Systemic mastocytosis with subcutaneous hemorrhage and edema in a Greyhound dog: case report and review of diagnostic criteria

Alexander Aceino,¹ Unity Jeffery, Julie Piccione, Carolyn L. Hodo

Abstract. Systemic mastocytosis, characterized by infiltration of multiple organs by neoplastic mast cells, is a well-described entity in human medicine with specific criteria for diagnosis, but is ill defined in veterinary literature. Hemostatic disorders are reported in humans affected by systemic mastocytosis but have not been well described in veterinary literature. A 5-y-old, spayed female Greyhound dog had a 1-mo history of progressive ventral cutaneous edema, hemorrhage, and pain. Cytology of an antemortem aspirate from the subcutis of the ventral abdomen was suggestive of mast cell neoplasia, but no discrete mass was present. The dog was euthanized and submitted for autopsy; marked subcutaneous edema and hemorrhage were confirmed. The ventral abdominal panniculus and dermis superficial to the panniculus carnosus were infiltrated by a dense sheet of neoplastic mast cells. The neoplastic cells contained toluidine blue–positive granules and formed aggregates within the bone marrow and several visceral organs, including the liver, spleen, heart, and kidney. Diffuse edema and hemorrhage is an unusual presentation of mast cell tumors in dogs. Antemortem tests, including complete blood count, coagulation profile, and viscoelastic coagulation testing, were suggestive of a primary hemostatic defect. We discuss here the diagnostic criteria used in humans, how these can be applied to veterinary patients, and the limitations of the current diagnostic framework.

Key words: canine; coagulation; edema; hemorrhage; mastocytosis; neoplasia; pathology.

Mastocytosis in human and veterinary literature encompasses a diverse group of diseases defined by the accumulation of neoplastic mast cells (MCs) in one or more organ systems. Unlike in human medicine, spontaneous mast cell tumors (MCTs) are common in veterinary medicine and account for up to 20% of cutaneous tumors in dogs.^{5,19} Despite the frequency with which cutaneous neoplastic MC diseases are encountered, few reports and descriptions of visceral or systemic mastocytosis exist in animals,^{2,11,13} and there is no published classification scheme for non-cutaneous MCTs in dogs. The World Health Organization (WHO) classification for human mastocytosis includes cutaneous mastocytosis, systemic mastocytosis, MC leukemia, and MC sarcoma.^{1,7,17,18} Systemic mastocytosis is classically defined by organ involvement other than, but not exclusive of, the skin, with the most common sites affected being the bone marrow, spleen, liver, lymph nodes, and gastrointestinal tract.^{17,18} According to the WHO guidelines,^{1,7,17,18} 1 major and 1 minor criterion, or 3 minor criteria, must be met for systemic mastocytosis to be diagnosed. The major criterion is defined as multifocal aggregates of ≥ 15 MCs in 1 or more extracutaneous organs; the 4 minor criteria include abnormal MC morphology, activation of a mutation at codon 816 of KIT, expression of CD2 and/or CD25 by MCs, and an increase in baseline serum tryptase >20 ng/mL.¹⁸ Further classification relies on the presence or absence of B findings (bone marrow infiltration, increased

serum tryptase levels, myeloproliferative disease, and hepatomegaly without liver dysfunction) and C findings (one or more cytopenias, palpable hepatomegaly with liver dysfunction, large osteolytic lesions or pathologic fractures, palpable splenomegaly, and malabsorption with weight loss).^{1,7,17,18} Rarely, spontaneous bleeding in the form of primary or secondary hemostatic disorders has been reported in humans with mastocytosis as a result of the release of tryptase, heparin, and heparin-like molecules by neoplastic MCs.^{3,10,16} To our knowledge, this finding has not been reported in veterinary patients with mastocytosis. We describe here the clinical, gross, and histologic findings in a Greyhound dog with presumed systemic mastocytosis and an apparent coagulation disorder.

A 5-y-old spayed female Greyhound dog was presented to the Texas A&M University Veterinary Medical Teaching Hospital (VMTH; College Station, TX) with an ~1-mo

Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences (Aceino, Jeffery, Hodo), and Texas A&M Veterinary Medical Diagnostic Laboratory (Piccione), Texas A&M University, College Station, TX; Department of Comparative Medicine, University of Texas MD Anderson Cancer Center, Bastrop, TX (Hodo).

