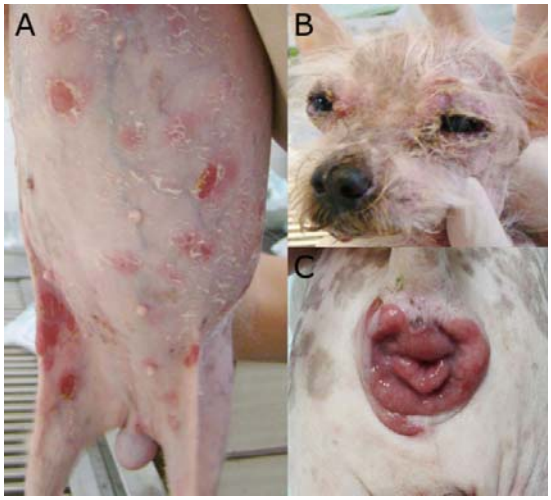

Fig. 1: Clinical features. Note the multifocal erosive plaques predominant in the inguinal and abdominal region (A). Erosion covered with crusts was also found in the mucocutaneous junction of the lips and eyelids (B). In addition, erosion and a palpable nodule were noted in the perianal region (C).

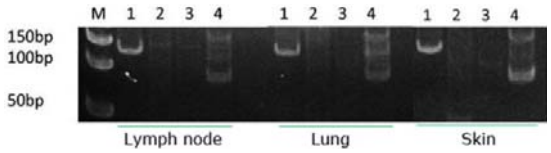

Fig. 2: PCR analysis for detecting antigen receptor rearrangement. DNA extracted from skin, lymph node and lung showed a single-sized amplificon for T-cell rearrangement (1=Control, 2 & 3=B cell origin; 4=T cell origin).

Generalized exfoliative erythroderma with alopecia and multifocal erosive plaques were obvious upon physical examination. Cutaneous signs were generalized, but were more severe in the abdominal and inguinal regions (Fig. 1a). Erosions covered with crusts were found in the mucocutaneous junctions, including the lips and eyelids (Fig. 1b). In addition, erosion and palpable nodules were observed in the perianal region (Fig. 1c). Together with the dermatological problems, enlarged submandibular lymph nodes (2.5 cm in diameter) and tachypnea (66 breaths per min) were also noted.

The cutaneous lesions and thoracic radiographs were inspected, and lymph node aspiration was performed to investigate possible tumor findings. Superficial and deep skin scrapings and cytologic examination were unremarkable; only a few bacterial cocci were found. Fungal culture was negative for canine dermatophyte infection. Systemic lupus erythematosus and cutaneous lymphoma were included in differential diagnosis, and blood work was performed to rule out possible metabolic and endocrine disorders. Marked leukocytosis (30.2×10^6 cells/mL: lymphocytes: 1.9×10^3cells/mL; reference range: $1.2\text{-}3.2\times10^3$cells/mL, monocytes: 0.8×10^3cells/mL; reference range: $0.3\text{-}0.8\times10^3$ cells/mL, granulocytes: 27.5×10^3cells/mL; reference range: $1.2\text{-}6.8\times10^3$cells/mL) and increased plasma alkaline phosphatase (ALKP) were found, but all other general biochemistry parameters were within normal range. Radiographic examination showed a severe diffuse interstitial pattern in all lung lobes. Tracheal lymph nodes were enlarged and a mass-like lesion on the left lung was found. Hepatomegaly was suspected on the radiographs, but

