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Management of Canine Mast Cell Tumors

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1. Incidence, Signalment, Etiology

The MCT is the most common skin tumor of the dog, and the second most common malignant tumor noted in the canine population. While MCTs are usually found in older dogs (mean age approximately 8-9 years), they have also been reported in younger dogs[1-4]. Several breeds appear to be at increased risk for the development of MCT including dogs of bulldog descent (boxer, Boston terrier, English bulldog), Labrador and golden retrievers, cocker spaniels, schnauzers and Sharpeis[1,4-6]. The etiopathogenesis of MCTs in the dog is unknown, as is the reason for the extremely high incidence in this species. The increased incidence of MCTs in certain breeds suggests the possibility of an underlying genetic cause[5] and studies are ongoing to identify these putative genetic risk factors. Interestingly, while dogs of bulldog ancestry are at higher risk for MCT development, it is generally accepted that MCTs in these dogs are more likely to be benign[4]. Additionally, it was recently demonstrated that Pugs develop multiple mast cell tumors that behave in a benign fashion. In contrast, anecdotal evidence suggests that Sharpeis develop MCTs that are biologically aggressive.

Kit is a receptor found on mast cells (as well as hematopoietic stem cells and melanocytes, among others) and Kit signaling is required for the differentiation, survival, and function of mast cells[7-13].Mutations in Kit have been demonstrated to occur in systemic mastocytosis in people and these mutations lead to excessive signaling, resulting in loss of growth control[14-20]. Several authors have recently identified the presence of Kit mutations in dog MCTs and these also resulting in uncontrolled signaling[21-24]. While up to 30% of all dog MCTs may have Kit mutations, these are not germ-line in nature (i.e., are not inherited) and occur during the process of tumor development. However, they do represent a target for therapy.

2. History and Clinical Signs

Most MCTs in the dog occur in the dermis and subcutaneous tissue[1,25]. Rarely, primary MCTs may present in other sites such as the oral cavity, nasopharynx, larynx, and gastrointestinal tract[26,27]. Visceral MCT involving the spleen, liver and/or bone marrow (often referred to as disseminated mastocytosis) is usually the result of systemic spread of an aggressive primary cutaneous MCT, although it can occur as an independent syndrome[28,29]. Cutaneous MCTs usually occur as solitary nodules, although roughly 10 to 15% of dogs will present with multiple tumors[30]. Approximately 50% of cutaneous MCTs occur on the trunk and perineal region, 40% on the limbs, and 10% on the head and neck[30-32]. Perhaps most importantly, the clinical appearance of MCTs can vary widely. MCTs arising in the subcutaneous tissue are often poorly circumscribed and may resemble lipomas. Cutaneous MCTs may also be present for various lengths of time. In general, MCT that are slow growing and present for at least 6 months are more likely to behave in a benign manner, while those that are rapidly growing large tumors are more likely to behave in a malignant manner[25]. Clinical signs of MCTs are due to the release of histamine, heparin and other vasoactive amines. Mechanical manipulation of the tumor during physical examination can induce degranulation leading to erythema and wheal formation (termed Darrier's sign) and occasionally, an owner will report that the tumor appears to change in size over short periods of time[32]. Gastrointestinal ulceration is also a potential complication of MCTs; between 35-83% of dogs with MCTs that underwent necropsy had evidence of gastric ulcers and plasma histamine concentrations were found to be elevated in dogs with MCT, primarily those with gross evidence of disease[33-35]. Elevated histamine levels presumably lead to stimulation of H2 receptors on

parietal cells, excessive gastric acid production and the development of ulcers.

3. Diagnosis

Cytologic evaluation of fine needle aspirates is probably the easiest method to diagnose a MCT. Poorly differentiated malignant mast cells may contain few, if any, granules in which case special stains (toluidine blue, geimsa) may be required. Excisional biopsy is required for histologic grading of the tumor. If cytologic diagnosis proves difficult, a needle or punch biopsy of the tumor can be obtained prior to surgery. This is preferable to a larger incisional biopsy, as local release of mast cell mediators may inhibit healing resulting in excessive bleeding.

