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Case Report

Systemic mastocytosis with terminal leukemic manifestation in two dogs

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Abstract

Systemic mastocytosis (SM) with leukemic manifestation is rarely reported in veterinary patients. Here, we describe two cases of systemic mastocytosis with leukemic spread of neoplastic mast cells in adult dogs, including their clinical signs, clinical and anatomic pathology, and immunohistochemical analysis of c-KIT as a diagnostic tool. In both cases, hematologic examination revealed normocytic and normochromic anaemia, leucocytosis with segmented neutrophils, and mastocytosis. Cytological analyses of the peritoneal fluid revealed many neoplastic mast cells. Case 1 was associated with a subcutaneous mast cell tumor and Case 2 was not associated with a cutaneous or subcutaneous mast cell tumor. Neoplastic mast cells were found in the lymph nodes, spleen, liver, and kidneys, and a diagnosis of visceral mastocytosis was established. SM with terminal leukemic manifestation was confirmed by the mastocythemia intensity associated with the morphological changes of the mast cells in the blood and bone marrow.

Key words: bone marrow, c-KIT, histopathology, mast cell leukemia, mastocytosis.

Introduction

In mammals, mast cells play a vital role in the immune system, by participating in hypersensitivity reactions, T-cell stimulation, and parasite defense (4). However, neoplastic proliferation of these cells is common in animals, especially dogs. Mast cell tumors (MCTs) are the most common skin tumor in dogs, comprising up to 21% of all canine cutaneous neoplasms (2). Conversely, systemic or visceral mast cell neoplasia is rare in dogs (3, 7, 9) and is generally characterized by a primary cutaneous mass of poorly differentiated cells (3).

In humans, mast cell leukemia (MCL) is very rare and is defined as the presence of more than 10% of neoplastic mast cells in the peripheral blood, accompanied by a hypercellular bone marrow, infiltrated by less-differentiated blasts (6). These diagnostic criteria are often extrapolated to the veterinary field, because there are none established for canine patients (7). Additionally, few reports have documented this condition in dogs by using thorough clinical and veterinary pathology evaluations, along with immunohistochemical (IHC) technique.

This report documents two cases of canine systemic mastocytosis (SM) with terminal leukemic manifestation, their clinical and pathological findings.

Case description

Case 1 was an 8-year-old, intact male Pinscher dog admitted to the Veterinary Teaching Hospital with abdominal distention, pale ocular and oral mucous membranes, and a subcutaneous mass near the preputial

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region. Abdominal ultrasonography revealed an enlarged spleen and large amount of abdominal effusion, precluding the evaluation of other abdominal organs.

Case 2 was a 5-year-old, spayed female Shar-Pei dog admitted to the same hospital 3 months later with prostration, diarrhea, occasional emesis, pale ocular and oral mucous membranes, and abdominal distention. Abdominal ultrasonography revealed several tumoral masses, suggestive of enlarged mesenteric lymph nodes, and abundant abdominal effusion, precluding a detailed ultrasonographic examination.

Complete blood count (CBC), serum biochemical, and abdominal fluid analyses were performed for both patients. The cytological smears were stained with Romanowsky stain. The CBC results are presented in Table 1. Peripheral blood smears revealed nucleus hypersegmentation of neutrophils and neoplastic mast cells. Case 1 showed mast cells with mild anisocytosis and abundant cytoplasmic granules. Case 2 showed mast cells with moderate anisocytosis and anisokaryosis, with scarce granules.

Table 1. Hematologic findings of case 1 and case 2.

Complete Blood Count (CBC)			
	Case 1	**Case 2**	**Reference values[1]**
Plasm characteristic	Normal	Normal	Colorless and clear
Plasmatic proteins (g/L)	68	60	60 - 80
Red blood cells (10^6 /µL)	4.5	3.8	5.5 - 8.5
Hemoglobin (g/dL)	109	90	120 - 180
Packed cell volume (%)	32	28	37 - 55
MCV* (fL)	71.1	73.7	60 - 77
MCHC* (g/dL)	341	321	320 - 360
RDW* (%)	12.4	13.9	12.0 - 15.0
Total leukocytes concentration (x10^9 cells/L)	48	52	6 - 17
Band neutrophils (x10^9 cells/L)	0.24	-	0 - 0.3
Segment neutrophils (x10^9 cells/L)	36.6	23.45	3 - 11.5
Eosinophils (x10^9 cells/L)	-	-	0.01 - 1.25
Lymphocytes (x10^9 cells/L)	0.98	1.88	1 - 4.8
Monocytes (x10^9 cells/L)	6.1	5.52	0.15 - 1.35
Mast cell (x10^9 cells/L)	4.88	21.26	0
Platelets (x10^3 cells/µL)	188	243	175 - 500

[1](Harvey, 2001).
*MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width.

