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Evaluation of two-centimeter lateral surgical margin for excision of grade 2 cutaneous mast cell tumors in dogs

R P. Fulcher

L L. Ludwig

P J. Bergman

Shelley Newman
University of Tennessee-Knoxville

Evaluation of a two-centimeter lateral surgical margin for excision of grade I and grade II cutaneous mast cell tumors in dogs

Ryan P. Fulcher, DVM; Lori L. Ludwig, VMD, MS, DACVS; Philip J. Bergman, DVM, PhD, DACVIM;
Shelley J. Newman, DVM, DVSc, DACVP; Amelia M. Simpson, DVM, DACVS; Amiya K. Patnaik, DVM, MS†

Objective—To evaluate completeness of excision and clinical outcome in dogs with cutaneous mast cell tumors (MCTs) excised with a lateral margin of 2 cm and a deep margin of 1 fascial plane.

Design—Prospective study.

Animals—16 client-owned dogs with 1 or more cutaneous MCTs.

Procedure—Excision of MCTs was performed with a 2-cm lateral margin and a deep margin of 1 fascial plane. Histologic tumor grading was performed; surgical margins were categorized as complete or incomplete. Follow-up information was obtained via repeat examination of the dogs by veterinarians or client-completed questionnaires.

Results—4 grade I and 19 grade II cutaneous MCTs were evaluated. Overall, 21 (91%) MCTs were completely excised; 2 grade II tumors had foci of mast cells at the 2-cm margin. Two dogs received adjunctive treatments following surgery. Follow-up information was available for all dogs (median follow-up period, 379 days; range, 51 to 538 days); no local recurrence was detected during this time. De novo MCTs were detected in 3 of 16 dogs at 37, 54, and 154 days after surgery. Via Kaplan-Meier analysis, median survival time and disease-free interval were both > 538 days (medians not yet reached). No prognostic variables were identified.

Conclusions and Clinical Relevance—Excision with a 2-cm lateral margin and a deep margin of 1 fascial plane may result in satisfactory excision of grades I and II MCTs in dogs, with recurrence rates similar to those reported previously. Use of these margins may minimize complications associated with larger local tumor resection. (*J Am Vet Med Assoc* 2006;228:210–215)

Mast cell tumors are the most common cutaneous neoplasm in dogs, comprising 7% to 21% of all cutaneous tumors and 11% to 27% of all malignant cutaneous tumors in that species.[1-3] Most MCTs develop in older dogs (mean age, 8.5 years), and there is no reported sex predilection.[2] Most commonly, MCTs develop in mixed-breed dogs; however, Boxers, Boston Terriers, Labrador Retrievers, Beagles, and Schnauzers are reported to be at high risk.[1] At the time of diagnosis, most dogs with cutaneous MCTs have a single mass; however, multiple masses are detected in approximately 3% to 14% of cases[1,4] and even in as many as 43% of cases.[5] Cutaneous MCTs are most often located on the trunk of the body (48% to 58%) and on the limbs (25% to 47%) but can develop anywhere on the skin.[6-8]

As many as 50% of cutaneous MCTs are considered malignant on the basis of their biological behavior.[9] The metastatic rate of MCTs may be as high as 96% for undifferentiated tumors and < 10% for well-differentiated tumors.[1,10] Metastasis most commonly involves regional lymph nodes, the spleen, and the liver; less frequently, metastasis involves the lungs, kidneys, heart, and bone marrow.[10]

With regard to MCTs in dogs, many prognostic factors have been identified, including age,[4,11] breed,[1] sex,[8] stage[7,12] and location of tumor,[2,7,13] tumor size,[8,14] the interval between detection of tumor and surgery,[8] plasma histamine concentration,[15] presence of tumor-related systemic signs,[16] evidence of metastasis at diagnosis,[13] and recurrence;[9] however, histologic grade of the tumor appears to be the most important.[17] Of all MCTs in dogs, grade I tumors account for 34% to 38%, grade II tumors account for 40% to 52%, and grade III tumors account for 14% to 22%.[4,17,18] However, there may be discordance between histologic grades assigned by different pathologists in as many as 50% of MCTs.[5]

