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

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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—Grade I and 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 resection. (J Am Vet Med Assoc 2006;228:210–215)

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)
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 study20 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. This had not been reported previously. 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 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 [0.9 mg/lb], 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 thiopentol (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). Cefazolin (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 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.

One fixation in neutral-buffered 10% formalin. Once the 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°, 90°, 180°, and 270° 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 (S.N.).

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 required by the investigational protocol. 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 tumor 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-
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 aspiration rates 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 8 2-cm marginal sections and 2 of the 4 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 subcutis 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. At the initial study evaluation, 2 of these dogs had multiple MCTs; 1 of these dogs had a history of 2 MCT excisions, 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 median 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. 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 and higher than findings in 2 previous studies from our institution (ie, among dogs with MCTs, 0% and 10% had multiple MCTs at initial evaluation, respectively). However, in another study 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.

The mean tumor diameter (1.3 cm) among tumors in the present study was smaller than that determined in other studies, 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. 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, 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, 21 of the 60 (33%) 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, 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 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 3 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 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, 4,12,16 morphometry, 7 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. 21 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 41 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 46 (mean interval after surgery to detection of de novo MCTs, 386 days) and earlier than it did in dogs of studies 12,41 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. 12,7,11,12,13,14 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, margins 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 cyto- or immunocytologic methods. 20

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. 5,11,12 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 20 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.

References


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 immunodiffusion assays (assays A and B) at serum IgG concentrations < 400 mg/dL, 400 to 800 mg/dL, and > 800 mg/dL.

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>IgG (mg/dL)</th>
<th>Mean bias (± SD)</th>
<th>Limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference method – assay H</td>
<td>&lt; 400</td>
<td>46.7 ± 97b</td>
<td>–143 to 237</td>
</tr>
<tr>
<td>400–800</td>
<td>258 ± 233b</td>
<td>–199 to 712</td>
<td></td>
</tr>
<tr>
<td>&gt; 800</td>
<td>385 ± 376b</td>
<td>–354 to 1,126</td>
<td></td>
</tr>
<tr>
<td>Assay B – assay A</td>
<td>&lt; 400</td>
<td>72.7 ± 91.2b</td>
<td>–101 to 251</td>
</tr>
<tr>
<td>400–800</td>
<td>–136.7 ± 239b</td>
<td>–605 to 332</td>
<td></td>
</tr>
<tr>
<td>&gt; 800</td>
<td>–615 ± 748b</td>
<td>–1,526 to 1,405</td>
<td></td>
</tr>
</tbody>
</table>

For each sample, the average of IgG concentrations obtained from the 2 radial immunodiffusion assays (assays A and B) was used as the reference method to which the other assay results were compared. Within a comparison, mean bias values with different superscript letters are significantly (P < 0.01) different.