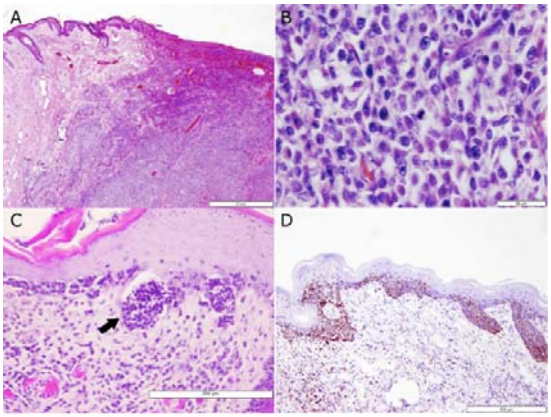

Fig. 3: Histopathological findings of the affected skin lesion. Neoplastic lymphoid cells were found infiltrated in the epithelia and subepithelial tissues including the adnexal structure (A).The neoplastic infiltrate consisted of small- to medium-sized lymphocytes and had round to oval nuclei with one or two prominent nucleoli (B). Epithelial tropism of lymphoid cells was noted, and formation of Pautrier's microabscesses (arrow) was also observed (C). Cells infiltrating the dermis, adnexal structure, and epidermis strongly expressed CD3 (D).

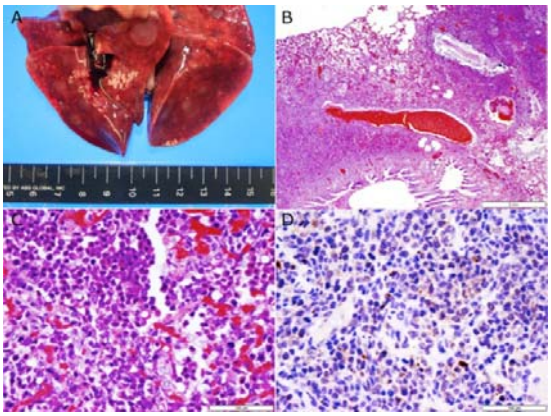

Fig. 4: Macroscopic and histopathological findings of a lung nodule. At necropsy, multifocal whitish nodules were noted on the surface of lung (A). Diffuse infiltration of round cells and multifocal mineralization were noted in the lung (B). The infiltrate was composed of similar neoplastic lymphoid cells as seen in the affected skin (C). These cells were also positive for CD3 by immunohistochemistry (D).

ultrasound examination was not performed because of severe respiratory distress. Fine needle aspiration (FNA) examination of the skin and submandibular lymph nodes showed aggregation of small- to medium-sized round cells with blue-gray cytoplasm and one or two predominant nucleoli. The tentative diagnosis was canine cutaneous lymphoma.

Diagnosis and prognosis: To confirm lymphocytic malignancy, FNA cells from the lymph node and skin were used for molecular diagnosis while waiting on histopathologic examination of the skin biopsy. PARR was performed at Chonbuk National University as described previously (Burnett *et al*., 2003). To ensure the DNA could be amplified, positive control primers were used to amplify the constant region gene of IgM (Cμ). Clonal rearrangements of T-cell receptor gamma (TCRγ) were amplified from fine-needle aspirated cells from lymph node and skin (Fig. 2). In addition, histopathological findings in the skin of the abdominal and perianal regions revealed that the hairy skin

had focally ulcerated, poorly demarcated, invasive, highly cellular masses in the dermis (Fig. 3a). The tumor was comprised of a uniform population of large round cells, resembling lymphoblasts with round nuclei, multiple prominent nucleoli, clumped chromatin, and scant to small amounts of cytoplasm (Fig. 3b). There was moderate nuclear pleomorphism and mitoses were common (2-3 per 400× field). Small to moderate-sized round cells obscured the dermal-epidermal junction, and intraepidermal infiltration of lymphoid cells (Pautrier's microabscesses) was also observed (Fig. 3c). Immunohistochemistry for CD3 was positive for both the tumor cells and the round cells in the epidermis (Fig. 3d), yet negative for CD79a (data not shown). Therefore, a definitive diagnosis of CETL was made, with a preliminary diagnosis of MF-type CETL based on the histopathological findings. Unfortunately, the dog expired the next day due to severe respiratory distress. At necropsy, multifocal whitish nodules were found distributed on the surface of lung (Fig. 4a). No specific findings except for hepatomegaly were found in the other organs. Histopathological findings revealed that the lungs were diffusely and intensely infiltrated by round cells similar to those seen in the affected skin (Fig. 4b and c). These cells were also positive for CD3 by immunohistochemistry (Fig. 4d). PARR was performed using DNA extracted from lung tissue and showed a single-sized T-cell amplicon (Fig. 2). PARR was negative in DNA extracted from the liver and heart tissues (data not shown).