Serum biochemical analysis in Case 1 revealed no abnormalities. Case 2, however, had hypoalbuminemia (48 g/L; reference values: 53–78 g/L).

The abdominal fluid of both animals had a modified transudate. In Case 1, there were predominantly neutrophils (65%) and, in lower concentration, moderately granulated mast cells (29%) with moderate anisocytosis.

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Case 2 had a predominance of hypogranulated mast cells (95%) with moderate-to-marked pleomorphism, lacy chromatin, prominent nucleoli, and increased nucleus:cytoplasm ratio.

Since both patients had CBC results consistent with mastocythemia and abdominal fluid analysis suggestive of visceral mastocytosis, bone marrow cytologic examination was performed to investigate potential MCL; the findings are presented in Table 2. Case 1 had densely granulated (85.4%) and moderate-to-poorly-granulated (14.6%) neoplastic mast cells with marked pleomorphism, lacy chromatin, and prominent nucleoli. In addition, there were neoplastic mast cells with multiple nuclei, nuclear molding and atypical mitoses. Case 2 presented poorly granulated (97%) neoplastic mast cells with similar cellular features.

Table 2. Bone marrow cytological exam of case 1 and case 2.

Cytological Evaluation of Bone marrow			
	Case 1	**Case 2**	**Reference values[1]**
Cellularity	90%	**	25 - 75 %
Megakaryocytes (cells/particles)	2	**	2 - 7
Myeloid:Erithroid ratio	*	4.9	0.75 - 2.53
Myeloblasts (%)	-	1.7	0.4 - 1.1
Promyelocytes (%)	-	1.1	1.1 - 2.3
Myelocytes (%)	1.8	3.4	3.1 - 6.1
Metamyelocytes (%)	3.0	4.6	5.3 - 8.8
Band neutrophils (%)	14.5	9.8	12.7 - 17.2
Segmented neutrophils (%)	24.8	9.2	13.8 - 24.2
Eosinophils (%)	0.6	-	1.8 - 5.6
Basophils (%)	-	1.1	0 - 0.8
Rubriblasts (%)	-	1.7	0.2 - 1.1
Prorubricytes (%)	-	-	0.9 - 2.2
Rubricytes (%)	-	2.9	19.2 - 35.1
Metarrubricytes (%)	-	1.7	9.2 - 16.4
Lymphocytes (%)	1.2	4.0	1.7 - 4.9
Macrophages (%)	-	0.6	0 - 0.4
Mast cells (%)	53.9	58.0	-

[1](Harvey, 2001).
*Erythroid cells absent.
**No particles were observed.

Despite supportive care with antibiotics, gastric mucosa protectors, and antiemetics, the clinical condition of both dogs deteriorated rapidly and euthanasia was elected in accordance to the recommendations of the Welfare Committee. Necropsy was performed in both cases.

On external examination, Case 1 presented pale oral and ocular mucosa and a 5 × 3 cm, subcutaneous inguinal mass, associated with local edema and hemorrhage. The left inguinal lymph node was mildly enlarged. On the cut surface, the parenchyma was replaced by a solid whitish, soft tissue mass. There was hepatosplenomegaly caused by the infiltration of multifocal millimetric whitish areas throughout the parenchyma. Both kidneys presented multifocal, whitish-yellow areas on the cortex. The duodenal mucosa was multifocally ulcerated, and the wall was thickened. Case 2 had similar lesions in the liver and spleen (Fig. 1), but no cutaneous or subcutaneous masses. The cervical, popliteal, tracheobronchial and mesenteric lymph nodes were also markedly enlarged and the parenchyma was replaced by a solid, whitish and soft neoplastic tissue (Fig. 2). Multifocal, soft, white nodules (up to 2 cm in diameter) were spread throughout the visceral peritoneum. The medullar cavity of the femur was infiltrated by a whitish and soft neoplastic tissue. Sections of the masses and several organs (including the bone marrow from the femur) were collected from both dogs and fixed in neutral 10% buffered formalin and routinely processed for histopathology examination. Giemsa staining and further immunolabelling for c-KIT (CD117 – Dako, USA) was performed using a polyclonal antibody (1:300 dilutions). For IHC analysis, a peroxidase system was applied, in which a secondary antibody is recognized by a polymer (Advance HRP enzyme, DakoCytomation, Carpinteria, CA, USA). Antigen retrieval was performed in a water bath at 98°C with a citrate buffer solution at pH 6 (DakoCytomation, Carpinteria, CA, USA). To block the endogenous peroxidase activity, the slides were incubated in a solution of 3% H2O2 in methyl alcohol. The reagents were applied manually, with a 16-hour incubation period for the primary antibody and 30 minute incubation period for the other reagents. The only exception was the diaminobenzidine chromogen, with which the sections were incubated for five minutes. A previously diagnosed well-differentiated MCT was used as the positive control. The negative control was assessed using normal serum as a substitute for the primary antibody.

