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Objective—To evaluate prognostic factors associated with outcome of dogs with multiple cutaneous mast cell tumors (MCTs) treated with surgery with or without adjuvant treatment.

Design—Retrospective case series.

Animals—54 dogs with a minimum of 2 simultaneous, histologically confirmed cutaneous MCTs that had been excised and had adequate staging and follow-up data.

Procedure—Medical records from 1998 to 2004 were examined. Outcome was assessed with the Kaplan-Meier product-limit method and log-rank analysis. Prognostic factors evaluated included signalment; number, histologic grade, location, size, local recurrence, and de novo development of MCTs; quality of surgical margins; clinical signs at the time of diagnosis; and use of adjuvant treatment.

Results—Medical records of 54 dogs with 153 tumors were included. Median follow-up time was 658 days. Median disease-free interval (1,917 days; range, 11 to 1,917 days) and median survival time (1,917 days; range, 14 to 1,917 days) were not yet reached. The 1-year and 2- to 5-year survival rates were 87% and 85%, respectively. The overall rate of metastasis was 15%. Factors that negatively influenced survival time in the univariate analysis included incomplete excision, local recurrence, size >3 cm, clinical signs at the time of diagnosis, and use of adjuvant treatment. Presence of clinical signs at the time of diagnosis was the only negative prognostic factor for disease-free interval detected in the multivariate analysis.

Conclusions and Clinical Relevance—Results suggested that multiple cutaneous MCTs in dogs are associated with a low rate of metastasis and a good prognosis for long-term survival with adequate excision of all MCTs. (J Am Vet Med Assoc 2006;228:91–95)

Mast cell tumors are the most common type of cutaneous tumor in dogs, representing 7% to 21% of all skin tumors in this species.1,2 The most common clinical finding is a solitary cutaneous MCT; however, approximately 11% to 14% of dogs have multiple cutaneous MCTs.3,4 Mast cell tumors have a biologically variable clinical course, ranging from benign to aggressive behavior and outcome. Many prognostic factors have been used to predict the biological behavior of MCTs. Variables such as histologic grade, anatomic location, growth rate, recurrence, and the presence of clinical signs are among the more commonly used prognostic factors.1,2-8

Clinical stage is also predictive of outcome.1,9 The current World Health Organization TNM classification of MCTs in dogs has multiple dermal tumors listed as stage III disease, suggesting a poor prognosis.10 Data supporting this current classification are lacking. There have been only a few references1-4 to multiple cutaneous MCTs in the veterinary literature, all of which suggest that there is no difference in outcome between patients with multiple MCTs and those with solitary MCTs.

We hypothesized that multiple cutaneous MCTs would have a benign clinical course, behaving more like discrete primary skin tumors rather than a form of metastasis or an advanced stage of disease. The purpose of the study reported here was to determine prognostic factors associated with outcome in dogs with multiple cutaneous MCTs treated with surgery with or without adjuvant treatment. The biological behavior of MCTs was also evaluated.

Criteria for Selection of Cases
Medical records from the CSU-VTH from January 1998 to December 2004 were retrospectively analyzed. Medical records of dogs with a histopathologic diagno-

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sis of a minimum of 2 anatomically distinct, simultaneous, cutaneous MCTs that were excised and that had adequate staging and follow-up information available were included in the study.

Procedures

Data collected from the medical records included age, weight, sex, and breed of dog; number, histologic grade, locations (truncal, appendicular, head, and neck), and size of MCTs; completeness of excision (complete or incomplete for each MCT excised); clinical signs at the time of diagnosis (cutaneous ulcers, signs of pain, swelling, or gastrointestinal tract signs consisting of vomiting, melena, or diarrhea); dates of development of new MCTs; local recurrence of MCTs; metastasis; and date and cause of death or outcome at the most recent follow-up examination. When available, information was also recorded on the results of staging procedures including CBC; serum biochemical analyses; results of cytologic examination of regional lymph nodes; radiography of the thorax and abdomen; ultrasonography of the abdomen; and results of cytologic examination of liver, spleen, or bone marrow aspirates. Extent of staging depended on location of tumors, clinician preference, and owner compliance for each case. Clinical stage at the time of definitive surgery was recorded as negative or positive for metastasis on the basis of results of diagnostic tests. Use of adjuvant treatment was recorded, including protocol used and response to treatment. Metastatic lesions were confirmed via cytologic or histologic examination. Follow-up information was obtained from the medical record or telephone interviews with the referring veterinarian or owner. Information on whether there was local recurrence of an MCT or development of a new MCT and what treatment was pursued was recorded. Patient status was recorded as alive without disease, alive with disease, dead attributable to disease, dead without evidence of disease, or lost to follow-up.