DISCUSSION

In this report, a case of canine CETL that had metastasized to the lung and lymph nodes is described. The clinical signs were multifocal plaques, nodules, and general alopecia, implying that the patient was between the plaque and tumor stages. Histopathological findings showed prominent infiltration of neoplastic lymphoid cells in the skin with epithelial tropism. Clonal rearrangement of TCRγ was confirmed by PARR. In addition, a single-sized amplificon for the TCRγ in skin was found in the lymph node and lung.

Early stage CETL is usually misdiagnosed as allergies, scabies, or seborrhea (Fontaine *et al.,* 2010), but CETL should be ruled out in every dog with exfoliative erythroderma, patches, ulcers, erosions and alopecia. The dog in our study had also been diagnosed with allergic dermatitis by a local veterinarian over a year prior to entering our clinic. It was when the tumor metastasized to other organs via lymphatic vessels that the owner finally noticed the enlarged lymph node and respiratory distress. Early diagnosis could be achieved by PARR analysis from an FNA sample. PARR can detect one malignant cell among 1 million cells (Yamazaki *et al*., 2008). In a study by Burnett *et al*. (2003), the sensitivity and specificity of PARR were shown to be 91 and 95%, respectively, in 77 dogs with lymphoid neoplasia. Indeed, CETL was suspected on the day of admission, and it was confirmed by PARR the next day, which was much faster than the results of the histopathologic examination.

Metastasis of CETL to the lymph nodes and other visceral organs has been only rarely reported in the tumor stage of MF (Moore *et al.,* 1994) and is usually restricted to a single organ, including the central nervous system, lung, diaphragm, and lymph node (Moore *et al.,* 1994; Czasch *et al.,* 2000; Bizikova *et al.,* 2009). In addition, Sèzary syndrome is characterized by the simultaneous proliferation of neoplastic lymphoid cells in the skin, peripheral blood, lymph node, and other organs exhibiting leukemic phase (Foster *et al*., 1997). Therefore, the present case is thought to have been either the tumor stage of MF or Sèzary syndrome. Histopathological findings of affected skin in Sèzary syndrome resemble those observed in classic MF. However, in Sèzary syndrome, small lymphocytes with hyperchromatic convoluted or sometimes cerebriform nuclei (Sèzary cells) are characteristically observed in the skin, peripheral blood, and lymph nodes (Foster *et al*., 1997). Unfortunately, the dog in the present case expired the day after admission, and blood samples collected at initial presentation were not sufficient to perform both blood smears to detect Sèzary cells and PARR to detect clonal T cell rearrangements in the peripheral blood. Therefore, it was difficult to determine whether or not the patient had Sèzary syndrome. Nevertheless, the present case showed some characteristic of Sèzary syndrome: marked leukocytosis, lymphadenopathy, and metastases of neoplastic lymphoid cells to other organs. These findings suggest that this may have been a case of Sèzary syndrome as a subtype of CETL.

Conclusion: This report presents a case of canine CETL with metastases to the lymph node and lung. Metastasis of neoplastic lymphoid cells to multiple organs has been rarely reported in the veterinary literature. For this case, detection of T cell rearrangement by PARR was a very effective and quick tool to diagnosis CETL with small samples from FNA. As CETL progresses very quickly, PARR analysis may be a useful tool for early diagnosis of CETL and lymphoid cells metastasized to other organs.

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