NEOPLASTIC DISEASE

SHORT TITLE: Cutaneous Mast Cell Tumour with Intrathoracic Metastasis

High-Grade Cutaneous Mast Cell Tumour with Widespread Intrathoracic Metastasis and Neoplastic Pericardial Effusion in a Dog

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Summary

An 8-year-old, neutered male French Bulldog was presented with a 2-day history of intermittent vomiting, reduced appetite and recent rapid development of multiple cutaneous masses over the head and neck regions. On presentation, the patient had a moderate volume of pericardial and bilateral pleural effusion. Echocardiography demonstrated irregular, heterogeneous thickening of the walls of the right ventricle and right atrium, consistent with

infiltrative intramyocardial disease. Cytological examination of fine needle aspirates from one of the cutaneous masses confirmed a mast cell tumour. Pericardial fluid analysis revealed a haemorrhagic neoplastic effusion due to mast cell neoplasia. Histopathological and immunohistochemical examination of tissues obtained post mortem, confirmed a high-grade cutaneous mast cell tumour with metastasis to the heart, pericardium, mediastinum and spleen. No metastatic disease was present in the submandibular lymph nodes or liver. Immunohistochemistry demonstrated KIT staining pattern 2. There was strong nuclear Ki67 labelling in an average of 65.0 cells per grid and an average of three positive AgNORs per nucleus in neoplastic cells. PCR for the activating duplication mutation in exons 8 and 11 of c-Kit were negative. To the authors' knowledge, this is the first report of a canine cutaneous mast cell tumour associated with neoplastic pericardial effusion and widespread intrathoracic metastasis.

Keywords: dog, intrathoracic metastasis; mast cell tumour; pericardial effusion

Mast cell tumours (MCTs) are the most common canine cutaneous neoplasia and vary widely in their biological behaviour. Cutaneous and subcutaneous locations are most common but other primary sites include tissues of the gastrointestinal tract, including oral cavity. There are several reports of presumed primary intrathoracic MCTs (Cowgill and Neel, 2008; Campbell *et al*, 2017; Cartagena-Albertus *et al*, 2019). Metastasis is often to local lymph nodes, liver, spleen and, rarely, bone marrow and lungs. The c-Kit gene is implicated in the pathogenesis of canine MCTs and mutations in this gene have been linked to tumour aggressiveness (Preziosi *et al*, 2004). The proto-oncogene c-Kit encodes KIT, a receptor tyrosine kinase, which is expressed on a variety of cells including mast cells. A variety of

somatic mutations in c-Kit have been described, most commonly in exon 11 (affecting the juxtamembrane domain of the protein), or in exons 8 or 9 (affecting the extracellular domain) (Ma *et al*, 1999). These mutations result in constitutive activation of KIT and subsequent proliferation, differentiation and survival of neoplastic mast cells. The prognosis of canine MCT depends on a number of factors, with tumour grade and stage of disease being the most influential. Cutaneous MCTs are routinely graded according to the Patnaik system (Patnaik *et al*, 1984), as grade 1, 2 or 3, or the Kiupel system (Kiupel *et al*, 2011), in which tumours are categorised as low or high grade, depending on their histological features. The histological grade of cutaneous MCTs is prognostic; it is well documented that dogs with Patnaik grade 3 tumours have a significantly shorter overall survival time compared to those with Patnaik grade 1 and 2 tumours (Patnaik *et al*, 1984; Takeuchi *et al*, 2013). Similarly, dogs with Kiupel high-grade tumours have significantly shorter overall survival times and progression-free survival times compared to those with low-grade tumours (Kiupel *et al*, 2011; Vascellari *et al*, 2013). Increasing stage of disease is also correlated with a worse prognosis, with a median survival time of just 110 days in dogs with distant metastatic (stage IV) disease (Murphy *et al*, 2006; Pizzoni *et al*, 2018).