Surgical excision remains the treatment of choice for cutaneous MCTs. Because neoplastic cells often extend beyond the observable or palpable tumor border,[19,20] MCTs have been traditionally excised with a 3-cm margin of tissue around the tumor and a deep margin of 1 fascial plane.[1] After MCT excision, local recurrence occurs in 0% to 50% of dogs[2,8,20,21] and distant recurrence or de novo development occurs in 11% to 38% of dogs.[6,11,20,21] Complete excision does not prohibit recurrence,[17] and completeness of excision may not affect local or distant recurrence rates or outcomes.[11,22] A recent study[11] of grade II MCTs treated with surgery alone revealed no significant difference in local or distant recurrence rates between completely excised and incompletely excised tumors, but the number of dogs in that study was small.

Inclusion of a 3-cm lateral margin of unaffected tissue during excision of an MCT results in a large tissue

MCT	Mast cell tumor

defect and may not be necessary for complete excision of the tumor. In addition, the amount of tissue deep to the tumor that must be removed for a complete excision is not definitively known. Our group has previously investigated surgical margins required for excision of cutaneous MCTs in dogs; tumors were each excised with a 3-cm lateral margin, and the excised tissue was evaluated histologically at the 1-, 2- and 3-cm lateral margins.[20] None of the specimens in that study[20] had mast cells detectable at the 2- or 3-cm lateral margin, suggesting that a 2-cm lateral margin may be adequate for complete excision of grade I and grade II MCTs. Tumor excisions were performed with a deep margin of 1 fascial plane in that study,[20] and tumor cells were detected within 1 mm of the deep margin of 2 of 20 grade II MCTs; however, despite the location of tumor cells close to the deep margin, local recurrence did not occur in either of those 2 dogs. The purpose of the study reported here was to evaluate the completeness of excision and clinical outcome in dogs with MCTs that were excised with a lateral margin of 2 cm and a deep margin of 1 fascial plane.

Materials and Methods

Sixteen client-owned animals were included in the study, each with 1 or more cutaneous MCTs (diagnosed on the basis of findings of cytologic examination of fine-needle aspirate specimens of the mass or masses). For inclusion in the study, dogs had to have ≥ 1 MCT located in an area of the body amenable to tumor excision with a 2-cm lateral margin. In some dogs, not all of the multiple MCTs could be excised according to protocol; these dogs were included in the study, but only those MCTs that were excised according to the investigational protocol were included in margin analysis. Dogs that had undergone excision of an MCT at an anatomic location separate from that of the present tumor were included, unless they had received noncorticosteroid chemotherapy or radiation therapy at any time prior to excision of the present tumor. Dogs were excluded from the study if they had non-MCT malignancy concurrently or had had non-MCT malignancy previously; had received chemotherapy (not including corticosteroids) for treatment of MCT or other malignancy at any time; or at any time had surgical treatment or radiation therapy of an MCT at the site of the present tumor. Some dogs were excluded because tumor locations (eg, scrotum or distal portion of an extremity) dictated that surgical margins would be greater or less than those required by the investigational protocol. Informed consent was obtained from owners of all dogs.