¹Corresponding author: Alexander Aceino, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, 4467 TAMU, College Station, TX 778453.
aaceino@cvm.tamu.edu

Figure 1. Systemic mastocytosis in a 5-y-old Greyhound dog. **A.** Peripheral blood smear with 2 basophils and 1 slightly disrupted, well-granulated mast cell. Modified Wright–Giemsa. Bar=20 μ m. **B.** Fine-needle aspirate of a swelling on the sternum with large aggregates of well-granulated, neoplastic mast cells. Modified Wright–Giemsa. Bar=10 μ m. **C.** The ventral trunk is diffusely and severely swollen and dark-red as a result of subcutaneous hemorrhage and edema. The cranial direction is to the left of the image. **D.** Hemorrhage is concentrated in the parietal subpleural space, predominantly cranially and caudally to the ribs, avoiding the body of the intercostal muscles.

history of waxing and waning ventral edema and bruising, intermittent vomiting and diarrhea, and episodic behavioral signs consistent with pain. Serial complete blood counts performed by the referring veterinarian were unremarkable until 2 d before presentation, when the results revealed a mild inflammatory leukogram characterized by minimal neutrophilia ($11.8 \times 10^9/\text{L}$, reference interval [RI]: $3.0\text{--}11.5 \times 10^9/\text{L}$) and a mild left shift (band neutrophils $0.8 \times 10^9/\text{L}$, RI: $0.0\text{--}0.3 \times 10^9/\text{L}$). There was also mild basophilia ($0.7 \times 10^9/\text{L}$, RI: $0.0\text{--}0.3 \times 10^9/\text{L}$) and occasional well-granulated MCs (2% of WBC differential, $0.3 \times 10^9/\text{L}$; Fig. 1A). The MCs were relatively uniform in appearance. There was also moderate regenerative anemia (hematocrit 0.23 L/L, RI: $0.32\text{--}0.55$ L/L; absolute reticulocyte count $145 \times 10^9/\text{L}$, RI: $<80 \times 10^9/\text{L}$). The platelet count was $217 \times 10^9/\text{L}$ (RI: $200\text{--}500 \times 10^9/\text{L}$). Other testing performed

by the referring veterinarian included biopsies of the bruised ventral skin (not including subcutis), which confirmed marked diffuse acute dermal hemorrhage and edema but did not identify an underlying cause. Fine-needle aspiration of the swelling on the sternum was examined by a board-certified clinical pathologist. The aspirated material contained MCs arranged as single cells and in small, dense aggregates (>10 cells). The MCs were well-granulated. Overall, the MCs displayed mild anisocytosis and anisokaryosis with a moderate, but slightly variable, N:C (nucleus:cytoplasm) ratio and no visible mitotic figures. A diagnosis of MC neoplasia was made based on the dense aggregation and number of MCs present (Fig. 1B). Serology was negative for *Babesia canis*, *B. gibsoni*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Rickettsia rickettsii*. A *Borrelia* spp. PCR and multiplex PCR testing for

Rickettsiales spp. including *Anaplasma phagocytophilum*, *Ehrlichia canis*, *E. chaffeensis*, *E. ewingii*, and *Rickettsia rickettsii* were negative.

Upon presentation to the VMTH, physical examination revealed pale mucous membranes, pitting edema of the forelimbs, and diffuse swelling, erythema, and ecchymoses over the sternal, ventral abdominal, and inguinal regions. Biochemistry testing was unremarkable. A complete blood count confirmed regenerative anemia (hematocrit 0.25 L/L, RI: 0.37–0.56 L/L; absolute reticulocyte concentration $139 \times 10^9/L$, RI: $<100 \times 10^9/L$), leukocytosis (total WBC $36.0 \times 10^9/L$, RI: $6.0\text{--}17.0 \times 10^9/L$) as a result of mature neutrophilia ($30.2 \times 10^9/L$, RI: $3.0\text{--}11.5 \times 10^9/L$) and monocytosis ($2.50 \times 10^9/L$, RI: $0.2\text{--}1.3 \times 10^9/L$), and thrombocytopenia ($123 \times 10^9/L$, RI: $200\text{--}500 \times 10^9/L$). Thrombocytopenia was interpreted as pathologic rather than a breed variant given that platelet counts $>200 \times 10^9/L$ had been reported previously for the patient. On blood smear examination, low numbers of MCs were present, but comprised $<1\%$ of leukocytes. There was increased fibrinogen (4.63 g/L, RI: 1.10–2.75 g/L) and D-dimers (4.06 nmol/L, RI: <2.03 nmol/L), decreased prothrombin time (PT; 6.2 s, RI: 6.4–9.5 s) and antithrombin III (71%, RI: $>114\%$), and activated partial thromboplastin time (APTT) within the RI (19.5 s, RI: 11.6–25.1 s). A point-of-care viscoelastic testing device (VCM; Entegriion) was used to further assess coagulation status and rule out hyperfibrinolysis; results were within normal limits. A CT scan of the head, thorax, and abdomen confirmed severe, diffuse, subcutaneous edema of the ventral body wall but did not identify any discrete masses. Given the severity of clinical signs, the owner elected euthanasia.

