

Lymphangiomatosis of the Systemic Skin in an Old Dog

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ABSTRACT. A 13-year-old, neutered male miniature dachshund was presented with a one-month history of bilateral symmetrical swelling in the pinnae and carpal, cubital and tarsal joints, and swelling in the tail. The lesions were histopathologically characterized by multiple dilated lymphatic vessels lined by a single attenuated layer of endothelial cells. The subcutis was predominantly involved. A number of spindle-shaped cells lining the irregular vessels were observed. Morphological atypia was not evident in these cells. Immunohistochemical analyses revealed that the proliferating endothelial cells were positive for factor VIII-related antigen and CD31. Based on the clinical presentation and histopathological features, the dog was diagnosed with lymphangiomatosis. Treatment with anti-inflammatory prednisolone improved the symptoms.

KEY WORDS: canine, lymphangioma, lymphangiomatosis, miniature dachshund.

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Lymphangiomatosis is a rare disorder characterized by diffuse or multifocal proliferation of lymphatic vessels involving soft tissue or parenchymal organs [4, 5, 7, 23]. Although this disease can be classified as the lymphatic counterpart of angiomas, it is not always possible to clearly distinguish lymphangiomatosis from angiomas, because the two disorders overlap. In humans, this disease occurs principally in children and rarely manifests after the first two decades of life [10, 22]. Therefore, the biologic nature of this condition is controversial, and it is often difficult to determine whether the lesions are true neoplasms, hematomas, or lymphangiectasias. Thus far, a few studies have reported a possible diagnosis of lymphangiomatosis in dogs [4, 5, 23]. As in humans, in dogs, this disease occurs at a young age. Here, we report the clinicopathological findings in an old dog diagnosed with lymphangiomatosis of the multiple skin lesions.

A 13-year-old, neutered male miniature dachshund was referred to the Veterinary Medical Center of the University of Tokyo with a one-month history of cutaneous swelling. Before referral, the dog had received diuretic and water deprivation treatment, but the lesions did not improve and eventually progressed. The vaccination status was current, and the dog had been receiving a monthly dose of heart-

worm preventative medication. Upon physical examination, the dog was good in general condition apart from the skin problem. Bilateral symmetrical swelling was observed in the pinna and carpal, cubital and tarsal joints, and swelling was present in the tail (Fig. 1A–D). The skin lesions were alopecic. Upon release of external pressure, the fluctuant swelling rapidly returned. The dog had no signs of pain or pruritus, and superficial lymph nodes were not enlarged.

Blood tests, including a complete blood count and measurement of blood urea nitrogen, creatinine, alanine aminotransferase, alkaline phosphatase, electrolytes and C-reactive protein, showed normal ranges. Total protein (6.2 g/dl) and albumin (3.1 g/dl) concentrations were within the reference ranges. Hemostatic defect was not observed. Radiographs revealed only pronounced soft tissue swelling. Cardiac and abdominal ultrasonography showed no identifiable underlying cause of the swelling. Percutaneous fine needle aspiration revealed a serosanguineous fluid with specific gravity 1.020 and protein density 1.8 g/l. Microscopic examination showed a few erythrocytes and lymphocytes, but no microorganisms.

The swelling lesions were surgically biopsied. The samples were fixed in 10% formalin and submitted for routine histopathological examination, which revealed that the subcutis in the lesions was distended by multiple dilated vessels (Fig. 2A). The vascular spaces were entirely empty, and the abnormal vessels were lined by a single layer of flattened endothelial cells with small hyperchromatic elongated nuclei (Fig. 2B and 2C). A number of spindle-shaped cells were observed around the vessels. The cells were grown while forming a luminal structure (Fig. 2C). Atypical features such as endothelial tufting, nuclear atypia, and mitotic

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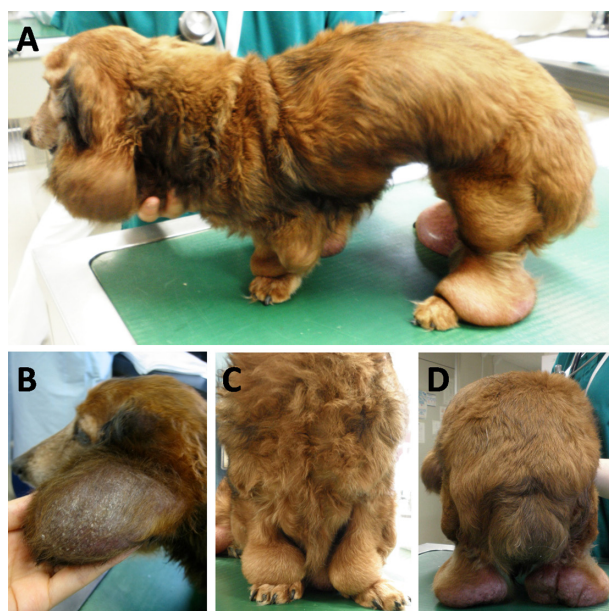