All dogs were premedicated with a combination of methadone (0.2 to 0.4 mg/kg [0.09 to 0.18 mg/lb], IM) and either atropine sulfate (0.02 mg/kg [0.009 mg/lb], IM) or glycopyrrolate (0.01 mg/kg [0.005 mg/lb], IM). Some dogs also received acepromazine (0.01 to 0.03 mg/kg [0.005 to 0.014 mg/lb], IM). Prior to induction of anesthesia, 1 dog was administered diphenhydramine (2 mg/kg [0.9 mg/lb], IM) alone and 1 dog was administered diphenhydramine (2 mg/kg, IM) and cimetidine (5 mg/kg [2.3 mg/lb], IM). Agents for anesthetic induction included diazepam (0.5 mg/kg [0.23 mg/lb], IV) in combination with propofol (3 mg/kg [1.36 mg/lb], IV) or thiopental (7 mg/kg [3.18 mg/lb], IV). Anesthesia was maintained with isoflurane (0.5% to 3%) delivered via an endotracheal tube; 1 dog also received a fentanyl infusion (0.03 to 0.06 μg/kg [0.014 to 0.027 μg/lb], IV). Cephazolin (22 mg/kg [10 mg/lb], IV) was administered perioperatively. Postoperatively, dogs that remained hospitalized received methadone (0.2 to 0.4 mg/kg, IM) every 6 hours for the first 12 to 24 hours for pain management. After discharge, most dogs were administered butorphanol (0.15 to 0.32 mg/kg [0.068 to 0.145 mg/lb], PO)

every 6 to 12 hours as needed for pain management; a transdermal fentanyl patch (approx 2 μg/kg/h) was applied to each of 3 dogs prior to discharge. Two dogs received carprofen (2.2 mg/kg [1 mg/lb], PO, q 12 h) or deracoxib (3 mg/kg, PO, q 24 h) in addition to opioid analgesics; 1 other dog was not administered opioids or nonsteroidal anti-inflammatory drugs after discharge.

Each tumor site was prepared for surgery routinely. A sterile surgical marker and ruler were used to delineate the gross tumor margin and to mark the skin 1 and 2 cm from this edge at 0°, 90°, 180°, and 270° around the tumor. These marks at the 2-cm margin were then connected. The skin, subcutaneous tissue, and fascia were incised along this margin. The fascia was held in its relative position at the skin edge with tissue forceps. The tumor was excised beneath the fascial plane by use of sharp and blunt dissection techniques. Once the tumor was removed, an additional biopsy specimen (approx 0.5 cm in length, width, and depth) of the deep tissue margin was obtained (designated as the additional deep margin) to ensure the ability to histologically evaluate the deep margin if tumor cells abutted the fascia. New surgical gloves and instruments were used for closure of the surgical site. The surgical margins were marked with surgical clips, and the incisions were sutured closed. For each dog, all detectable MCTs were removed during a single anesthetic episode.

Each excised MCT was prepared by use of a previously described technique.[20] Briefly, the cut surfaces were inked with yellow dye[a] and were allowed to dry. The excised tumor was then placed on a piece of cardboard and held in place with several needles to maintain its original shape during fixation in neutral-buffered 10% formalin. The additional deep margin was fixed in neutral-buffered 10% formalin.

Once fixation was complete, the tumor was sectioned by use of modification of a previously described technique.[20] Briefly, a 1-cm-long, full-thickness section of tissue was taken at locations 0°, 45°, 90°, 135°, 180°, 225°, 270°, and 315° around the 2-cm margin. Four similarly sized sections were obtained from the 1-cm margin at the 0°, 90°, 180°, and 270° positions. Two full-thickness sections were obtained from the center of the tumor mass at right angles to each other to confirm the cytologic diagnosis and histologically assess the margin deep to the tumor. Lastly, 1 section of the additional deep margin was collected. All sections were stained with H&E, and Giemsa stains were used when needed. All tissue sections were evaluated histologically by 1 pathologist (SJN); tumors were graded by use of the Patnaik grading system.[17] All surgical margins were categorized as complete or incomplete, where incomplete resection was characterized as the presence of mast cells within 1 mm of the surgical margin.

