Surgically planned versus histologically measured lateral tumor margins for resection of cutaneous and subcutaneous mast cell tumors in dogs: 46 cases (2010–2013)

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Objective—To compare preplanned lateral surgical margins and measured lateral histologic margins for cutaneous and subcutaneous mast cell tumor (MCT) resections in dogs.

Design—Retrospective case series.

Sample—51 biopsy specimens from dogs (n = 46) with MCTs.

Procedures—All canine patients that underwent curative-intent surgical resection of cutaneous or subcutaneous MCTs from January 1, 2010, through June 30, 2013, with complete medical records including signalment, body condition score (BCS), surgery report (with measured surgical margins), and histopathology report were included. The surgically measured tumor margins in each quadrant were grouped and compared with the corresponding histologic margins. Specimens from dogs with truncal MCTs and a BCS of 7 to 9 on a scale from 1 to 9 (ie, high) were compared with those of dogs with a BCS of 4 to 6 to evaluate effect of BCS on tissue margins.

Results—51 specimens were included. Surgically mapped lateral margins differed significantly from histologically reported margins in all 4 quadrants. The mean histologic margins were 35% to 42% smaller than the surgical margins for the combined 51 specimens. A higher BCS did not significantly influence the magnitude of the decrease in lateral margins measured histologically. No significant difference was found for the magnitude of the differences between any of the 4 lateral margins.

Conclusions and Clinical Relevance—Results of this study suggested that surgical and histologic margins may differ significantly for canine cutaneous and subcutaneous MCTs. This may be a result of tissue shrinkage following excision and fixation, extension of the MCT beyond palpable margins, or both. Histologic measurements may significantly underestimate the tumor-free margins in dogs with cutaneous and subcutaneous MCTs. (J Am Vet Med Assoc 2015;247:184–189)

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ABBREVIATIONS

| BCS | Body condition score |
| MCT | Mast cell tumor |

One of the primary goals of the surgical treatment of patients with cancer is to obtain tumor-free margins. Surgical margins of 2 to 3 cm have been proposed for wide resection of subcutaneous and cutaneous MCTs in small animal patients, with these margins determined on the basis of gross appearance and palpation, although 1 recent report proposed proportional margins. Most clinical studies report the use of 1- to 2-mm, histologically determined tumor-free margins as the definition of clean margins for MCT resection, although 1 study required ≥ 5 mm to meet this criterion. In a recent poll of veterinary surgical oncologists, 4 mm appears to be the most commonly used cutoff value for histologically clean margins when assessing the results of histopathologic examination of MCTs excised with the goal of obtaining wide margins.

Studies in human patients with tumors include reports of significant shrinkage of skin tissue samples, with tumor-containing skin shrinking by a mean of 11% and tumor-free margins shrinking by 19% in 1 recent study evaluating excision of basal cell carcinomas in 42 patients. An older study found that most of this skin tissue shrinkage phenomenon appears to take place prior to fixation in formalin. The mean tissue specimen surface area reported in that study was 237 mm² for the planned surgical excision, 184 mm² for the excised tissue, and 163 mm² for the final fixed tissue specimen. A different study found that a substantial portion of the shrinkage occurs during the fixation of the specimens. Interestingly, those authors reported a vast difference in the amount of shrinkage between the mean tumor margins (10.28 to 6.78 mm or 34%) and the tumor diameter (41.74 to 39.88 mm, or 4.5%) when freshly excised and fixed samples were compared. Whereas tissue shrinkage has been most often described for lateral margins after excision of...
skin specimens and for superficial tumors that include a skin margin, it has been observed in other tissues as well (in human patients for samples of oral mucosa,13 tongue mucosa,14 and the liver15 as well as for porcine intestinal specimens as reported in a recent experimental study16). A 2005 study17 evaluating shrinkage of skin samples in dogs reported a similar finding of tissue shrinkage from the initial surgical planning versus the skin samples in dogs reported a similar finding of tissue shrinkage from the initial surgical planning versus the skin samples in dogs.