4. Staging

a. CBC, biochemistry profile, urinalysis: These tests are part of a minimum data base and should be included in the work-up of any animal suspected to have cancer. Dogs with MCTs (especially those with systemic disease) may have anemia secondary to GI bleeding.
b. Buffy coat smear: It was originally believed that while the buffy coat smear was not an extremely sensitive test, it was fairly specific for mast cell neoplasia. However, it is now clear that this is not the case, as several studies have demonstrated that dogs with many different kinds of disease, including pneumonia, parvovirus, pancreatitis, skin disease and gastrointestinal diseases may have mast cells circulating in the periphery[36-38].
c. Bone marrow aspiration: In the normal bone marrow, mast cells are found infrequently. While bone marrow evaluation is more likely to detect systemic involvement than the buffy coat smear[29], it is usually easier to find evidence of systemic involvement in other organs (liver, spleen). Therefore, routine bone marrow aspiration is not recommended on most patients.
d. Lymph node aspiration: All regional lymph nodes should be carefully examined for signs of enlargement and any suspicious nodes should be aspirated for cytologic examination. Also, as metastatic nodes may palpate within normal size, it is recommended that all accessible regional lymph nodes be examined by aspiration cytology. Malignant mast cells in metastatic lymph nodes are often found in clusters/aggregates rather than singly, aiding in a diagnosis of metastasis. If possible, lymph node aspiration should be performed prior to surgery, as post-op inflammation can result in mast cell migration to local nodes and thus confuse the interpretation.
e. Evaluation of the abdominal and thoracic cavities: Thoracic radiographs may be included as part of staging, although pulmonary involvement is uncommon. Abnormalities reported include lymphadenopathy (sternal, hilar), pleural effusion, and anterior mediastinal masses, although these are rare[29]. Evaluation of the abdominal cavity is important in dogs with MCTs, as spread to the liver and spleen and abdominal lymph nodes may be noted. It is recommended that fine needle aspiration of the liver and spleen be performed if abnormalities are detected during ultrasound examination, or if the dog possesses negative prognostic indicators[39-41].
f. Clinical staging system for canine MCTs: A revised staging system for dermal mast cell tumors has been proposed. This is shown in Table 1.

5. Prognostic Factors

a. Histologic grade: The histologic grade of a MCT is determined after excisional biopsy of the tumor, and cannot be assessed simply by cytologic evaluation of fine needle aspirates. It is the most consistent and reliable prognostic factor and correlates significantly with survival, but it will not predict the behavior of every MCT. Furthermore, there is disagreement in tumor grading among pathologists; in one study there was significant variation among pathologists in grading the MCTs ($P < 0.001$), although this was found to be less so if all pathologists strictly employed the system described by Patnaik[6,42,43]. Grade 1: these MCTs are considered to behave in benign manner and complete surgical excision is usually curative[6,25,44,45]. Grade 2: These represent at least 45% of all MCTs reported and their biologic behavior is more difficult to predict [6,25,30,44]. Many dogs with complete excision of a Grade 2 MCT are cured and radiation therapy following incomplete excision of solitary Grade 2 MCTs can cure greater than 80% of affected patients[45,46]. However, it is important to note that Grade 2 MCTs have the ability to spread to local lymph nodes, as well as distant sites, and a proportion of dogs that

undergo definitive therapy (surgery and radiation) may go on to develop metastatic disease. Furthermore, some dogs that present with Grade 2 MCTs will already have evidence of metastatic disease making appropriate staging important. Given the wide variation in biologic behavior among Grade 2 tumors there is now an effort to identify subcategories of Grade 2 tumors that may be more likely to behave in an aggressive manner using additional prognostic indicators described below. Grade 3: These represent between 20-40% of all MCT reported[6,25,30,44]. They often behave in a biologically aggressive manner, exhibiting metastasis early on in the course of disease. The mean survival time of dogs with Grade 3 MCT has been reported as 18 weeks when treated with surgery alone 25. In one study, the percentage of dogs with Grade 3 MCTs surviving at 1500 days was reported as 6%, and in another study, the percentage of dogs surviving at 24 months was 7 %, indicating that these tumors are particularly malignant[6,47]. With the recent addition of post-operative chemotherapy, survival times of Grade 3 MCT patients may be improved.

b. Clinical stage: Recent evidence suggests that the historical staging system for MCTs is not reflective of tumor biology, and a new system has been proposed (Table 1). In two studies, the presence of mast cells in the regional lymph node was a negative prognostic factor for survival and disease-free interval[48,49]. However, this may not be the case as an additional study revealed that dogs with Grade 2 tumors and lymph node metastasis treated with radiation post surgery achieved long-term survival[50]. Lastly, while it would seem intuitive that dogs with multiple cutaneous mast cell tumors do not do well, two separate studies have demonstrated that this does not necessarily affect prognosis[48,51].

c. Anatomic location: MCTs that develop in the oral cavity, nail bed, inguinal, preputial, and perineal regions were originally reported to behave in a more malignant fashion regardless of histologic grade[45,52]. However, two reports now demonstrate that at least for definitive evidence for MCTs in the inguinal, preputial, and perineal regions this is likely to be untrue and dogs with tumors in these locations do not necessarily fare poorly[53,54]. MCT that originate in the viscera (GI tract, liver, spleen) or bone marrow carry a grave prognosis[29,55].