The DFI was defined as the time from original diagnosis to the development of a new or recurrent MCT or development of metastasis. The survival time was defined as the interval from diagnosis to death. In the study reported here, the time to development of a new MCT was defined as the interval between diagnosis and the appearance of a new, anatomically distinct cutaneous MCT. The time to local recurrence was the interval between surgery and evidence of local recurrence at a site from which an MCT had previously been excised. Adjuvant treatment was categorized as single-agent prednisone, chemotherapy (vinblastine or lomustine), radiation therapy (curative or palliative intent), or multimodal treatment (combination of therapies). The number of adjuvant treatments administered and response to treatment were also recorded. Histologic review of MCTs was performed when available. Forty-six cases were reevaluated by 1 pathologist (EJE) to standardize histologic grading among patients, according to the guidelines of Patnaik et al,3 on the basis of the amount of cellular differentiation, mitotic rate, and depth of invasion.

Statistical analysis—Survival time and DFI were assessed by use of the Kaplan-Meier product-limit method. Log-rank analysis3 and the Fisher exact test17 were used for analyzing prognostic variables. Patients were censored from the analysis if they were lost to follow-up, alive at the time of data analysis, or died of a disease that was not associated with an MCT or treatment. Variables analyzed in the univariate analysis as prognostic factors included patient age, weight, breed, and sex; number of MCTs at the initial evaluation; and histologic grades, locations, and sizes of MCTs. The quality of surgical margins, previous history of an MCT, presence of metastatic disease, local recurrence of MCTs, formation of new MCTs, clinical signs at diagnosis (gastrointestinal tract or cutaneous), and the use of adjuvant treatment were also analyzed. The variables determined to be significant (P < 0.05) in the univariate analysis were then evaluated by multivariate analysis.8 For all final analyses, values of P < 0.05 were considered significant.

Results

There were 656 individual cases of MCTs in dogs evaluated at the CSU-VTH from January 1998 to December 2004. Of those, 61 dogs had multicentric cutaneous MCTs, resulting in a prevalence rate of 9%. Fifty-four dogs with 153 MCTs were identified that fit the inclusion criteria. Median age of dogs was 9 years (range, 2 to 15 years). Median weight of dogs was 31.3 kg (69 lb; range, 7.2 to 54.5 kg [16 to 120 lb]). The most common breeds were mixed-breed dogs (n = 15), Labrador Retrievers (11), and Boxers (6). Sixteen other breeds were represented, and 11 dogs were brachycephalic. There was a predominance of spayed female dogs (n = 36) with a ratio of 3.5 females to every male. There were 6 sexually intact females, 11 castrated males, and 1 sexually intact male. The sex of all dogs evaluated for MCTs from January 1998 to December 2004 was evaluated; a female-to-male ratio of 1:4.1 was detected. The risk for females to develop multiple cutaneous MCTs, compared with solitary MCTs, was found to be significantly (P = 0.006) increased by use of the Fisher exact test.17 The median number of simultaneous MCTs at the time of the initial evaluation was 2 (range, 2 to 6). Mast cell tumors from 46 of 54 dogs were available for histologic review. Histologic diagnosis in the remaining 8 dogs was made by various pathologists. Grading differed from the original diagnosis in 8 dogs. All MCTs in 1 dog were grade 1, all MCTs in 33 dogs were grade 2, all MCTs in 5 dogs were grade 3, and MCTs in 15 dogs were a combination of tumor grades. Overall, 83% (n = 38) of dogs had either grade 1 or 2 MCTs. All MCTs in 29 dogs were removed with complete excision, all MCTs in 9 dogs were removed with incomplete excision, and MCTs in 16 dogs were removed with complete and incomplete excision. All MCTs in 76% (32/42) of dogs measured < 3 cm. All MCTs in 15 dogs were in a truncal location, all MCTs in 4 dogs were in an appendicular location, and all MCTs in 35 dogs were in a combination of locations, including the head and neck. Eleven dogs had clinical signs at the time of diagnosis. Of these, 2 dogs had gastrointestinal
tract signs (anorexia, vomiting, melena, or diarrhea) and 9 had clinical signs associated with the local MCT (erythema, signs of pain, swelling, or ulceration). Nineteen dogs had a previous history of having an anatomically distinct, solitary cutaneous MCT. A significant difference in DFI or survival time was not detected in dogs with a previous history of an MCT. Additionally, none of the dogs with multiple MCTs had any evidence of disease recurrence at the initial site at the time of initial evaluation.