For each dog, follow-up information regarding tumor recurrence or metastasis was obtained via repeat examination by a veterinarian or through client communication. End points for follow-up were local MCT recurrence, de novo MCT development, metastasis, and death. Local recurrence was defined as development of an MCT at or within 2 cm of the original surgical site. De novo development was defined as development of a cutaneous MCT at an anatomic location > 2 cm from the original tumor. Metastasis was defined as evidence of MCT in a noncutaneous tissue. Local recurrence, de novo development, and metastasis were confirmed via cytologic or histologic examination of tissue specimens, and any mass reported by the owner that was not cytologically or histologically examined was categorized as an MCT for purposes of this study. The disease-free interval was defined as the time from tumor excision to identification of local recurrence, de novo development, or metastasis. Survival time was defined as the time from tumor excision to death due to any cause.

For each of the 16 dogs, data recorded and evaluated included signalment; prior history of MCT, including treatment dates and histologic grade (if known); staging of the present tumor (if performed) and time of staging (either preoper-

atively or postoperatively); number of MCTs present; presence of MCTs that were excised but not included in the study; tumor size in 3 dimensions; tumor diameter (defined as the largest dimension through the tumor); largest tumor diameter for each dog (for dogs with > 1 MCT); tumor size score (the sum of tumor measurements in the 3 dimensions); largest tumor size score for each dog (for dogs with > 1 MCT); location of each tumor (defined as forelimb [distal to the scapulo-humeral joint], hind limb [distal to the hip joint], trunk, or head and neck); tumor location group for each dog (defined as forelimb only, hind limb only, trunk only, or multiple locations); surgery date; histologic grade of the tumor or tumors; highest histologic grade of tumor on each dog (for dogs with > 1 MCT); detection of mast cells at the 1-cm, 2-cm, deep, and additional deep margins; postoperative adjunctive treatments; local MCT recurrence; de novo MCT development; metastasis; disease-free interval; death; cause of death; and survival time.

Kaplan-Meier survival time and Kaplan-Meier disease-free interval were calculated. For survival analysis, dogs were censored as of the date they were last known to be alive or the date they died as a result of other disease. For disease-free interval analysis, dogs with no local MCT recurrence or de novo MCT development were censored as of the date they were last known to be disease-free.

Breed, sex, sexual intactness, MCT location group, highest histologic grade, and completeness of excision at the 2-cm lateral margin were evaluated as potential prognostic variables for the Kaplan-Meier disease-free interval via log-rank testing. Cox proportional hazards analysis was used to evaluate age, sex, weight, number of MCTs identified at the initial evaluation, number of MCTs included in the study, the largest tumor size score, the largest tumor diameter, and tumor location group as potential prognostic variables for the disease-free interval. Statistical analyses were performed with a χ^2 analysis (categorical vs categorical), Kendall rank correlation (continuous vs continuous), Kruskal-Wallis test (categorical vs continuous), and Mann-Whitney U test (categorical vs continuous) by use of a computer software package.[b] For all statistical tests, a value of $P < 0.05$ was considered significant.

Results

Sixteen dogs with 23 cutaneous MCTs met the criteria for inclusion in this study. The median age was 7 years (mean, 6.9 years; range, 3 to 11 years). Nine dogs were female (6 spayed and 3 sexually intact), and 7 dogs were male (6 castrated and 1 sexually intact). Overall, 9 breeds were represented: Pug (n = 4), American Pit Bull Terrier (3), and mixed breed (3) were most common, and 6 other breeds had a single representative each. The mean body weight was 25.1 kg (55.22 lb; median, 22.7 kg [49.94 lb]; range, 5.9 to 62.3 kg [12.98 to 137.06 lb]).

One dog had a prior history of MCT. Previously, this dog had 2 MCT excisions, each at different anatomic locations; each of those tumors was a grade II tumor, and each was completely excised. At the time of inclusion of this dog in the study, there was no evidence of local recurrence at either excision site.