Upon postmortem examination, the ventral neck, thorax, abdomen, and all 4 limbs were diffusely and severely swollen and dark red with areas of patchy alopecia and extensive subcutaneous hemorrhage and edema (Fig. 1C). No cutaneous or subcutaneous masses were observed, despite thorough examination. The skeletal muscle overlying the ventral thorax and abdomen was friable, edematous, and red, as a result of the degree of edema and necrosis. There were areas of hemorrhage cranial and caudal to each associated rib (Fig. 1D) and on the thoracic surface of the diaphragm. Incidentally, an incomplete peritoneal-pericardial diaphragmatic hernia was observed. Despite the patient's clinical history of vomiting and diarrhea, no gastrointestinal lesions were observed. Toxicologic testing for anticoagulants including warfarin, bromadiolone, brodifacoum, diphacinone, chlorophacinone, coumatetralyl, difenacoum, and difethialone was performed by the Texas A&M Veterinary Medical Diagnostic Laboratory (College Station, TX) and was negative.

Tissues were fixed in 10% neutral-buffered formalin, processed routinely, sectioned at 5 μ m, and stained with hematoxylin and eosin (H&E). Microscopic examination of 12 of 14 sections of skin from the ventral neck, thorax,

and limbs revealed an unencapsulated, densely cellular, poorly demarcated, invasive neoplasm expanding the deep panniculus superficial to the panniculus carnosus (Fig. 2A). The neoplasm was composed of sheets of round cells with distinct cell borders, a moderate amount of finely granular, eosinophilic cytoplasm, and a round-to-oval nucleus with finely stippled chromatin and indistinct nucleoli (neoplastic MCs; Fig. 2B). Anisocytosis and anisokaryosis were mild. The mitotic count was <1 per 10 high-power (400 $\times$) fields (2.37 mm² total area). Rare single-cell necrosis was observed. Erythrocytes were admixed with the neoplastic population and extended into the superficial dermis. The dermis and panniculus were diffusely expanded by edema. Additional organs examined microscopically included liver, lungs, spleen, kidneys, heart, esophagus, bone marrow, colon, and skeletal muscle. Neoplastic MCs were also observed in the liver, spleen, heart, esophagus, and bone marrow.

Toluidine blue staining was performed to better visualize and identify MCs within visceral organs and confirmed aggregates of 15 or more MCs in the liver and spleen (Fig. 2C). MC percentage in the bone marrow (calculated based on 100-cell count of a toluidine blue-stained representative section) was 6%, which is considerably below the 20% threshold considered consistent with MC leukemia in the WHO criteria.^{1,7,18}

Neoplastic cells from subcutaneous tissues had positive immunolabeling for c-kit (Biocare Medical). Labeling was distributed approximately equally between intense focal or stippled cytoplasmic labeling and diffuse cytoplasmic labeling representative of KIT-staining patterns II and III, respectively (Fig. 2B).⁹ The neoplastic round cells in the subcutis were negative for multiple myeloma oncogene 1 (MUM-1) immunolabeling performed by the Michigan State University Veterinary Diagnostic Laboratory (MSU-VDL; East Lansing, MI). Fibrin thrombi were not seen on H&E staining of any tissue. To increase sensitivity for microthrombi, phosphotungstic acid hematoxylin (PTAH) staining was performed on sections of liver, spleen, kidney, and skin, which did not reveal any thrombi.⁴ Given the presence of MC aggregates in bone marrow and multiple visceral organs and the lack of a discernible subcutaneous or cutaneous mass, a presumptive diagnosis of systemic mastocytosis was made.