On histopathologic analysis, Case 1 had an inguinal subcutaneous mass comprising densely arranged neoplastic mast cells, with moderate amounts of intracytoplasmic granules, nuclear indentation, and karyomegaly. Up to 20 mitoses were visible in 10 high-power fields (HPFs, 400x). Atypical pleomorphic cells with up to four nuclei were commonly observed. The bone marrow had hypercellular areas, predominantly composed of blast cell mantles with fine cytoplasmic granules, which compressed the remnants of the normal haematopoietic cells or adipocytes. The spleen, liver, kidneys, and visceral lymph nodes had most of the parenchyma replaced by neoplastic mast cells with similar features. Histopathologic analysis of Case 2 revealed visceral and superficial lymph nodes, and the spleen, liver (Fig. 3), and small intestine severely effaced by mantles and cords of neoplastic cells. These cells were round, with moderate amount of eosinophilic cytoplasm, and large, indented, eccentric nuclei filled with loose chromatin. Anisocytosis and anisokaryosis were marked. Fifteen mitoses were visible in 10 HPFs. Binucleated and trinucleated cells were common. The bone marrow was diffusely hypercellular, and multifocally infiltrated by densely packed groups of highly pleomorphic cells (Fig. 4). Giemsa strongly stained neoplastic cells in Case 1 (Fig. 5). Most of the neoplastic mast cells within the examined tissues, including the bone marrow, of both dogs had variable intensity of membrane and cytoplasmic c-KIT labelling. In Case 1, most of the cells presented a KIT I pattern and a lower number presented a KIT II pattern. Occasionally, individual neoplastic cells showed a KIT III pattern. In Case 2, most cells had a KIT II pattern (Fig. 6), especially those arranged in the dense cords or groups. Several individual neoplastic cells showed a KIT III pattern.

Considering the diagnostic panel (CBC, blood and bone marrow cytology, necropsy, histopathology, and IHC analysis), a diagnosis of SM with terminal leukemic manifestation was established for both cases.



Figure 1. Liver in Case 2. The edges are round and the lobes are enlarged. Inset: On cut surface there are whitish areas throughout the liver parenchyma. Spleen, located caudal to the liver in the abdominal cavity, also is enlarged.

Discussion

In human medicine, mastocytosis is defined as an abnormal accumulation of mast cells in one or more organs. The diagnostic criteria of SM established by the World Health Organization mainly include the observation of multifocal, dense infiltrates of mast cells in the bone marrow and/or other extracutaneous organ(s) (6, 17). In contrast, MCL is very rare and requires a 20% threshold of neoplastic mast cells within the bone marrow and/or >10% in the peripheral blood in addition to visceral infiltration (6).

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In veterinary literature, localized extracutaneous MCT has been occasionally reported (3, 5, 15, 16, 18). Unlike cats, dogs rarely develop visceral mastocytosis (4, 7, 9) and, when it occurs, it is commonly associated with a primary cutaneous MCT (3, 18). In a previous study of canine SM, 14 of 16 dogs had a primary cutaneous MCT and only two had SM without a skin tumor (12), as found in Case 2.



Figure 2. Mesenteric lymph nodes in Case 2. They are markedly enlarged with parenchymal replacement by a soft, white, tumoral mass interspersed by red areas (necrosis and hemorrhage).

