



CASE REPORT

Histopathologic and Immunohistochemical Features of Disseminated Cutaneous Hemophagocytic Histiocytic Sarcoma in a Maltese Dog

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ABSTRACT

A 14-year-old, spayed female, Maltese with multiple nodular skin lesions was presented to a local animal hospital. The nodules were infiltrated by discrete round to polygonal neoplastic cells with large nuclei. Cells having phagocytic activities and multinucleated cells were also present. Immunohistochemically, neoplastic cells were positive for vimentin, S100, lysozyme and CD18 and negative for pancytokeratin, CD3, CD79 $\alpha$ , and CD138. Considering histopathologic and immunohistochemical features, the tumor was diagnosed as disseminated cutaneous hemophagocytichistiocytic sarcoma. This is the first report of this disease in a Maltese dog.

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INTRODUCTION

Canine histiocytic sarcomas (HS) are highly aggressive malignant neoplasms with high mortality rate and consist of neoplastic histiocytes, which can be divided into dendritic cells (DCs) and macrophages. Neoplastic cells may show hemophagocytic activities provided the cells are derived from monocyte/macrophage lineage, and display pleomorphism and finely vacuolated to granular cytoplasm. Primary sites for HS are widespread in the body and include the spleen, bone marrow, lung, brain, lymph nodes, subcutaneous tissues and skin (Affolter and Moore, 2002). HS is known to have a predilection for certain breeds such as the Bernese mountain dog, Golden retriever, Labrador retriever, Flat-coated retriever, Sharpei, Beagle (Thio *et al.*, 2006; Valli, 2007). A few cases of primary hemophagocytichistiocytic sarcoma (HHS) in other breeds have been reported (Uno *et al.*, 1993). Several immunohistochemical stains are necessary for the definite diagnosis of HS because it displays similar histopathological features to other round cell tumors such as malignant plasmacytoma, and large cell lymphoma (Valli, 2007). In this case, we report a very unusual type of HHS in a Maltese dog.

**Case description:** A 14-year-old, spayed female, Maltese dog was presented to a local animal hospital for evaluation of multiple cutaneous masses. More than 30

masses were distributed over the body surface, involving the gingiva. According to the owner, several small nodules had been recognized initially, which disseminated rapidly over about 3 weeks. The number of masses had appeared to decrease at one point but 50-60 lesions had then emerged over a period of 3 months. There was no evidence of metastasis to internal organs at the time of biopsy submission. On presentation, the dog had a normal appetite but showed extreme nervousness. Results of a complete blood cell count indicated moderate anemia and dehydration. The serum chemistry values were within normal ranges.

A fine needle aspiration biopsy of one mass revealed that these nodules consisted of large discrete round tumor cells in varying sizes with pleomorphic nuclei. The cells had slightly basophilic cytoplasm containing a variable number of small vacuoles. For definite diagnosis of these masses, the lesions located in the scapular and gingival regions were removed surgically and referred to the College of Veterinary Medicine, Department of Veterinary Pathology, Konkuk University.

**Diagnosis:** The tumor samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Hematoxylin and eosin (HE) stained slides were examined for evaluation of morphologic features. With immunohistochemical examination, antibodies to vimentin (1:1500 dilution; Biogenex, San Ramon, CA), pancy-

keratin (1:300 dilution; Dako Denmark A/S, Glostrup, Denmark), S100 protein (1:200 dilution; Biogenex), lysozyme (1:200 dilution; Dako), CD3 (1:100 dilution; Dako), CD18 (1:100 dilution; University of California, Davis, CA), CD79 $\alpha$  (1:100 dilution; Dako), and CD138 (1:50 dilution; Dako) are the most widely used to detect all surface antigens for categorization of the diagnosis. Immunohistochemistry (IHC) was performed on previously diagnosed canine splenic HHS (n=2), cutaneous histiocytoma (n=8), and plasmacytoma (n=5) tissues as positive controls. Slides were deparaffinized, rehydrated with PBS and incubated in 3% hydrogen peroxide for 20min. Heat-induced antigen retrieval was performed by boiling in Tris-EDTA (pH 9.0) for vimentin and pancytokeratin, and by boiling in citric acid (pH 6.0) for CD3, CD79 $\alpha$  and S100. Enzymatic pre-treatment was performed with proteinase K for CD18, CD138, and lysozyme. After applying the primary antibodies at room temperature for 3h (for pancytokeratin, S100, lysozyme, CD18, and CD79  $\alpha$ ) or at 4°C overnight (for vimentin, CD3, and CD138), slides were washed in PBS 3 times, and incubated with horseradish peroxidase secondary antibodies (DAKO REAL™ Envision kit; Dako) for 20min. All the slides were counterstained with Gill's hematoxylin.