MC neoplasias in both humans and dogs are frequently associated with gain-of-function mutations in the tyrosine kinase receptor KIT, encoded by the proto-oncogene *c-KIT*; these mutations are thought to be related to neoplastic transformation.^{12,17,19} A key feature in $>80\%$ of human systemic mastocytosis cases is the presence of a point mutation in codon 816 of exon 17 of *c-KIT* (D⁸¹⁶V).¹⁷ Detection of this mutation in affected tissues or peripheral blood is 1 of the 4 minor criteria for diagnosis.^{1,7,18} In dogs, although no specific mutations have been associated with systemic mastocytosis, mutations in exon 8 and exon 11 of

Figure 2. Systemic mastocytosis in a 5-y-old Greyhound dog. **A.** The superficial dermis of the ventral abdomen is markedly expanded by edema and hemorrhage, and the deep dermis and panniculus is infiltrated by sheets of neoplastic mast cells that extend to the margins of the section. H&E. 2 $\times$. **B.** The panniculus is infiltrated by sheets of neoplastic mast cells. H&E. Bar=20 μ m. Inset: neoplastic cells have positive stippled and diffuse cytoplasmic immunolabeling for c-KIT. c-KIT immunohistochemistry. Bar=20 μ m. **C.** The liver is infiltrated by aggregates of neoplastic mast cells. H&E. Bar=50 μ m. Inset: granules within the neoplastic cells stain with toluidine blue (magenta color). Bar=20 μ m.

c-KIT have been identified in cutaneous MCTs and are associated with higher tumor grade and with favorable response to tyrosine-kinase inhibitors.^{6,20} PCR testing of formalin-fixed, paraffin-embedded haired skin for the exon 8 and exon 11 mutations was performed on tumor tissue from our case by MSU-VDL, and neither mutation was detected. Further investigation would be needed to elucidate whether specific point mutations in *c-KIT* or other genetic factors are associated with systemic mastocytosis

in veterinary patients. Interestingly, in addition to the KIT immunolabeling pattern, other prognostic indicators used in cutaneous MCTs such as mitotic count, apparent degree of cellular differentiation, and abundant toluidine blue staining, would not have predicted a poor prognosis in our patient, and may not be useful in the diagnosis of systemic mastocytosis. The pathogenesis of systemic mastocytosis in dogs may differ from that of the more common cutaneous MCTs and warrants further research.

A striking and unique feature of our case is the amount of hemorrhage and edema observed. Edema results from MC degranulation releasing mediators, such as histamine, which increase vascular permeability. Hemorrhage resulting from hemostatic abnormalities in patients with mastocytic diseases is described occasionally in humans.^{3,10,16} Cutaneous mastocytosis can promote hyperfibrinolytic and hypocoagulable states by the actions of tryptase and heparin, similar to the mechanisms of anaphylaxis.^{8,14} Several case reports of human patients suggest that neoplastic MCs can also release heparin-like molecules that are resistant to heparinase and other anti-heparin treatments.^{3,10,16} In human mastocytosis cases, the dominant effects are typically on secondary hemostasis and so result in prolongations of PT and APTT,^{3,10,16} which were not seen in our case. However, a subset of human systemic mastocytosis patients have clinical evidence of bleeding and normal or minimally prolonged coagulation times, suggestive of a defect of primary hemostasis.³ This defect has been linked to reduced von Willebrand factor concentration and/or activity, consistent with *in vitro* evidence that heparin can bind and inhibit von Willebrand factor.¹⁵ Unfortunately, von Willebrand factor was not measured in our case. Nevertheless, available laboratory testing was most consistent with a predominantly primary hemostatic defect, as there was no prolongation of PT or APTT and no evidence of hyperfibrinolysis on viscoelastic testing. Further circumstantial evidence for reduced von Willebrand concentration or function as a contributor to the bleeding is the fact that platelet concentrations (although lower than normal) were above the $50 \times 10^9/\text{L}$ threshold associated with spontaneous bleeding. Congenital von Willebrand disease was considered unlikely in our case given the patient's age and lack of previous episodes of spontaneous bleeding (e.g., at time of spay). The combination of thrombocytopenia, elevated D-dimers, and reduced antithrombin could also be consistent with consumptive coagulopathy, such as hyperfibrinolytic syndrome or thrombotic disseminated intravascular coagulation, but this was considered less likely because coagulation times were within RIs, viscoelastic testing did not support hyperfibrinolysis, and fibrin microthrombi were not detected with PTAH staining.

An interesting observation is how well differentiated the neoplastic MCs appeared in the cytologic and histologic preparations. The MCs were heavily granulated in the fine-needle aspirate and in the blood film as well as toluidine blue-stained histopathology slides. Variation in cell and nuclei sizes was minimal, and only a few mitotic figures were seen while performing a mitotic count.