Ten of the 16 dogs had 1 cutaneous MCT at the initial evaluation. Six dogs had multiple MCTs: 4 dogs each had 2 MCTs, 1 dog had 3 MCTs, and 1 dog had 4 MCTs. Fourteen of the 16 dogs had all of their MCTs included in the study. Two dogs each had 1 additional MCT that was excised but not included in the study. Of these, 1 dog had 2 MCTs, of which 1 (a completely excised grade I tumor) was excluded from the study because the tissue was not processed according to the investigational protocol. The other dog had 4 MCTs, 1

of which (an incompletely excised grade II tumor) was adjacent to another raised cutaneous mass that was diagnosed cytologically as an MCT but was diagnosed via histologic evaluation as a focal acute hematoma; these 2 masses were excised together with a single elliptical incision that did not follow the study protocol.

Staging procedures varied among dogs. Preoperative staging procedures were performed in 9 of the 16 dogs and included thoracic radiography (n = 6); abdominal ultrasonography (6); cytologic evaluation of a bone marrow aspirate (4); abdominal radiography (4); assessment of a buffy coat smear (3); and cytologic evaluation of fine-needle aspiration specimens of the regional lymph node (2), liver (1), and spleen (1). Four of 16 dogs (including 1 dog with preoperative staging) had staging procedures performed postoperatively, including abdominal ultrasonography (n = 2), cytologic evaluation of a bone marrow aspirate (1), thoracic radiography (1), assessment of a buffy coat smear (1), and cytologic evaluation of fine-needle aspirates of the liver (1) and spleen (1). Mastocytosis or metastasis was not evident in any dog in which staging procedures had been performed, regardless of whether assessments were made pre- or postoperatively.

The 23 MCTs were located on the trunk of the body (n = 10 [44%]), hind limb (7 [30%]), forelimb (3 [13%]), and head and neck (3 [13%]). Of the MCTs on the extremities, all were proximal to the tarsus on the hind limb or proximal to the elbow joint on the forelimb. Considering every tumor on a given dog, 6 dogs had MCTs on the trunk of the body only, 5 had MCTs on the hind limb only, and 5 had MCTs in multiple location categories; no dog had MCTs exclusively on the forelimb. Mean tumor diameter was 1.3 cm (median, 1.1 cm; range, 0.4 to 3.1 cm). The mean largest tumor diameter for each dog was 1.5 cm (median, 1.15 cm; range, 0.4 to 3.1 cm). The mean tumor size score was 3.3 cm (median, 2.8 cm; range, 1.0 to 8.8 cm). The mean largest tumor size score for each dog was 3.8 cm (median, 3.25 cm; range, 1.0 to 8.8 cm).

Four of the 23 (17%) MCTs were grade I tumors, and 19 (83%) were grade II tumors. There were no grade III tumors. Overall, 21 of the 23 (91%) MCTs were completely excised. All grade I tumors were completely excised at the 1- and 2-cm margin. For 13 of the 19 grade II MCTs, no mast cells were detected at the 1-cm margin; for 17 of those 19 tumors, no mast cells were detected at the 2-cm margin. All tumors were completely excised at the deep margin, and in all instances, the additional deep margin was devoid of mast cells.

In 2 grade II tumors from 2 dogs, excisions were deemed incomplete because of the presence of mast cells at the 2-cm margin. There was a perivascular infiltrate comprised of eosinophils and occasional well-granulated mast cells and lymphocytes in 1 of the eight 2-cm marginal sections and 2 of the four 1-cm marginal sections obtained from 1 of those 2 tumors; however, there was no apparent connection between these foci and the primary mass. The dog from which this tumor had been excised had no additional treatments performed, and there was no evidence of local MCT recurrence or de novo MCT development during a follow-up period of 375 days. The second incomplete MCT excision had a small focus of

well-granulated mast cells and eosinophils in the sub-cutis in 1 of the eight 2-cm marginal sections and all of the 1-cm marginal sections. The dog from which this tumor had been excised had a second surgery performed 2 weeks after the excision to remove a 2-cm margin around the initial surgical site. There was no evidence of gross disease in this dog at the time of the second surgery, and histologic evaluation of the excised tissue revealed no evidence of MCTs. This dog also had no evidence of local recurrence or de novo MCT development during a follow-up period of 433 days.