## RESULTS

Grossly, skin lesions were reddish, firm and nodular with regional alopecia. Several nodules were hemorrhagic (Fig. 1). Microscopically, round to polygonal individual tumor cells with large nuclei were densely accumulated in the dermis and inflammatory infiltrates. Cells with characteristic morphology of phagocytosis, such as abundant foamy cytoplasm containing red blood cells, hemosiderin, leukocytes, and cell debris were commonly observed. Some cells were obviously large (20-30  $\mu$ m in diameter) and had multiple nuclei. There was marked anisocytosis and anisokaryosis due to these large cells and multinucleated giant cells (MGCs). Nuclei were round to oval, vesicular, and hyperchromatic with 1-3 prominent irregular nucleoli (Fig. 2A), and there were 1-2 mitotic figures per 40 high-power field. Extra-vascular erythrocytes caused by intra-tissue hemorrhage during surgery were abundant (Fig. 2B). On the basis of the histologic features, 2 main diagnoses, HHS and phagocytic plasmacytoma, were considered.

Immunohistochemically, all of the neoplastic cells in the present case were positive for vimentin, S100 protein, CD18, and lysozyme and negative for cytokeratin and CD138 (Fig. 3A). Specific CD18-positive staining was observed in the cytoplasm and membrane of neoplastic histiocytes and MGCs in the biopsy samples (Fig. 3B). All neoplastic cells uniformly expressed lysozyme with a granular pattern in the cytoplasm (Fig. 3C). The neoplastic cell population was negative for both CD3 and CD79 $\alpha$ . The results of IHC staining of splenic HHS, cutaneous histiocytomas and plasmacytomas are described in Table 1.

Negative CD3 and CD79 $\alpha$  staining indicated that these cells did not belong to T-cell or B-cell lineages. Phagocytic plasmacytoma was also excluded due to lack of staining for CD79 $\alpha$  and CD138. Positive CD18 staining

**Table 1:** Immunohistochemical findings of hemophagocytichistiocytic sarcoma, histiocytoma and plasmacytoma detected in various breeds of dogs

Antibody	Present case	No. positive/No. tested (%)		
		Splenic HHS (n=2)	Histiocytoma (n=8)	Plasmacytoma (n=5)
Cytokeratin	-	0/2 (0)	0/8 (0)	0/5 (0)
Vimentin	+	2/2 (100)	8/8 (100)	5/5 (100)
S100	+	2/2 (100)	3/8 (37.5)	2/5 (40)
Lysozyme	+	2/2 (100)	6/8 (75)	1/5 (25)
CD3	-	0/2 (0)	0/8 (0)	0/5 (0)
CD18	+	2/2 (100)	7/8 (87.5)	1/5 (25)
CD79 $\alpha$	-	0/2 (0)	0/8 (0)	5/5 (100)
CD138	-	0/2 (0)	0/8 (0)	5/5 (100)

HHS: Hemophagocytichistiocytic sarcoma.

could not distinguish HHS from HS, but positive staining for lysozyme revealed this tumor was composed of histiocytes derived from macrophages having phagocytosis ability. Furthermore, the results of IHC stains and morphologic features were exactly identical to those of splenic HHS. Overall, histopathologic features and IHC staining results indicated that the cutaneous nodules were disseminated cutaneous HHS.

## DISCUSSION

Histiocytic proliferative diseases in dogs comprise cutaneous histiocytoma, cutaneous histiocytosis, systemic histiocytosis and HS (Moore *et al.*, 2006). Cutaneous histiocytoma, a proliferation of Langerhans cells, is the most prevalent benign skin tumor in young dogs. Histiocytosis is defined as a reactive proliferation of non-neoplastic histiocytes. Cutaneous histiocytosis is a proliferation of benign histiocytes within skin, whereas systemic histiocytosis is characterized by infiltrates of histiocytes with involvement of multiple organs (Mastrorilli *et al.*, 2012).

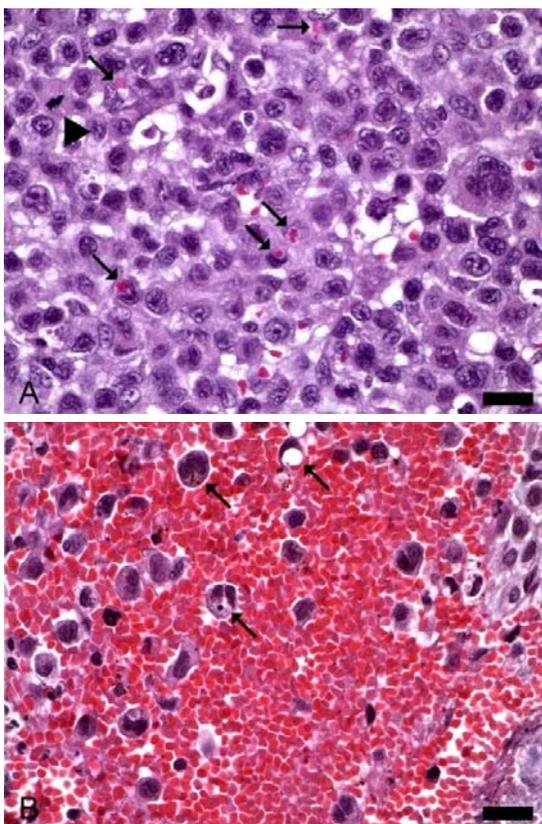
Malignant tumors of histiocytic cells are very rare disorders in Maltese dogs, and reported most frequently in specific breeds such as flat-coated retriever and the Bernese mountain dog (Hedan *et al.*, 2011). Recently, disseminated cutaneous HS in a Great Dane (Mastrorilli *et al.*, 2012) and splenic HHS in a flat-coated retriever (Soare *et al.*, 2012) were described. HHS is regarded as a proliferation of neoplastic macrophages which expressed CD11d/CD18 and MHC class II molecules and have phagocytic and degradative properties (Moore *et al.*, 2006).