Complete staging including CBC; serum biochemical analyses; cytologic examination of regional lymph node aspirates; radiography of the thorax; ultrasonography of the abdomen; and cytologic examination of liver, spleen, and bone marrow aspirates had been performed in 29 dogs. Variable staging including a minimum of a CBC, serum biochemical analyses, and cytologic examination of regional lymph node aspirates had been performed in the remaining dogs. The overall rate of metastasis was 19% (n = 8). Three dogs had a cytologic diagnosis of regional lymph node metastasis at the time of initial evaluation; an additional 5 dogs developed metastases during the course of their disease. Four of the 5 dogs developed metastasis to the regional lymph node, with only 1 dog having evidence of systemic dissemination. All cases of metastatic disease were confirmed cytologically. Surgery was the primary mode of treatment for all dogs, and 20 of 34 dogs received adjuvant treatment postoperatively. Five dogs were treated with prednisone only, 8 received vinblastine or lomustine chemotherapy (ranging from 2 to 16 cycles), 2 dogs received curative-intent radiation therapy, and 5 dogs received a combination of treatments (surgery, chemotherapy, and palliative or curative-intent radiation therapy). Curative-intent radiation therapy ranged from 14 to 16 fractions of 45 to 48 Gy; palliative radiation therapy ranged from 1 to 4 fractions of 6 to 8 Gy.

Forty-four percent (n = 24) of dogs developed de novo MCTs. The median time to development of a de novo MCT was 770 days (range, 11 to 1,917 days). Of the 24 dogs with de novo MCTs, 23 received additional treatment consisting of surgery only in 12 dogs, vinblastine- or lomustine-based chemotherapy in 5 dogs, and the remainder received a combination of modalities (surgery, chemotherapy, or radiation therapy). Histologic grade for de novo MCTs was only available for 10 dogs and did not differ from the original tumor grade in the small number of available cases. Eleven of 24 dogs that developed de novo MCTs had >1 de novo MCT develop (range, 2 to 6 new MCTs). Ten (19%) dogs had local recurrence of an MCT, which was confirmed cytologically or histologically. In 8 of these 10 dogs, the local recurrences occurred in MCTs that had been excised with incomplete surgical margins.

For all dogs, the median DFI was not yet reached at 1,917 days (range, 11 to 1,917 days; Figure 1). The median survival time was also not yet reached (range, 14 to 1,917 days). The 1-year survival rate was 87%, and the 2- to 5-year survival rate was 85% (Figure 2). At the date of the most recent follow-up examination, 27 dogs were alive, 3 of which had MCT disease. Twenty-three dogs were dead; death in 9 of those dogs was associated with MCT disease. Three dogs died of unknown causes, and the remainder died of causes that were not associated with MCT disease. Four dogs were lost to follow-up. Of the MCT disease–related deaths, 8 of 9 dogs had metastatic disease either at the time of diagnosis (n = 3) or the time of death (5). Additionally, all MCTs in 4 of those dogs were grade 3 tumors, MCTs in 6 dogs had been incompletely excised, 2 dogs had MCTs that measured > 3 cm, and 6 dogs had clinical signs at the time of diagnosis.

In the univariate analysis, the following variables had a significant negative influence on survival time: incomplete excision of surgical margins (P = 0.001), local recurrence of an MCT (P = 0.000), size > 3 cm (P = 0.01), clinical signs at the time of diagnosis (P = 0.003), and use of adjuvant treatment consisting of chemotherapy and multimodality therapy instituted after the first surgery (P = 0.000). Of those, incomplete excision of surgical margins, clinical signs at the time of diagnosis, and use of adjuvant treatments were also found to negatively influence DFI (Table 1). In the multivariate analysis, the only significant prognostic variable for DFI was clinical signs at the time of diagnosis (P = 0.023; Figure 3).
Discussion

The reported incidence of multiple cutaneous MCTs in the veterinary literature ranges from 11% to 14% to as high as 29%. In our study, an incidence of 9% was detected, suggesting an uncommon form of this disease. The median age and weight of dogs in our study were similar to the signalment reported for solitary MCTs. A sex predilection has not been reported for MCTs; however, our results indicated that spayed female dogs had an increased risk for development of multiple cutaneous MCTs. Overall, there was a predominance of grade 1 or 2 MCTs (83%) that measured < 3 cm (76%).