Fig. 1. Appearance of the skin lesions at the first presentation: (A) entire body, (B) pinnae, (C) forelimbs, and (D) hindlimbs and tail.

figures were not seen. Alcian blue and periodic acid-Schiff (PAS) stains did not reveal any mucosubstances in the lesions. Immunohistochemical analyses for factor VIII-related antigen (Dako, Glostrup, Denmark), CD31 (Thermo Fisher Scientific, Waltham, MA, U.S.A.), Iba1 (Wako, Osaka, Japan), and Ki-67 antigen (MIB-1; Dako) were performed by the Envision polymer method (Dako), described previously



Fig. 3. Appearance of the entire body after 3 months of treatment. Note that relief of the symptoms was achieved, compared with Fig. 1.

[12, 13]. Both the endothelial cells and the spindle-shaped cells were positive for factor VIII-related antigen (Fig. 2D) and CD31 (Fig. 2E), but negative for Iba1 and Ki-67. Taken together, these findings were suggestive of a specific variant of lymphangiomatosis, although the method for the diagnosis in dogs was not yet established.

Treatment with oral prednisolone (1 mg/kg once daily) was initiated, because surgical resection, which would have entailed extensive excision, was considered maladaptive. Although cutaneous swelling worsened soon after treatment, the disease eventually abated. Three months later, partial remission was achieved (Fig. 3), and oral prednisolone was subsequently tapered. The dog's swelling has been maintained in partial remission with prednisolone (0.5 mg/kg every other day) for more than 8 months.

The differential diagnosis of lymphangiomatosis includes angiomas, lymphangioma, and, most importantly, angiosarcoma. In the present case, atypical features such as endothelial redundancy and nuclear atypia were absent,

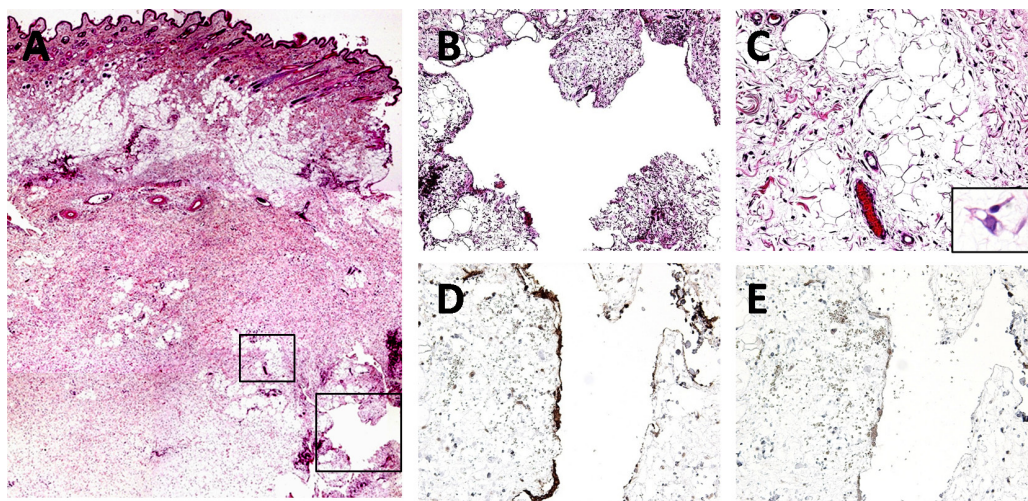


Fig. 2. Histopathological and immunohistochemical findings. (A) The subcutis with interconnected dilated lymphatic vessels ($\times 20$, HE). (B) The abnormally dilated lymphatic vessels in the subcutis [magnification of right box in (A), $\times 40$, HE]. (C) The abnormally dilated lymphatic vessels lined by a single layer of endothelial cells and the spindle-shaped cells surrounding the vessels [magnification of left box in (A), $\times 100$, HE]. Insert: the spindle-shaped cells forming luminal structures ($\times 400$, HE). (D) Immunohistochemistry for factor VIII-related antigen. The endothelial cells and the spindle-shaped cells were positive ($\times 100$). (E) Immunohistochemistry for CD31. The endothelial cells and the spindle-shaped cells were positive ($\times 100$).

