The Journal of Veterinary Medical Science

NOTE

Pathology

Multiple cutaneous pleomorphic leiomyosarcoma in a dog

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J. Vet. Med. Sci.
81(11): 1564–1566, 2019
doi: 10.1292/jvms.19-0295

Received: 30 May 2019
Accepted: 2 August 2019
Advanced Epub:
12 September 2019

ABSTRACT. A male dog developed multiple cutaneous masses at 15 different sites between the ages of 11.5 and 13-years. The masses were surgically removed and histopathologically examined. In gross appearance, the cut surfaces of the masses were white with partially red areas. Microscopy revealed that the tumors were located at the dermis and were composed of spindle cells and pleomorphic cells with abundant eosinophilic cytoplasm, accompanying giant cells. These cells were occasionally arranged in bundles with minimal stromal collagen. Immunohistochemical analysis revealed that the neoplastic cells were strongly positive for vimentin, partially positive for smooth muscle actin and desmin, and negative for cytokeratin. Based on these pathological findings, the tumor was diagnosed as multiple cutaneous pleomorphic leiomyosarcoma.

KEY WORDS: cutaneous, dog, pleomorphic leiomyosarcoma

Leiomyosarcomas of the skin are rare in humans and animals [4, 5]. Although some cases of multiple, highly differentiated smooth muscle tumors have been reported in dogs [6, 7], cats [2], horses [1], and ferrets [10], cutaneous smooth muscle tumors are typically solitary, comprising a single lesion [5, 7]. To the best of our knowledge, these reports include cases that describe multiple masses occurring densely at one area or single masses with multinodular architecture [1, 2], and cases describing masses at various cutaneous sites that are extremely rare in veterinary practice. In the present study, we describe the histopathological and immunohistochemical features of multiple cutaneous pleomorphic leiomyosarcomas in a dog; the leiomyosarcomas were poorly differentiated and were difficult to distinguish from anaplastic sarcoma with giant cells.

A male shih tzu dog had developed multiple cutaneous masses comprising nodules 1–3 cm across at 15 different body sites, including at the muzzle (1), face lift (1), neck (2), dorsal trunk (9), limb (1), and foot pad (1). The masses developed between the ages of 11.5 and 13 years. These masses developed and enlarged sequentially and were surgically removed on four occasions. Local recurrence at surgical sites was not observed. Eleven masses: those at the muzzle (1), face lift (1), neck (1), dorsal trunk (6), limb (1), and foot pad (1), which were removed during the first three procedures, were diagnosed as soft tissue sarcomas without immunohistochemistry at a private diagnostic laboratory. Four masses, from the neck (1) and dorsal trunk (3), were removed on the fourth occasion and sent to our laboratory for histopathological evaluation. Ultrasound and radiological investigation of the dog when it was 12-years-old revealed two nodular masses in the lungs of 2 cm in size and no neoplastic lesions in the intra-abdominal organs. Enlargement of lymph nodes was not observed. The lung masses did not enlarge any further up until the dog's death. The dog died from debilitation at the age of 13 years, and necropsy was not performed at the owner's request.

In gross appearance, the masses were firm and the cut surfaces of the masses were white with partly red areas (Fig. 1A). The tissues were fixed in 10% buffered formalin. They were trimmed, embedded in paraffin, sectioned at 4-μm thicknesses, and stained with hematoxylin and eosin (HE) and Masson's trichrome stain.

On microscopic analysis, 15 masses from various sites showed similar histologic features. The tumors were located at the dermis and involved hair follicles (Fig. 1B). They were poorly demarcated and invaded into the surrounding tissues (Fig. 1C). No vascular or lymphatic invasion was observed. Focal necrosis and hemorrhage were observed in the masses. The tumors were composed of spindle cells and pleomorphic cells with abundant eosinophilic cytoplasm occasionally accompanying giant cells (Fig. 1D). These giant cells were frequently observed in invasive areas, and the neoplastic cells were occasionally arranged in bundles with minimal stromal collagen (Fig. 1D). Marked anisocytosis and anisokaryosis were observed. Nuclei were oval to elongated, and mitotic activity was 12 per 10 high-power fields (2.37 mm^2), with bizarre mitotic structures occasionally observed. The neoplastic cells were stained red by Masson's trichrome stain (Fig. 1E). Canine soft tissue mesenchymal tumor is graded according to three components/criterion [8, 9]: degree of differentiation (poorly differentiated), mitotic count (12 per 10 high-power fields), and