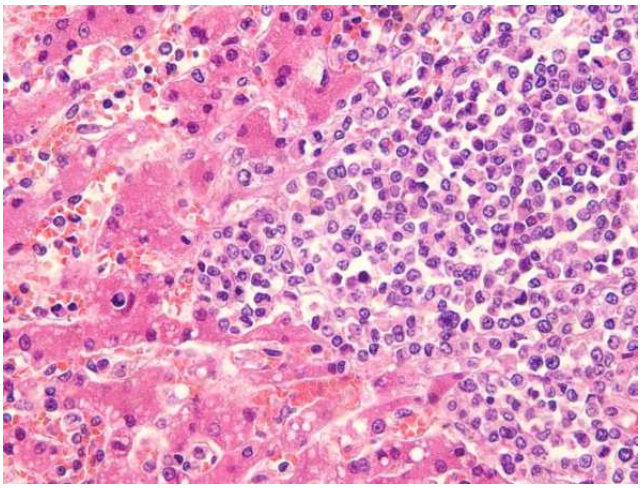


Figure 3. Liver in Case 2. Infiltration and partial replacement of hepatocytes by dense groups of highly pleomorphic hypogranulated mast cells. HE, 400x

Moreover, standardized diagnostic criteria for canine MCL are lacking. One study (9) reported a patient with an abdominal mass associated with mastocythemia (42% mast cells), highly increased plasma histamine concentration, and bone marrow cytology with 300–400 mast cells/1,000 nucleated cells. Another study (7) reported a dog with marked mastocythemia (43% mast cells), associated with moderate anisocytosis, karyolysis, and degranulation. Similarly, we found that circulating neoplastic mast cell concentrations were variably lower (10.2% in Case 1 and 40.8% in Case 2).

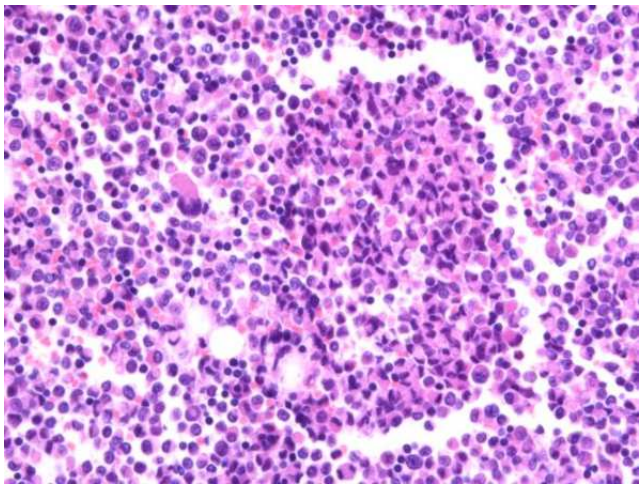


Figure 4. Bone marrow of the femur in Case 2. Hypercellularity, characterized by infiltration and partial replacement of haematopoietic tissue by dense groups of highly pleomorphic hypogranulated mast cells (central field). Hematoxylin and eosin staining. HE, 400x.

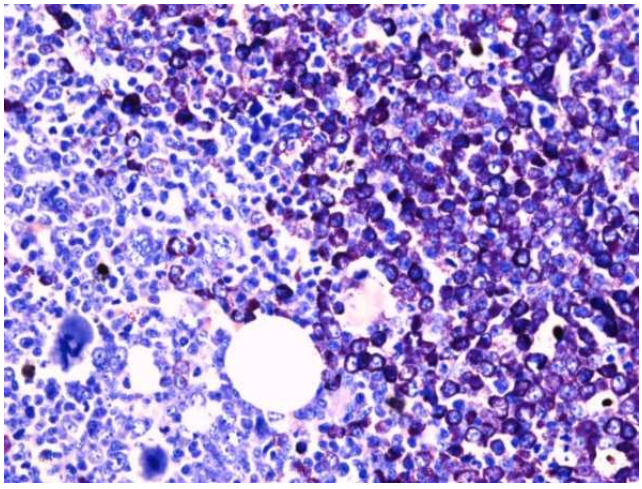


Figure 5. Bone marrow of the femur in Case 1. Mantle of neoplastic mast cells (central-right field) with cytoplasm containing Giemsa-positive granules replacing and comprising haematopoietic tissue. HE, 400x.