One dog received adjunctive chemotherapy (consisting of administration of prednisone, vinblastine, and cyclophosphamide) after surgery. The dog had an incomplete resection of a grade II MCT that was not included in the study because it was adjacent to another raised cutaneous mass (diagnosed cytologically as an MCT but histologically as an acute focal hematoma) and could not be excised according to the investigational protocol (the masses were excised together with a single elliptical incision). Within a follow-up period of 224 days, there was no evidence of local recurrence or de novo MCT development in this dog.

The median follow-up interval for all dogs was 379 days (range, 51 to 538 days). None of the dogs had local recurrence of an MCT. De novo MCTs developed in 3 of the 16 dogs (a mixed-breed dog, a Viszla, and an American Pit Bull Terrier); these tumors were detected after disease-free intervals of 37, 54, and 154 days. All de novo tumors developed in dogs with complete excisions of grade II MCTs, and all de novo tumors were histologically classified as grade II. At the initial study evaluation, 2 of these dogs had multiple MCTs; 1 of these dogs had a history of 2 MCT excisions (each a grade II tumor) at 5 months and 4 years prior to inclusion in our study. Treatment for these de novo tumors included complete surgical excision in 2 dogs and cytoreductive surgery combined with full-course radiation therapy in another dog.

The Kaplan-Meier median disease-free interval was > 538 days (median not yet reached); 13 of 16 dogs were estimated to remain disease-free at 538 days. Dogs were either disease-free at the time of last follow-up or had de novo MCT development. No prognostic variables for the disease-free interval were identified. Kaplan-Meier medial survival time was > 538 days; all dogs were alive at the end of the study period.

Discussion

The median age (7 years) and sex distribution (approx 1.3 females to 1 male) of the dogs in the present study were similar to groups used in prior studies.[4,6,11,14,20] The American Pit Bull Terrier appears to be overrepresented in our study; however, the proportion of this breed in the population of dogs evaluated at The Animal Medical Center is unknown and may indeed be higher than other previously reported populations.

Of the 16 dogs, 6 (38%) had more than 1 cutaneous MCT. This proportion is considerably higher than the 3% to 14% described in most reports[1,4] and higher than findings in 2 previous studies[17,20] from our institution (ie, among dogs with MCTs, 0% and 10% had multiple MCTs at initial evaluation, respectively). However, in another study[5] of dogs with MCTs, 6 of 14 (43%) dogs had multiple MCTs. It is unknown whether dogs with multiple MCTs are at higher risk for local recurrence or de novo MCT development; however, no variables were identified as prognostic for a disease-free interval in our study.

Tumors were located on the trunk, extremities, head, and neck. Undoubtedly, the selection criteria used in the present study affected the distribution of tumor locations because tumors in certain anatomic locations (eg, the scrotum or distal portions of the extremities) were excluded. Given that the tumor location may be prognostic for the biologic behavior of MCTs, there may be an effect on outcome as a result of the exclusion of certain sites, such as distal portions of the extremities and the muzzle.[7,13]

The mean tumor diameter (1.3 cm) among tumors in the present study was smaller than that determined in other studies,[4,6,21] in which the mean value was approximately 2.0 cm. The mean tumor size score was 3.3 cm, which was also smaller than a previously reported value.[20] This difference may be a consequence of the small sample size of this study and our prior study. Perhaps earlier detection of MCTs by owners (who are generally becoming increasingly aware of their pets' overall health) may have contributed to the apparent trend in the literature toward smaller tumor size at diagnosis. Whether the smaller tumor size among the dogs in our study influenced the clinical outcomes is unknown. However, tumor diameter and tumor size score were neither related to completeness of excision nor prognostic for disease-free interval.