Reports of systemic mastocytosis are rare in veterinary medicine, and proper diagnosis based on human guidelines is often not possible. As mentioned above, the diagnosis of systemic mastocytosis in human literature depends on the fulfillment of 1 major and 1 minor, or 3 minor, criteria, with further subclassification relying on B and C

findings.^{1,7,17,18} The diagnosis of systemic mastocytosis in veterinary literature currently must rely primarily on fulfillment of the WHO major criterion of having multifocal dense infiltrates of MCs (≥ 15 MC) in extracutaneous organs. Given the lack of identification of a specific point mutation and the irregularity of serum total tryptase testing, it is difficult to fulfill the minor criteria in veterinary species. Subclassification based on the B and C findings is achievable but would require additional testing that is expensive and/or not widely available and thus not often performed. Further investigation into the pathogenesis and clinical manifestations of systemic mastocytosis in veterinary patients is needed, and formation or adaptation of a classification system may be warranted.

Acknowledgments

We thank Dr. Aline Rodrigues Hoffman for consultation on this case.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

Funding for publication charges was provided by the Texas A&M University Department of Veterinary Pathobiology. The authors received no other financial support for the research, authorship, and/or publication of this article.

ORCID iDs

Alexander Aceino <https://orcid.org/0000-0002-4878-2983>

Carolyn L. Hodo <https://orcid.org/0000-0003-4113-7081>

References

1. Arber DA, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 2016;127:2391–2405.
2. Bundza A, Dukes TW. Cutaneous and systemic porcine mastocytosis. *Vet Pathol* 1982;19:453–455.
3. Carvalhosa AB, et al. A French national survey on clotting disorders in mastocytosis. *Medicine* 2015;94:e1414.
4. Cotovio M, et al. Detection of fibrin deposits in horse tissues by immunohistochemistry. *J Vet Intern Med* 2007;21:1083–1089.
5. Dobson JM, et al. Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. *J Small Anim Pract* 2002;43:240–246.
6. Gleixner KV, et al. Synergistic antiproliferative effects of KIT tyrosine kinase inhibitors on neoplastic canine mast cells. *Exp Hematol* 2007;35:1510–1521.
7. Horny HP, et al. Mastocytosis. In: Swerdlow SH, et al., eds. *World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues*. (Vol. 2, WHO Classification of Tumours. Rev. 4th ed.). IARC Press, 2016:61–70.

8. Kauhanen P, et al. Effects of skin mast cells on bleeding time and coagulation activation at the site of platelet plug formation. *Thromb Haemost* 1998;79:843–847.
9. Kiupel M, et al. The use of KIT and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors. *Vet Pathol* 2004;41:371–377.
10. Koenig M, et al. An unusual cause of spontaneous bleeding in the intensive care unit—mastocytosis: a case report. *Cases J* 2008;1:100.
11. Lamm CG, et al. Disseminated cutaneous mast cell tumors with epitheliotropism and systemic mastocytosis in a domestic cat. *J Vet Diagn Invest* 2009;21:710–715.
12. Letard S, et al. Gain-of-function mutations in the extracellular domain of KIT are common in canine mast cell tumors. *Mol Cancer Res* 2008;6:1137–1145.
13. O’Keefe DA, et al. Systemic mastocytosis in 16 dogs. *J Vet Intern Med* 1987;1:75–80.
14. Smith TF, et al. Cutaneous mastocytosis with bleeding: probable heparin effect. *Cutis* 1987;39:241–244.
15. Sobel M, et al. Heparin inhibition of von Willebrand factor-dependent platelet function in vitro and in vivo. *J Clin Invest* 1991;87:1787–1793.
16. Sucker C, et al. Fatal bleeding due to a heparin-like anticoagulant in a 37-year-old woman suffering from systemic mastocytosis. *Clin Appl Thromb Hemost* 2008;360–364.
17. Valent P, et al. Mastocytosis: pathology, genetics, and current options for therapy. *Leuk Lymphoma* 2005;46:35–48.
18. Valent P, et al. Mastocytosis: 2016 updated WHO classification and novel emerging treatment concepts. *Blood* 2017;129:1420–1427.
19. Welle MM, et al. Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. *Vet Dermatol* 2008;19:321–339.
20. Zemke D, et al. Mutations in the juxtamembrane domain of c-KIT are associated with higher grade mast cell tumors in dogs. *Vet Pathol* 2002;39:529–535.