which suggested that the proliferating cells were benign. Immunohistochemical analyses revealed that the proliferating cells were positive for factor VIII-related antigen and CD31, which indicated that the proliferating cells originated from endothelial cells. Although the distinction between blood vessels and lymphatic vessels was difficult by these endothelial cell markers, the multiple abnormal vessels were thought to be lymphatic, because of the absence of erythrocytes in the vascular spaces. However, we were unable to directly determine this, because of lack of antibodies to lymphatic endothelial cell markers in dogs. The distinction between lymphangiomas and lymphangiomatosis is problematic. By convention, the lesions in lymphangioma are limited, solitary, and well-defined mass [7, 23]. In this case, the multiple lesions and the histopathological findings could correspond to lymphangiomatosis.

Other differential diagnosis is lymphedema, which is a chronic disorder characterized by an abnormal accumulation of fluid in the interstitial space [8]. Abnormal fluid accumulation in lymphedema may result from a primary defect in the lymphatic system, or can be secondary to other diseases, irradiation, or surgical procedures [9]. This case had no previous history of infections, malignancy, trauma, irradiation, or surgical interventions except for castration. Because of the extent of involvement and the lack of secondary causes, secondary lymphedema was ruled out. In addition, the lesions in the dog could not be primary lymphedema, caused by a developmental abnormality of the lymphatic system, because of the delayed onset and histological findings.

The present report documents the first case of lymphangiomatosis in an old dog. Although lymphangiomatosis in humans and dogs generally occurs in juveniles, the onset of the symptoms in this case has occurred at an advanced age. The reason for delayed onset of lymphangiomatosis is unknown. The abnormal endothelial cells were negative for Ki-67 stain, indicating that proliferation of the cells was not active. Generally, the diagnosis of lymphangiomatosis at birth is uncommon, since it seems that a latent period is required for symptoms to develop. Very slow growth of the abnormal endothelial cells may be associated with the delayed onset.

Only 3 previous cases diagnosed as lymphangiomatosis have been reported in the veterinary literature; first case was lymphangiomatosis of the pelvic limb [5], second case was lymphangiomatosis of the inguinal and caudal mammary regions [4], and third case was lymphangiomatosis of the liver [23]. The first case was untreated, and the dog was euthanized. The second case was treated with surgical intervention, and the dog was disease-free. The third case was treated with anti-inflammatory doses of prednisolone to prevent hepatic fibrosis, but the outcome was not described. The prognosis of lymphangiomatosis in dogs is not well understood. In humans, the prognosis of this disease is determined by the site and extent of involvement. Human patients with involvement of the liver, spleen, lung or thoracic duct usually have a poor prognosis [17]. On the other hand, human patients with soft tissue involvement have a good prognosis, since these lesions respond to limited sur-

gical resection [10]. Treatment options other than surgical resection include radiation [1, 6, 19], glucocorticoids [11, 22], cyclophosphamide [22] and interferon-alpha [14, 15, 18, 21] in humans. In the present case, anti-inflammatory doses of prednisolone were administered, because surgical resection was not considered a valid option. Although the efficacy of glucocorticoids in the treatment of lymphangiomatosis remains unclear, this patient has experienced some relief of the symptoms. Various anti-inflammatory drugs, including glucocorticoids, have been found to be antiangiogenic through a direct inhibition of the vascular endothelial growth factor (VEGF) gene expression [2, 16]. This mechanism can be due to an interaction between the glucocorticoid receptor with transcription factors, such as activator protein 1 (AP-1) and nuclear transcription factor kappa B (NF- κ B) [3]. Lymphangiogenesis, like angiogenesis, occurs through sequential steps that involve: production of VEGF, matrix metalloproteinase (MMP) expression, and endothelial cell migration, proliferation and organization into functional vessels [20]. Based on that, the mechanisms of prednisolone to improve the symptoms in this case appear to be multifactorial and likely include: (i) inhibition of VEGF expression, (ii) inhibitory effect on MMP activity and (iii) alteration of VEGF receptor signal transduction which could affect endothelial cell proliferation. However, the exact role of prednisolone in the management of lymphangiomatosis is uncertain, because little is known about its biologic nature and pathogenesis. Further studies are required to characterize this rare disorder.

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