The objective of the study reported here was to evaluate and compare the difference between preplanned lateral surgical margins and measured lateral histologic margins for cutaneous and subcutaneous MCTs in dogs. We hypothesized that histologic margins would be significantly smaller than preplanned surgical margins and that patients with a higher BCS would have less shrinkage of the margins.

Materials and Methods

Patient records for all dogs with well-defined, freely moveable, subcutaneous or cutaneous MCTs undergoing curative-intent surgery at the Veterinary Teaching Hospital, North Carolina State University, from January 1, 2010, until July 1, 2013, were reviewed. The only patients included were those with a complete, detailed surgery report with the measured and obtained size of the 4 lateral margins and description of the deep margin and a full histopathologic report that included description and measurements of all 4 lateral margins and the deep margin. Patients that did not undergo surgery by or under the direct supervision of a board-certified surgeon were excluded.

Patient signalment, body weight, and BCS were recorded. The histologic deep margins, the tumor location (cutaneous vs subcutaneous), and the tumor grade defined by means of the Patnaik scale were recorded.16 All specimens were trimmed following a similar protocol for skin masses, according to the recommendations of Kamstock et al.19 Planned surgical margins were measured in millimeters with a sterile flexible ruler in 4 to 8 locations around the palpable margin of the MCT, and the measured points were connected to create either a circle or an ellipse drawn on the patient’s skin with a sterile marker. The skin was incised and the resection completed along this drawn line. Following excision of the MCT and surrounding tissues, deep margins were inked, and lateral margins were either inked or marked with suture to orient the pathologist. The descriptions of dorsal, cranial, ventral, and caudal were used for truncal tumors, whereas the descriptions proximal, distal, medial (cranial), and lateral (caudal) were used for appendicular specimens. For analysis purposes, all margins with similar directionality were grouped together (ie, proximal and dorsal, ventral and distal, cranial and medial, and caudal and lateral).

After formalin fixation, the sample area, if not yet inked, was painted with surgical ink on all surgical margins and air dried for 2 to 3 minutes; excess ink was removed by blotting with paper towel, and samples were trimmed to capture the central deep margin and 4 peripheral margins at 90° positions (dorsal, ventral, cranial, and caudal). Margins were measured in millimeters with a clear transparent ruler, recording the distance from the margin of the neoplasm, as identified by light microscopy, to the inked margin of the section. The reported surgical and histologic margins were compared.

Statistical analysis—A 2-tailed t test for paired samples was performed to evaluate for statistically significant differences between margins by pairwise directional differences (eg, ventral vs caudal). Pairwise difference in specimen shrinkage for the 4 lateral margins of truncal MCTs was compared by means of a nonparametric Wilcoxon rank sum test. Because of skewness in the measurement data in the high-BCS category, a nonparametric Wilcoxon test was performed on the high-BCS patient subgroup to evaluate the null hypothesis that a high BCS would not influence the amount of shrinkage (shrinkage amount was the difference in millimeters). Significance was set at P < 0.05 for all analyses. Statistical analyses were performed with commercial software.

Results

Patient data—Fifty-one specimens from 46 canine patients met the study selection criteria. All patients had a discrete palpable cutaneous or subcutaneous MCT. All MCTs were diagnosed prior to surgery on the basis of results of cytologic examination of fine-needle aspirates. All surgeries were de novo wide excisions without prior biopsies, with diagnosis made on the basis of results of cytologic examination of prior fine-needle aspirate samples. Forty-one MCTs were located on either the trunk or the appendicular lateral skin proximal to the elbow or stifle joint, 6 were distal to the elbow or stifle joint, 2 were on the ear base, and 1 each was on the nose and the lip. Identified patients had a median body weight of 26.5 kg (58.3 lb; range, 1.3 to 68.6 kg [2.9 to 150.9 lb]; mean ± SD, 27.3 ± 11.6 kg [60.1 ± 25.5 lb]). The median BCS was 6 (range, 3 to 8; mean ± SD, 5.8 ± 1.2), and median age at initial examination was 8.0 years (range, 2 to 15 years; mean ± SD, 8.2 ± 2.7 years). Forty-eight specimens were obtained by means of circumferential (circular) excision, and 3 truncal specimens were obtained in elliptical fashion (long axis dorsoventrally oriented in 2 patients and craniocaudally oriented in 1).