An 8-year-old, neutered male French bulldog was referred to the Royal Veterinary College's Queen Mother Hospital for Animals with a two-day history of intermittent vomiting, hyporexia and recent rapid development of multiple cutaneous masses (within the preceding 3 days). On presentation, physical examination revealed tachycardia (140 beats per minute), muffled heart sounds, tachypnoea (32 breaths per minute), dyspnoea and multiple cutaneous masses on the dorsal head, neck and cranial thoracic regions. Haematological abnormalities included a mild neutrophilia (12.91×10^9/L; reference interval [RI], 3.0 — 11.5 $\times 10^9$/L), moderate lymphopaenia (0.29×10^9/L; RI, 1.0—4.8×10^9/L), and moderate

haemoconcentration (packed cell volume (PCV) 60%; RI, 37 × 55, haemoglobin concentration 20.8 g/dl; RI, 12—18). Blood smear examination was unremarkable. Serum biochemistry examination revealed only a moderate hypochloraemia (88.8 mmol/L; RI: 100—116 mmol/L). Ultrasound assessment revealed moderate pericardial and bilateral pleural effusion although no peritoneal effusion was present. Echocardiography demonstrated irregular, heterogeneous thickening of the right ventricular free wall and right atrial wall, consistent with infiltrative intramyocardial disease. Electrocardiography confirmed sinus arrhythmia with first-degree atrioventricular block.

Fine needle aspirates, obtained from one of the cutaneous masses, contained abundant individual neoplastic mast cells, occasional erythrocytes, low numbers of lysed cells, and a pale blue background with lipid spaces and abundant mast cell granules. Mast cells had round to oval nuclei (up to 20—25 µm), finely granular chromatin, multiple prominent nucleoli and a moderate amount of pale blue cytoplasm, commonly with low numbers of mast cell granules and occasional crisp vacuoles. There were mild anisokaryosis and occasional mitotic figures. Other cells included occasional dense clumps of plump spindle cells (likely fibroblasts), occasional eosinophils, neutrophils and rare macrophages. These findings were consistent with mast cell neoplasia.

Upon induction of anaesthesia, prior to further investigations, the dog suffered a cardiopulmonary arrest and open-chest cardiopulmonary resuscitation was required for a successful return of spontaneous circulation. Before closing the emergency thoracotomy site, a pericardial effusion sample and tissue samples from the pericardial/mediastinal fat were obtained for cytological and histopathological examination, respectively, due to their abnormal gross appearance. The heart also appeared grossly abnormal with widespread multifocal cream-coloured raised nodules expanding from the myocardium.

The pericardial fluid appeared red, turbid and had a total nucleated cell concentration of 5.90×10^9/L, protein concentration of 30 g/L and a PCV of 14%. Cytological examination of direct smears of the fluid (Fig. 1) found low nucleated cellularity, moderate numbers of erythrocytes, occasional lysed cells and a clear background. Nucleated cells were dominated by poorly granulated, highly anaplastic mast cells with large nuclei (up to 30 µm), smooth to coarse chromatin, multiple prominent nucleoli and a small to moderate amount of pale to dark blue cytoplasm with low numbers of purple granules. Anisokaryosis was mild to moderate and occasional binucleation was seen. Rare mitotic figures were also observed. Other cells included low numbers of neutrophils, lymphocytes, occasional macrophages and scattered eosinophils. These findings were consistent with a haemorrhagic neoplastic effusion due to mast cell neoplasia. The mast cells in the effusion matched the appearance of those in the previously described cutaneous mass.

After two days of management in the Intensive Care Unit the dog suffered a second cardiopulmonary arrest and resuscitation was not attempted. A cosmetic necropsy was requested by the owners.