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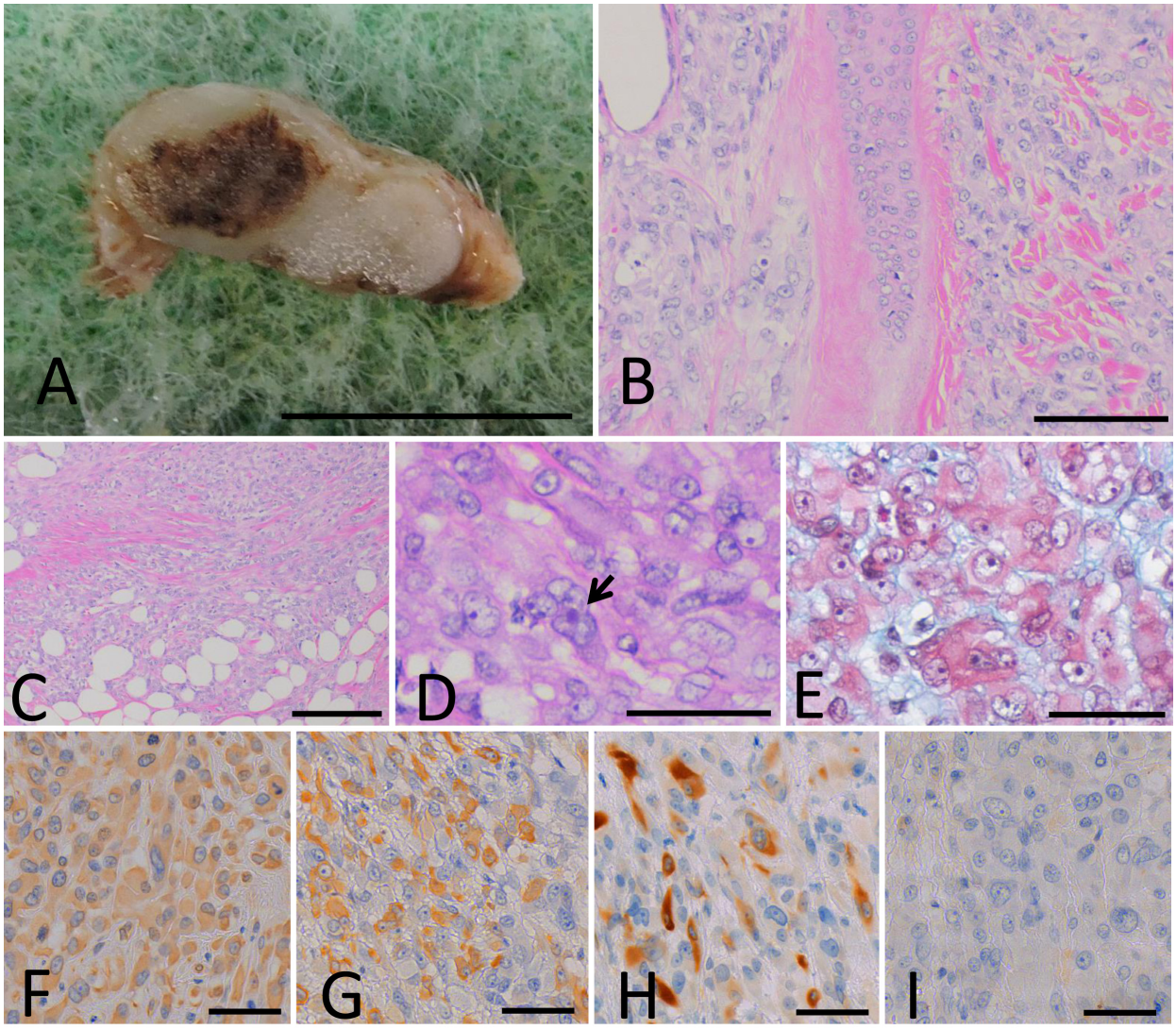

Fig. 1. (A) Gross findings. The cut surface of the mass is white with a partly red area. Bar, 1 cm. (B–D) Histological findings. (B) The tumor is located at the dermis and involves a hair follicle. Hematoxylin and eosin stain. Bar, 50 μm. (C) The tumor invades into subcutaneous tissues. Hematoxylin and eosin stain. Bar, 100 μm. (D) Spindle cells and pleomorphic tumor cells are arranged in bundles accompanying giant cells (arrow). Hematoxylin and eosin stain. Bar, 20 μm. (E) The neoplastic cells are stained red with Masson's trichrome stain. Bar, 20 μm. (F–I) Immunohistochemical findings. The neoplastic cells are positive for vimentin (F), partially positive for smooth muscle actin (G) desmin (H), and negative for cytokeratin (I). Immunoperoxidase reaction with diaminobenzidine chromogen and hematoxylin counterstain. Bar, 20 μm.

amount of necrosis (<50% necrosis); the histologic grade of the tumor was grade 2.