Thirty-three dogs were classified as having a normal BCS (4 to 6 on a scale from 1 to 9), and 11 were classified as having a high BCS (7 to 9). One dog had a BCS of 3, and 6 dogs had no BCS recorded at the time of surgery. Truncal MCTs were excised from 26 dogs with a normal BCS and from 6 dogs with a high BCS. Fourteen MCTs were subcutaneous and 37 were cutaneous. Of the cutaneous MCTs, 3 were classified as grade I, 31 were classified as grade II, and 1 was classified as grade III.

Tumor margins—Mapped lateral surgical margins for all tumors in all sites in all directions ranged from 10 to 35 mm for all 4 quadrants (mean ± SD, 21.7 ± 6.9 mm; median, 20 mm). The mapped surgical lateral margins divided by direction were a mean of 21.9 ± 7.2 mm (dorsal: median, 20; range, 10 to 35 mm), 21.9 ± 7.2 mm (ventral: median, 20; range, 10 to 35 mm), 21.4 ± 6.9 mm (cranial: median, 20; range, 10 to 35 mm), and 21.6 ± 6.7 mm (caudal: median, 20; range, 10 to 35 mm) for the 4 individual quadrants for all specimens combined. The reported histologic margins for all specimens ranged from 1 to 30 mm (dorsal: mean, 12.6 ±
6.6 mm; median, 12 mm), 1 to 35 mm (ventral: mean, 12.4 ± 6.9 mm; median, 12.3 ± 6.9 mm; median, 10.5 mm), and 1 to 32 mm (mean, 13.3 ± 6.9 mm; median, 13 mm).

The mean ± SD of all the surgically planned margins in all the specimens (n = 51) was 21.4 ± 6.9 mm to 21.9 ± 7.2 mm, whereas the mean ± SD of the histologic margins was from 12.3 ± 6.9 mm to 13.3 ± 6.9 mm (Table 1). The mean histologic margins were 58% to 65% smaller than the original surgical margins, representing a decrease in the margin of 35% to 42%. The dorsal, cranial, ventral, and caudal margins for all 51 specimens combined differed significantly (P < 0.001) between pre-excised and fixed specimens.

No significant difference was found between any of the lateral margins, for all specimens combined, for all truncal specimens, and for all truncal specimens for the lateral margins, for all specimens combined, for any possible combination. No significant difference was found in any of the lateral margins between dogs with a normal BCS and dogs with a higher BCS (Table 2).

The size difference in the dorsal and cranial margins was significantly (P < 0.05) different for the 6 appendicular MCTs versus the 41 truncal MCTs, but the other 2 margins did not significantly differ (Table 3).

The histologically measured deep margins for all 51 specimens ranged from 0 to 22 mm, with a mean ± SD of 7.3 ± 6.2 mm (median, 5 mm; range, 0 to 22). The deep margin of truncal specimens in dogs with a normal BCS had a mean ± SD thickness of 7.7 ± 6.7 mm (median, 7; range, 0 to 20 mm); the deep margin in dogs with a high BCS was 8.8 ± 4.1 mm (median, 10 mm; range, 1 to 15 mm). Appendicular specimens had a mean ± SD deep margin of 2.1 ± 2.1 mm (median, 1 mm; range, 0 to 5 mm).

**Discussion**

In the present study of canine patients that underwent curative-intent surgical resection of cutaneous or subcutaneous MCTs from 2010 through 2013, median histologically reported margins were 38% to 43% smaller, compared with planned and obtained surgical margins. This could translate to a 20-mm in vivo surgical margin being reported as a 12-mm margin histologically, suggesting that histologically reported tumor...
free margins may underestimate the surgically obtained margins for excision of cutaneous and subcutaneous MCTs in dogs by up to 40%. Whereas anecdotal reports previously supported this phenomenon, no objective study has previously documented the extent of this effect in veterinary small animal patients.