d. Growth rate: Tumors present for long periods of time may be more likely to be benign. In one study, 83% of dogs with tumors present for longer than 28 wks prior to surgery survived for at least 30 wks, compared to only 25% of dogs with tumors present for less than 28 wks[25].

e. Breed: Boxers have a high incidence of MCTs, but these tend to be more well differentiated and carry a better prognosis[4,25]. The same has been shown to be true for pugs[56]. However, every MCT should be treated as potentially malignant, regardless of breed.

f. Markers of Proliferation: Several proliferative indices have been evaluated in an attempt to predict the outcome of canine MCTs. Perhaps the most useful is Ki-67, a protein found in the nucleus the levels of which appear to correlate with cell proliferation. In one study, the mean number of Ki-67 positive nuclei was significantly higher for dogs that died of MCTs than for those that survived. For dogs with Grade 2 tumors, the number of Ki-67 was significantly associated with outcome[47]. This was recently confirmed by an additional study that demonstrated the Ki-67 score can be used to divide Grade 2 MCTs into two groups with markedly different expected survival times[57]. A recent study showed that mitotic index (MI, number of mitoses per 10 high power fields) may be extremely useful for predicting the biologic behavior of canine MCTs[58]. When dogs presenting with metastatic disease were excluded from analysis, those with tumors possessing a MI $\leq$5 had a median survival time of 80 months, compared to 3 months for those possessing a MI >5 suggesting that MI is a strong predictor of overall survival for dogs with MCTs. Other proliferation markers such as assessment of agryophilic nucleolar staining organizing regions (AgNORs) and PCNA have been used to try to determine biologic behavior of MCTs, although theses may not be as reliable[44,59,60]. Lastly, one study sought to establish a new grading scheme for MCTs using Kit immunohistochemical staining patterns as an indicator of tumor aggressiveness. While there was some evidence that dogs with certain cytoplasmic Kit staining patterns did live as long as those with other patterns, no group reached a median survival time and most dogs in each of the 3 groups were apparently cured of disease post surgery[61].

g. Kit mutations: As previously mentioned, mutations in Kit have been found in canine MCTs and research suggests they are associated with an increased risk of local recurrence, metastasis, and death of affected dogs[24,62].

6. Treatment

a. Surgery: Wide surgical excision is indicated for all canine MCTs. Historically, it has been recommended that the margins need to be at least 3 cm in each direction; deep margins are as important as the lateral margins. Recent studies demonstrated that all Grade 1 MCTs were completely excised with 1 cm of normal tissue around the MCT (lateral margins) and 1 fascial plane included in the excision (deep margin)[63,64]. Additionally, 75% and 68% of grade II MCT were completely excised with a 1 cm lateral margin and one fascial plane as the deep margin. Similarly, 100% and 89% of Grade 2 MCT were completely excised with a 2 cm lateral margin and one fascial plane for the deep margin. Neither of the studies evaluated Grade 3 MCTs. Because tumor grade is usually not known prior to surgery, it appears prudent to still recommend a 3 cm lateral margin and one fascial plane for the deep margin when feasible. All of the excised tissue should be submitted and margins should be labeled so the pathologist is able to specifically identify any areas of incomplete excision. However, even histologically clean margins do not guarantee that a tumor will not recur. In one study, 83% of dogs with Grade 1 MCT, 44% of dogs with Grade 2 MCT, and 6% of dogs with Grade 3 MCT were alive 1500 days after surgical excision6. In another study, 100% of dogs with Grade I, 44% of dogs with Grade 2, and 7% of dogs with Grade 3 were alive two years after surgical excision47. Lastly, a proportion of Grade 2 tumors that are incompletely excised will not recur post surgery. In a recent study, the estimated proportions of Grade 2 tumors that recurred locally at 1, 2, and 5 years were 17.3%, 22.1%, and 33.3% respectively65. Eleven (39.3%) dogs developed MCT at other cutaneous location and the median overall survival was 1426 days.

b. Radiation Therapy: Substantial data suggests that radiation therapy is effective at eliminating remaining microscopic disease following incomplete excision of Grade 1 and 2 MCT (greater than 90% three year control rate)[46,66]. Unfortunately, dogs with Grade 3 tumors do not fare as well; while the radiation may be effective at preventing local recurrence, many dogs develop metastasis. Radiation therapy has also been used to treat solid MCTs (macroscopic disease) when surgery was not an option. Varying degrees of success have been found; in one study, a 50% one-year control rate was obtained49. However, radiation therapy should not be utilized as the primary therapeutic modality if surgical intervention is an option. Palliative radiation has been used to treat dogs with non-resectable high grade MCTs, although systemic effects of degranulation following radiation may lead to vomiting, hypotension and death.