Overall, 21 of the 23 (91%) MCTs in the dogs of the present study were completely excised. This proportion compares well with the finding of another study,[6] in which 54 of 60 (90%) MCT excisions were complete, 3 (5%) were complete but close (ie, neoplastic cells were detected within 1 mm of the margin), 1 (2%) was incomplete (ie, neoplastic cells were detected at the margin), and the classification of 2 (3%) was unknown following surgery. However, in that study,[6] 21 of the 60 (35%) tumors had incomplete excisions prior to definitive surgical treatment, indicating that the rate of complete excision following initial surgical treatment may be as low as 65%. In our prior investigation of surgical margins associated with MCTs,[20] all 23 tumors were excised completely at the 3-cm lateral margin. However, the excisions of 2 (9%) tumors were considered complete but close on their deep margin (ie, neoplastic cells present within 1 mm of the surgical margin). A report[23] that included descriptions of the completeness of surgical margins of 214 cutaneous MCTs in dogs that were treated with excisional surgery indicated that only 42% had complete margins, 19% had narrow margins (ie, neoplastic cells within 5 mm of the margin), and 39% had incomplete margins (ie, neoplastic cells at the margin). In this light, complete excision of 91% of the MCTs in the present study seems acceptable.

Two of the 23 (9%) excisions were categorized as incomplete because there were foci of mast cells at the 2-cm margin. Previous investigations[20,24,25] in dogs revealed clumps of mast cells within grossly normal marginal tissues surrounding cutaneous MCTs. In the present study, the relationship between the mast cells at a marginal section and the resected tumor is unknown. Although these

cells were assumed to be neoplastic for the purposes of our analyses, their true nature is unknown, as standard light microscopy alone cannot determine the neoplastic potential of such cells. Other techniques, such as immunohistochemistry,[4,12,18,26] morphometry,[5] and biochemistry,[25] may be used to elucidate the nature of these cells or may help identify neoplastic cells at the excisional margin that could have otherwise gone unnoticed.[27]

Of the 2 dogs with an incomplete MCT excision in our study, 1 underwent a second excisional procedure, in which a 2-cm margin of tissue was excised around the scar from the initial surgery. Evidence of gross or microscopic disease was not detected in the resected tissue, as has been reported on other occasions.[23] This finding brings into question whether the initial surgery was truly an incomplete resection and further supports the idea that the foci of mast cells detected histologically may not have been associated with the primary tumor. In 433 days and 375 days, respectively, since the surgery, there had been no local recurrence of MCT in this dog or in the other dog with an incomplete tumor excision that received no additional treatment.

The preparation of tissue specimens, sectioning methods, and margin examinations used in our study presumably differed from routine preparation and analysis performed in most commercial laboratories. Although the present study involved extensive measures to maintain the relationship of the tissues from skin to fascia, demarcate the surgical margin with tissue dye, and exhaustively evaluate the surgical margins, such practices are not standard, even at our institution. Because different tissue fixation and sectioning techniques could alter margin assessment, careful consideration should be applied to the interpretation of margin results obtained from standard sample processing.

The additional deep margin was evaluated in our study because of our previous experience[20] involving detection of a complete but close, deep margin associated with excision of an MCT. However, in the present study, no MCTs extended to the deep fascial layer, so evaluation of this additional deep margin appears to have been unnecessary. Although it seems unlikely that tumor cells would cross a fascial plane, findings of the present study cannot confirm this claim.

De novo development of an MCT was detected in 3 of the 16 dogs at 37, 54, and 154 days after surgery. This finding compares well with those of earlier studies[6,11] in which, following tumor excision alone, development of an MCT in a different cutaneous location occurs in 11% to 38% of dogs. The de novo development of MCTs in the dogs of the present study occurred earlier than it did in dogs of our other study[20] (mean interval after surgery to detection of de novo MCTs, 386 days) and earlier than it did in dogs of studies[6,21] by other investigators (in which the interval after surgery to detection of a de novo MCT at a different body location ranged from 240 days to 18.5 months). It is unknown why de novo development of MCTs was detected earlier in the present study, and a lack of standardized staging procedures among these studies makes it difficult to comment on potential differences between the study populations.