Most previous study on canine HHS focused on distinguishing macrophages and DCs (Affolter and Moore, 2002; Fant *et al.*, 2004). In the present study, we performed IHC examination to differentiate histiocytes and plasma cells because avid phagocytosis could rule out epidermal and dermal DCs. The results of IHC staining and characteristic morphologic features strongly suggest that the neoplastic cells are derived from macrophages, not from epidermal or dermal DCs or plasma cells. As a result, utilization of IHC staining using anti-CD18, CD79 $\alpha$ , CD138 and lysozyme antibodies allowed unequivocal identification of HHS.

Malignant tumors of histiocytes are uncommon in non-predisposed breeds. This histopathological and immunohistochemical diagnosis of the HHS in a Maltese is considered extremely rare, and this is the first report of it.

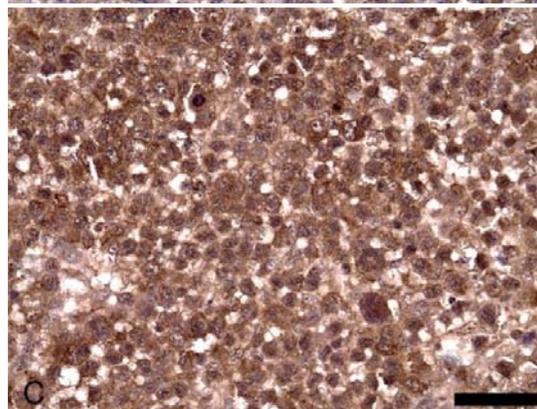
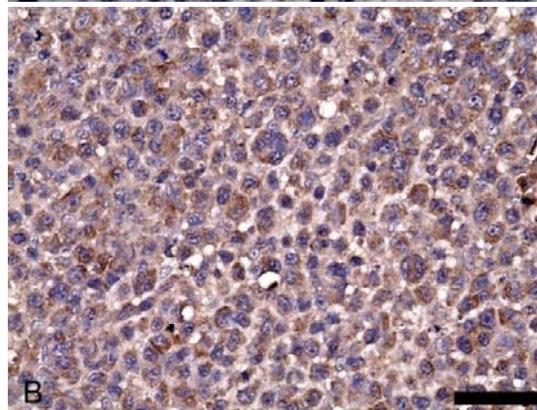
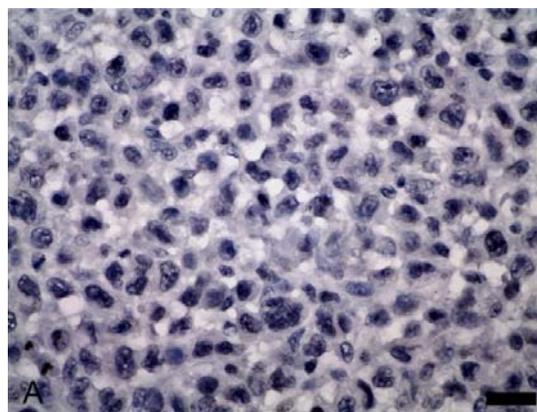


**Fig. 1:** Multifocal, raised, firm, erythematous nodules are present in a dog. The largest lesion is located on the right lateral neck region.



**Fig. 2:** Histopathologic features of the dog's lesion stained with hematoxylin and eosin. A) Dermal lesions, scapular region; dog. There is a multinucleated giant cell at the right center. Some neoplastic cells contain several ingested erythrocytes (arrow). A mitotic figure is noted at the left upper area (arrowhead). The nuclei are round to oval and have one or more nucleoli and B) Gingival lesions in dog, binucleated and neoplastic cells containing hemosiderin, cell debris, erythrocytes, and phagocytic vacuoles in the cytoplasm are located among extravascular erythrocytes caused by hemorrhage during surgery (arrow). Bar=20  $\mu$ m.

**Author's contribution:** Ha-Young Lim carried out most of the work in writing. Young-Gyu Jang and Jung-Hyung Ju contributed equally to this paper with the first author. Jung-Hyang Sur provided information to analyze the findings. All authors approved the manuscript.



**Fig. 3:** Immunohistochemical staining of the dog's dermal lesion. A) Dermal lesions, scapular region; dog. With CD138 staining, none of the infiltrating neoplastic cells are labeled, B) Dermal lesions, scapular region; dog. CD18+ neoplastic histiocytic cells including multinucleated giant cells are infiltrated in the dermis and C) Dermal lesions, scapular region; dog. Most of the malignant histiocytes are stained extensively with anti-lysozyme antibody. Gill's hematoxylin counterstain. Bar: A=20  $\mu$ m; B & C=50  $\mu$ m.

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