On gross examination the heart had multiple, random, white, raised nodules ranging from 2 × 2 mm to 11 × 10 mm that extended into the myocardium (Fig. 2). Skin samples from the right dorsal cervical and submandibular regions had moderately demarcated, 5 × 10 mm to 10 × 10 mm nodules. Histologically, the skin nodules consisted of poorly demarcated, highly infiltrative, moderately cellular, high-grade mast cell tumours. Mast cells were organized in loose sheets that dissected collagen bundles and there were multifocal areas of dermal necrosis and vasculitis. Very rare eosinophils were observed scattered between the neoplastic mast cells. The neoplastic cells had moderately distinct borders, a moderate amount of pale, eosinophilic cytoplasm with one or multiple paracentral, pleomorphic nuclei. There were 34 mitoses, three binucleated cells and six indented bizarre nuclei in 10 high-

power fields (total area 2.37 mm^2), equating to high-grade tumours. Sections from the heart (Fig. 3), pericardium, mediastinum and spleen contained neoplastic foci composed of poorly-differentiated mast cells similar to those described in the skin. Mast cells were not metachromatic with toluidine-blue or modified Wright-Giemsa stains in any of the examined sections. The submandibular lymph nodes, lung or liver did not contain macroscopic or microscopic evidence of neoplastic mast cells.

Due to the poorly differentiated appearance of the cells, immunohistochemistry for CD3, CD79, AE1AE3 (multikeratin), vimentin and KIT were performed to exclude other round cell tumours. Additionally, Ki67 immunolabelling and argyrophilic nucleolar organising regions (AgNOR) staining were performed. Immunohistochemistry demonstrated negative labelling of the neoplastic cells for CD3, CD79 and AE1AE3 antigens, whereas vimentin immunolabelling was positive. Immunohistochemical labelling for KIT was also positive and showed both a loss of peri-membrane labelling and perinuclear or stippled cytoplasmic labelling in greater than 10% of neoplastic cells, consistent with KIT labelling pattern 2 (Preziosi *et al*, 2004) (Fig. 4). There was strong Ki67 nuclear labelling in an average of 65.0 cells per grid and an average of three positive AgNORs per nucleus in neoplastic cells. The AgNOR × Ki67 index was 192.3. A polymerase chain reaction technique for the detection of activating duplication mutations in exons 8 and 11 of c-Kit was negative. The cytological, histological and immunohistochemical findings were consistent with mast cell neoplasia.

There are no previous reports of metastasis of a cutaneous MCT to intrathoracic tissues such as the pericardium and mediastinum. A few post-mortem descriptions of cardiac involvement do exist (Walter and Rudolph, 1996; Ware and Hopper, 1999), but these reports do not provide any insight into the clinical presentation of the cases. As mast cell tumours

often metastasise to regional lymph nodes prior to systemic dissemination, it is interesting that with the presence of masses on the head in the present case, no metastatic disease was detected in the submandibular lymph nodes. As the metastatic pattern of head and oral tumours can be unpredictable (Skinner *et al*, 2017), it is possible the masses present on this patient's head drained through the retropharyngeal lymph nodes instead, which were not sampled. The intrathoracic metastatic disease may have developed secondary to the cutaneous tumours present on the patient's neck or thorax, which would have drained via other regional lymph nodes, such as the superficial cervical or axillary lymph nodes and which were also not sampled.

A neoplastic pericardial effusion secondary to mast cell neoplasia has not been previously reported. Exfoliation of neoplastic cells into a body cavity can cause highly cellular effusions, with lymphoma being the most common cause of neoplastic effusions in dogs and cats (Dempsey and Ewing, 2011). Cavity effusions in dogs with MCTs are uncommon and seen primarily in cases with visceral involvement; both pleural and peritoneal effusions have been reported (Cowgill and Neel, 2008; Harris *et al*, 2013). Effusions secondary to mast cell neoplasia in dogs can be highly eosinophilic (Harris *et al*, 2013), likely due to the development and chemotaxis of eosinophils secondary to prostaglandin D2, leukotriene, IL-3, IL-5 and GM-CSF secreted from activated mast cells (Stone *et al*, 2010). Only occasional eosinophils were seen in the pericardial effusion of this case and very low numbers were observed on histopathology of the affected tissues.