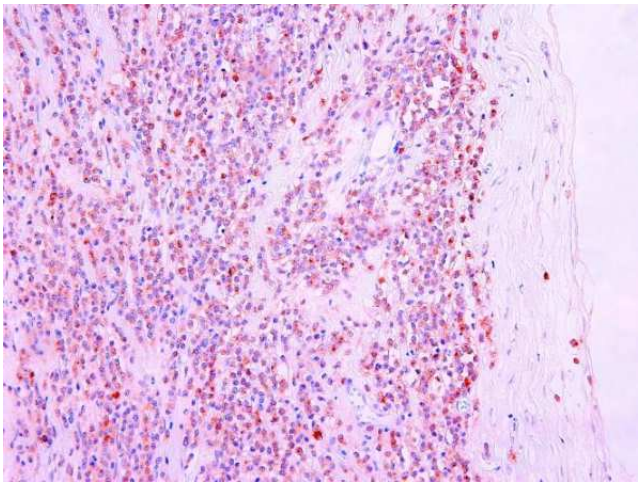


Figure 6. Lymph node in Case 2. Neoplastic mast cells with intense focal and dense aggregates of brown immunolabelling within the cytoplasm (KIT II). Some individual neoplastic cells have diffuse brown immunolabelling inside cytoplasm (KIT III). c-KIT IHC analysis. Chromogen DAB, Mayer's Haematoxylin counter stain, 200x.

Apparently, anemia is a common finding in animals and humans with SM (4, 6, 12, 16), possibly because of mechanisms involved in chronic diseases (16), myelophthisis (8), and/or blood loss through gastrointestinal (GI) ulceration (2). Leukocytosis in Case 1 was mainly attributed to mature neutrophilia, possibly associated with stress, GI tract ulcers, and/or tumor necrosis (12). However, in Case 2, high numbers of circulating mast cells were the main cause of leukocytosis. Thrombocytopenia was often documented in other case reports, and was most likely due to systemic heparin release and disseminated intravascular coagulation (12, 16). However, platelet numbers were within the normal range for both patients in our study. Histopathologic analysis revealed that the multifocal involvement of the bone marrow does not reduce platelets precursors.

In SM, biochemical analysis results may be unremarkable (3, 16) or may reveal hypoalbuminemia (4, 6), as seen in one of our patients. In this study, the decreased plasma proteins may have resulted from liver damage due to neoplastic infiltration and/or blood loss in the GI tract.

Since mastocythemia alone is not necessarily related to SM (11), bone marrow evaluation is essential for an accurate diagnosis (16). Infiltration of mast cells in this organ is frequent in canine SM (15, 16), and may represent up to 58% of the cases, characterized by ≥10 mast cells/1,000 nucleated cells (12). Bone marrow hypercellularity is expected in MCL (14), as seen in Case 1. The absence of marrow particles in Case 2 precluded its cellular evaluation. Additionally, in both animals of the present report, more than 50% of the cellular components were mast cells. This proportion markedly exceeded the threshold established for MCL diagnosis in humans (6). Decreased numbers of maturing erythroid and myeloid cells were probably directly associated with neoplastic proliferation and consequent myelophthisis (16). For both dogs in this study, the histopathology revealed neoplastic mast cells with multifocal distribution in the bone marrow. Therefore, it may be more accurate to interpret this finding as metastases from viscera and consequent terminal mast cell leukemia.

Although the histological grading system is a prognostic indicator in canine cutaneous MCT (2, 3), its usefulness is questionable for the extracutaneous form. Both animals in this study had disseminated infiltration of highly pleomorphic mast cells, with nuclear atypia, and abundant mitoses—all features of malignancy.

c-KIT is a IHC marker for mammalian mast cells (10). It is normally expressed in the cell membrane, but may accumulate within the cytoplasm of neoplastic mast cells (13). c-KIT labelling intensity and distribution are related to prognosis in cutaneous MCT (1, 10, 13). In our study, in Case 2, the vast majority of neoplastic cells within the different tissues were highly undifferentiated and KIT II pattern predominated. In cutaneous MCT, these patterns are strongly related to a more aggressive tumoral behavior, characterized by an increased rate of recurrence and decreased survival (10).

The frequency of both conditions may be underestimated in veterinary patients, since histopathologic, histochemical, IHC analyses, and bone marrow examination are frequently required for final diagnosis. Because SM with leukemic manifestation is often related to a grave clinical condition and poor prognosis in dogs, further studies regarding their early detection and pathophysiology are warranted.

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