The median number of MCTs was 2, with 6 being the highest amount of simultaneous MCTs detected in 1 dog partly attributable to our surgically excisable inclusion criteria. Results of the study reported here likely represent the prognosis for this specific subset of dogs and may not be applicable to dogs with > 6 cutaneous MCTs.

Clinical signs associated with the gastrointestinal tract have been reported to be a negative prognostic factor for survival. In our study, 20% (n = 11) of dogs had clinical signs at the time of diagnosis. Clinical signs were defined as gastrointestinal tract signs or those associated with a local MCT such as ulceration, edema, or signs of pain. In the multivariate analysis, presence of clinical signs at the time of diagnosis was the only variable that negatively influenced DFI. The overall rate of metastasis was 15% (n = 8), which is comparable to that of intermediate-grade solitary MCTs. Seven dogs had evidence of metastatic disease in the regional lymph node; only 1 dog had cytologic evidence of systemic metastasis, suggesting that multiple cutaneous MCTs have a low rate of metastasis with metastasis developing most commonly in a regional lymph node.

Twenty-four (44%) dogs developed a de novo MCT in a different location during the course of their disease (median, 770 days). However, survival time was not affected by the formation of a new MCT, which is consistent with results of other studies. Determining whether the new MCTs truly arise de novo or whether each MCT is a form of metastasis has been evaluated. In that study, PCR assays were used to detect a clonal origin for multiple distant cutaneous MCTs in 2 dogs. Further investigation into this clinical dilemma is warranted.

Wide excision of all cutaneous MCTs was the primary mode of treatment for all dogs. This method of treatment provided a median DFI that was not yet reached at 1,917 days, and 1-year and 2- to 5-year survival rates of 87% and 85%, respectively. Because of the nonstandardized adjuvant treatment used and case selection bias, it was not possible to determine the effect of adjuvant treatment for the management of multiple MCTs.

The current TNM advanced staging classification for multiple MCTs in dogs may not be accurate on the basis of results of our study and others. Thamm et al retrospectively evaluated 18 dogs with multiple MCTs treated with chemotherapy and found no difference in their survival time, compared with dogs with solitary MCTs treated with chemotherapy. Results of another study indicate that the median survival time of dogs with multiple grade 2 MCTs treated with surgery and prednisone alone was 1,507 days. McCaw et al evaluated 10
dogs with multiple MCTs and found no difference in their response rate after treatment with prednisone, compared with dogs with solitary MCTs. Results of another study indicate that there was no difference in prognosis for dogs with multiple cutaneous MCTs detected at the time of diagnosis or that developed at a later date, compared with dogs with solitary cutaneous MCTs. Results of our retrospective study indicated that survival times in dogs with multiple simultaneous cutaneous MCTs treated with wide excision may be comparable to those in dogs with solitary intermediate-grade MCTs.

References

Selected abstract for JAVMA readers from the American Journal of Veterinary Research

Measurement of urinary glycosaminoglycans in dogs
David C. Grant et al

Objectives—To measure urine concentrations of sulfated glycosaminoglycans (GAG), determine optimal storage conditions for urine samples, establish a reference range, and determine whether there is correlation between 24-hour total urine GAG excretion and the GAG-to-creatinine ratio (GCR).

Animals—14 healthy adult dogs.

Procedure—Single urine sample GAG concentrations and GCRs were measured in samples collected from 14 healthy dogs at the start of the 24-hour collection period. Twenty-four–hour total urine GAG excretions were determined from urine collected during a 24-hour period in the same 14 dogs. Total sulfated GAG concentrations also were measured in urine from these dogs after the urine had been stored at 4°C and –20°C for 1, 7, and 30 days.

Results—Urine GAG concentrations were not significantly different from baseline values after urine was stored at 4°C for up to 1 day and –20°C for up to 30 days. Neither single urine sample GAG concentration [R2, 0.422] nor GCR [R2, 0.084] was an adequate predictor of 24-hour total urine GAG excretion.

Conclusions and Clinical Relevance—Results of this study provide data that can be used to establish a reference range for 24-hour total urine GAG excretion in dogs and adequate conditions for sample storage. Contrary to findings in humans, there was no significant linear correlation between 24-hour total urine GAG excretion and single urine sample GCR in dogs, limiting clinical use of the single urine sample test. (Am J Vet Res 2006;67:51–55)