c. Chemotherapy: The use of adjuvant chemotherapy is indicated following excision of Grade 3 MCTs, metastatic MCTs, non-resectable high grade tumors, or for any other MCT with negative prognostic indices. While radiation therapy, is the treatment of choice for incompletely excised Grade 1 and 2 MCTs, evidence suggests that post-op chemotherapy may prevent local recurrence (unpublished) and therefore should be considered for patients who are not candidates for radiation, if such therapy is not available or if the owners cannot afford the cost therapy.

i. Corticosteroids: The reported response rate of canine MCT to prednisone is 20-70%, with [67,68]. Partial remissions are more common than complete remissions, and at least some of the observed response may be due to a decrease in tumor-associated edema. This decrease is likely due to stabilization of mast cell granules and a reduction in mast cell mediator production.

ii. CCNU (lomustine): In one study, 8/19 dogs (42%) with measurable MCTs had an objective response (1 CR, 7 PR) to single agent lomustine for a median duration of 77 days69. Preliminary unpublished data suggests that CCNU given in the adjuvant setting post surgery (either alone or with prednisone and vinblastine) can significantly prolong survival times of dogs with high grade tumors or tumors with negative prognostic indicators. CCNU can induce hematopoietic and hepatic toxicity including severe neutropenia, thrombocytopenia and liver failure. Patients receiving this drug should be monitored very closely and the CCNU should be discontinued if there is evidence of either toxicity. In general, CCNU is dosed at 50-70 mg/m2 orally every 3-4 weeks. A CBC and liver panel should be performed prior to each dose.

iii. Vinca Alkaloids: Single agent response rates of vincristine, vinblastine and vinorelbine are 7%, 12% and 13%, respectively, suggesting that vinca alkaloids are not effective as sole agents for the treatment of MCTs70-72. Vinblastine has been combined with prednisone in other studies, inducing objective responses ranging from 27-47%48,73. A combination of vinblastine, cyclophosphamide and prednisone resulted in a 64% (7/11) response rate in 1 study[74]. The dose of vinblastine is 2-3 mg/m2

given every 1-3 weeks. The major toxicity of this drug is neutropenia and occasional gastrointestinal upset is noted. This drug is a vesicant and thus must be given through intravenous injection. It is often used in an alternating manner with CCNU.

iv. Kit inhibitors: Orally bioavailable mall molecule inhibitors of Kit (SU11654 and AB1010) have recently been demonstrated to have activity against canine MCT and clinical trials with such inhibitors are ongoing[75]. Additionally, the commercially available Kit inhibitor Gleevec (imatinib mesylate) has been used to treat MCTs and unpublished data indicated that this drug has biologic activity in dogs with aggressive mast cell disease. However, Gleevec can cause idiosyncratic hepatotoxicity and is extremely expensive, limiting its use. Evidence suggests that such Kit inhibitors may be particularly useful for dogs with tumors exhibiting Kit mutations.

7. Supportive Care

a. H2 antagonists: As histamine stimulates gastric acid production by parietal cells, MCT may cause GI ulceration. To prevent this, any of the standard H2 antagonists may be utilized including cimetidine, ranitidine, or famotidine. Alternatively, proton pump inhibitors such as omeprazole may be utilized; these inhibitors are probably more useful in the setting of gross mast cell disease where standard H2 antagonists may be less effective.

b. H1 antagonists: Massive mast cell degranulation can lead to hypotensive shock and death. Therefore, all patients with gross mast cell disease should be placed on the H1 antagonist diphenhydramine.

Table 1. Proposed clinical staging for canine dermal mast cell tumors

Estadio	Tumor (es)	GL Regional	Metástasis
IA	1 tumor, confinado en piel, <3cm, bien circunscrito	Negativo	negativo
IB	>1 tumor, confinado en piel, <3cm, bien circunscrito, distancia entre lesiones >10cm	Negativo	Negativo
II	1 o más tumores cutáneos, bien de >3cm o poco circunscritos o con satélites	Negativo	Negativo
III	Cualquiera	Positivo	Negativo
IV	Cualquiera	Cualquiera	Positiva

Table 3. Schematic for treatment of canine mast cell tumors

Grade I-escisión completa:	no más tratamiento
Grade I –escisión incompleta:	escisión más amplia si no es posible la cirugía; puede pensarse en no más tratamientos
Grade II- escisión completa:	quimioterapia solo si hay factores de pronóstico negativo
Grade II-escisión incompleta:	escisión más amplia o radioterapia si no es posible la cirugía; puede pensarse en no más tratamientos si no hay factores de pronóstico negativo; quimioterapia si hay factores de pronóstico negativo
Grade III-escisión completa:	quimioterapia
Grade III-escisión incompleta:	quimioterapia+/- radioterapia

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