The absence of grade III MCTs in the dogs of our study makes application of the results to all types of MCTs in dogs impossible. Whether grade III tumors could have met the inclusion criteria for our study is unknown, as many develop in anatomic locations that are not amenable to wide excision[1,2,7,13]; also, the extent of invasion of grade III MCTs into adjacent tissues is largely unknown, making concise definition of wide surgical margins impossible at this time. Further studies are needed to determine how far neoplastic cells extend from the gross tumor margin in grade III MCTs; presumably, margin recommendations for grade III MCTs may be different than those for the other tumor grades. If surgical recommendations vary in accordance with histologic grade, preoperative grade assessment may become necessary, either through histologic means or perhaps through cytologic or immunocytologic methods.[28]

Terminology describing outcome analysis of MCT excision is not standardized in the veterinary literature. There is some consensus regarding the use of the term local recurrence. However, descriptions of tumors that develop subsequently at sites other than the original location vary and include terms such as de novo development, distant recurrence, and metastasis.[6,11,21] Similarly, the disease-free interval has been used in several ways in the literature.[6,20,21] These differences highlight the difficulty regarding assessment of prognosis, determination of etiopathogenesis, and prediction of development of MCTs in dogs. Further standardization of the terminology used to describe clinical outcomes and study of the relationship between 1 MCT and another in a given dog will be necessary to decrease these difficulties. Perhaps immunocytologic and genetic evaluations of MCTs[29] will provide data with which to elucidate the relationships among MCTs in a dog with multiple lesions and between a resected MCT and its recurrence.

In dogs of the present study, excision of cutaneous MCTs with a 2-cm lateral margin and deep margin of 1 fascial plane resulted in complete excision in 91% of grades I and II MCTs. Although mast cells detected at the surgical margins in the incomplete MCT excisions were assumed to be part of the neoplastic process, it is unknown whether those cells reflect a true neoplastic process or focus. In the dogs of our study, excision of tumors with margins set by our investigational protocol resulted in a local recurrence rate and de novo development rate that compared well with prior reports. Although the case number was small, our data suggest that excision of MCTs at these investigational margins results in similar clinical outcomes in dogs with wider excisions. However, extrapolation of these results to grade III MCTs and MCTs on the muzzle, scrotum, or distal portions of the extremities in dogs is difficult. Further investigation into the surgical margins required for complete excision of grade III MCTs is warranted.

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Correction: In "Evaluation of five commercially available assays and measurement of serum total protein concentration via refractometry for the diagnosis of failure of passive transfer of immunity in foals," published November 15, 2005 (*J Am Vet Med Assoc* 2005;227:1640–1645), the comparisons in Table 6 were incorrect. The table with the corrected comparisons is reprinted below.

Table 6—Summary statistics (mg/dL) of the differences between the reference method* and a handheld quantitative colorimetric immunoassay[h] (assay H) or the difference between 2 radial immunodiffusion[a,b] assays (assays A and B) at serum IgG concentrations < 400 mg/dL, 400 to 800 mg/dL, and > 800 mg/dL.

Comparisons	IgG (mg/dL)	Mean bias (± SD)	Limits of agreements
Reference method – assay H	< 400	46.7 ± 97[a]	–143 to 237
	400–800	256 ± 233[b]	–199 to 712
	> 800	385 ± 378[b]	–354 to 1,126
Assay B – assay A	< 400	72.7 ± 91.2[a]	–101 to 251
	400–800	–136.7 ± 239[b]	–605 to 332
	> 800	–61.5 ± 748[b]	–1,528 to 1,405

For each sample, the average of IgG concentrations obtained from the 2 radial immunodiffusion assays[a,b] (assays A and B) was used as the reference method to which the other assay results were compared.

[a,b]Within a comparison, mean bias values with different superscript letters are significantly ($P \leq 0.01$) different.