The cutaneous mass in the described patient was consistent with a Patnaik grade 3/Kiupel high-grade MCT. This is unusual as French bulldogs are more likely to develop Patnaik grade 1 cutaneous MCTs (Śmiech *et al*, 2017). Immunohistochemistry for KIT and vimentin was important to confirm the histopathological diagnosis of metastatic mast cell neoplasia in this case because the mast cell granules were difficult to observe on HE sections.

Additionally, toluidine blue and Giemsa staining did not demonstrate metachromasia. However, the mast cell granules, despite being sparse, stained well with modified Wright-Giemsa stain during the cytological assessment of the cutaneous masses and pericardial effusion, emphasising the importance of cytological examination of mast cell neoplasia. Activating mutations in exons 8, 9 and 11 of the c-Kit gene are common in canine MCTs, with one study demonstrating mutations in 26.2% of tumours (Letard *et al*, 2008). C-Kit mutations are also significantly associated with higher grade MCTs (Vozdova *et al*, 2019) and are associated with decreased disease-free interval, increased risk of tumour recurrence and decreased overall survival time (Webster *et al*, 2006). Despite the high-grade designation in this case, PCR for the activating duplication mutations in exons 8 and 11 of c-Kit were negative. C-Kit gene sequencing was not performed and it is possible that other activating mutations were present in this MCT.

Proliferation markers, such as Ki67 and AgNOR, have been assessed in canine MCTs and are associated with prognosis (Webster *et al*, 2007). Mast cell tumours with a Ki67 index >23 positive cells per grid have a significantly increased risk of local recurrence, metastasis and MCT-related death. Similar findings were observed in MCTs with increased AgNOR counts. An AgNOR × Ki67 index >54 has been associated with increased risk of local recurrence and MCT-related death (Webster *et al*, 2007). The KIT expression pattern is also associated with prognosis. Normally, KIT is expressed in the cell membrane of mast cells (Reguera *et al*, 2000), which is classified as KIT staining pattern 1. In some neoplastic mast cells KIT is expressed focally (staining pattern 2) or diffusely (staining pattern 3) throughout the cytoplasm. Increased cytoplasmic KIT expression is associated with increased risk of local recurrence and reduced overall survival (Kiupel *et al*, 2004). In the case presented here, the Ki67 index was very high (65 cells per grid) in addition to an elevated AgNOR count (3.0 AgNORs per neoplastic cell), high AgNOR × Ki67 index of 197.3 and KIT staining pattern

2. These results demonstrate an aggressive phenotype, which correlates with the cytological appearance and histopathological grade of the tumour, as well as the dog's clinical presentation.

In summary, to the authors' knowledge, this is the first report describing a canine cutaneous MCT associated with a neoplastic pericardial effusion and widespread intra-thoracic metastasis.

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Conflict of Interest Statement

The authors declare no conflicts of interest with respect to the research, authorship or publication of this article.

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FIGURE LEGENDS

Fig. 1. Dog, pericardial effusion, mast cell tumour. Poorly granulated neoplastic mast cells have nuclei up to 20 μm in diameter, smooth chromatin, prominent nucleoli and small to moderate amounts of mid—dark blue cytoplasm, with low numbers of fine purple granules and punctate vacuoles. Note mitotic figure (arrow). Modified Wright's stain. Bar, 20 μm.

Fig. 2. Dog, heart, mast cell tumour. Multiple random white nodules expand the right and left ventricles, left auricle, aorta and pulmonary artery. Bar, 2 cm.

Fig. 3. Dog, heart, mast cell tumour. Sheets of neoplastic mast cells dissect myocardial fibres. There is moderate to marked anisocytosis and anisokaryosis and frequent mitoses. No visible eosinophils. HE. Bar, 20 μm.

298 Fig. 4. Dog, heart, mast cell tumour. Neoplastic mast cells exhibit a cytoplasmic perinuclear

299 KIT labelling pattern (KIT labelling pattern 2). IHC. Bar, 20 μm.