Immunohistochemistry was performed using the immunoenzyme polymer method, and primary antibodies used are shown in Table 1. Histofine MAX-PO (Multi) (Nichirei Bioscience Inc., Tokyo, Japan) was used as a secondary antibody. Following immunoreaction, the sections were stained with diaminobenzidine, followed by counterstaining with Mayer's hematoxylin. The neoplastic cells from the masses were strongly positive for vimentin, partially positive for smooth muscle actin (SMA) and desmin, and negative for cytokeratin (AE1/AE3) (Fig. 1F–I). The neoplastic cells were not surrounded by collagen IV+ and laminin+ basement membrane.

The differential diagnoses for this neoplasm included leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma, glomus tumor, and myofibroblastic sarcoma. The tumor exhibited consistent immunohistochemical staining for SMA; thus, rhabdomyosarcoma, fibrosarcoma, and malignant fibrous histiocytoma were ruled out. The lack of a collagen IV+ and laminin+ basement membrane ruled out glomus tumor. However, because myofibroblastic sarcoma can also be positive for vimentin, SMA, and occasionally desmin [4], it cannot be rule out solely by immunohistological findings alone. In the present case, the histopathological features, including connection to hair follicles, scant stroma and large amounts of acidophilic cytoplasm, which stained red with Masson's trichrome stain, were considered to be suggestive of leiomyosarcoma rather than myofibroblastic sarcomas. Leiomyosarcomas are common in the gastrointestinal tract, spleen, uterus, and urinary bladder [9]; however, this

Table 1. Primary antibodies used in the present study

Antibody	Clone	Dilution	Source	Antigen retrieval
Cytokeratin	AE1/AE3	1:200	Dako Denmark A/S., Glostrup, Denmark	MW, 95°C, 10 min
Smooth muscle actin	1A4	1:400	Dako Denmark A/S., Glostrup, Denmark	No treatment
Desmin	D33	1:100	Dako Denmark A/S., Glostrup, Denmark	AC, 121°C, 20 min
Vimentin	V9	1:200	Dako Denmark A/S., Glostrup, Denmark	MW, 95°C, 10 min
Collagen IV	Polyclonal	1:1,000	LSL, Tokyo, Japan	Pepsin, 37°C, 30 min
Laminin	Polyclonal	1:100	Progen Biotechnik, Heidelberg, Germany	Pepsin, 37°C, 30 min

MW, microwave, citrate buffer (pH 6.0); AC, autoclave, citrate buffer (pH 6.0); Pepsin, 0.4% pepsin (SigmaAldrich Co., St. Louis, MO, U.S.A.).

neoplasm was not observed in these sites following ultrasound or radiological examination, and no vascular or lymphatic invasion was detected by histopathological examination. Consequently, we considered it to be cutaneous in origin rather than being a metastatic lesion. All the cutaneous masses from the various sites exhibited similar histological and immunohistological features; therefore, we considered it to be multiple occurrence. Canine leiomyosarcomas may originate from arrector pili muscles or vessel walls and can be classified into piloleiomyosarcomas, angioleiomyosarcomas, and pleomorphic leiomyosarcomas [4, 7]. In the present case, on the basis of the histological findings, we diagnosed the tumor as multiple cutaneous pleomorphic leiomyosarcoma. Its origin was not clear, but it may have originated from the arrector pili muscles because all the masses involved hair follicles.

Cutaneous leiomyosarcomas are typically solitary, single lesions, and metastatic lesions have not previously been reported in dogs [4, 7]. In humans, multiple cutaneous leiomyomas are known to occur as part of hereditary leiomyomatosis syndrome accompanying renal cell carcinomas resulting from mutation in the fumarate hydratase gene [11], and malignant transformation from a cutaneous pilar leiomyoma to leiomyosarcoma has been previously reported [3]. Although the genetic background was unclear and renal tumor was not observed in the present case, a genetic predisposition may be related to these multiple cutaneous leiomyosarcomas in dogs. Although multiple poorly differentiated pleomorphic leiomyosarcomas have not been reported in animals, these tumors may get overlooked because they could be diagnosed as pleomorphic sarcoma without immunohistopathological analysis in veterinary pathology. Immunohistopathological investigation of cutaneous pleomorphic sarcomas may be required to evaluate the occurrence of multiple pleomorphic leiomyosarcoma.

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