In the present study, the closest lateral margins per specimen were grouped and assessed because we felt this was the main margin parameter that will dictate clinically whether a revision is advised. We evaluated all 4 lateral margins individually as well, to identify any potential directionality in the contractile behavior of skin and skin-composite samples. In 1966, Irwin20 reported that skin tension lines in dog skin influence the directionality of the donor site defect, but no data exist to investigate this for excised samples. Results of the present study did not suggest a directionality in the magnitude of the lateral margin size difference for any of the lateral margins.

A previous experimental study17 evaluating samples of normal tissue from dogs found significant shrinkage of specimens as a result of sample handling and processing. However, the amount of shrinkage between surgically obtained and histologically measured margins has not been reported in veterinary medicine. Similar to our findings, a previous study12 of human patients found that the mean closest free margin of specimens decreased 34% after formalin fixation. Tissue shrinkage has not been reported in veterinary medicine.

Margins were also measured only in areas of the specimen in which the tissue architecture remained intact and not from areas where processing fragmented the tissue (eg, as occurred in some adipose-rich areas). Margins were also measured only in areas of the specimens where the surgical margin marking ink could be visualized by microscopy. Because some of the specimens were assessed prior to publication of the 2-tier grading system by Kiupel et al,21 only the Patnaik et al18 grade is reported.

In this study, a high versus normal BCS of the patient did not significantly influence the magnitude of the difference of the lateral margins in truncal MCT resections. The single patient with a low BCS and truncal MCT was not statistically compared. Nontruncal MCTs were not compared for influence of BCS owing to less accumulation of subcutaneous fat in the distal limb region. This finding is contrary to our hypothesis that different tissues and tissues with a different lipid content contract less and therefore shrink less, as previously reported by others.10,11,22,23 However, we suggest that this result may be attributed to a type II statistical error because there were only 6 patients in the high-BCS group in the present study.

In this study, the margin difference for appendicular versus truncal specimens differed significantly for 2 of the 4 margins, which was an interesting finding. Previous studies in human patients have suggested that there may be a difference in tissue contractility for truncal versus appendicular skin13 as well as for truncal versus head and neck skin.24 Nonetheless, we suggest that additional investigation with use of a standardized specimen collection protocol is necessary to further evaluate this phenomenon in dogs.

In this study, the deep margin was reported historically but was not specifically measured or noted in the surgery report either during planning or after excision. Only the tissue type of the deep margin (eg, 1 fascial plane) was recorded. Therefore, we did not perform any statistical analyses to assess the change in deep margin depth after specimen excision and fixation.

In 1 prior study4 evaluating adequacy of surgical margins and clinical outcome in dogs with cutaneous MCTs, the development of metastasis was reported. There was no local recurrence in patients with cutaneous MCTs excised with a lateral histologic margin < 10 mm and a deep margin < 4 mm (1 grade II and 3 grade III MCTs); however, the surgically planned lateral margins were not reported.8 In another retrospective study9 evaluating recurrence, the authors stated that complete excision (1- to 2-mm tumor-free margin) of grade II cutaneous MCTs in 31 dogs resulted in no local recurrence in 89% of patients. Another group10 evaluated subcutaneous MCTs in dogs with a similar definition for complete excision (tumor-free margin > 1 mm) and reported earlier local tumor recurrence for incompletely excised tumors. All subcutaneous tumors were unencapsulated and were grouped according to tumor growth pattern (circumscribed, infiltrative, or combined).8 Local tumor recurrences for patients with infiltrative subcutaneous MCTs and incomplete surgical margins had a predicted time to recurrence of 70 days, compared with 1,000 days for infiltrative MCTs resected with complete margins, which was significantly different.10 Contrary to those reports, Michels et al11 found no significant difference in recurrence rates for tumor-free versus non–tumor-free margins in 31 dogs with cutaneous MCTs; however, group sizes were small (20 dogs with tumor-free vs 11 with non–tumor-free margins). A larger study9 of 214 surgically excised pathology specimens from canine cutaneous MCTs reported 90 cases with complete margins (> 3-mm tumor-free lateral margins) and only 5 cases of local recurrence for that subgroup. The 340 MCTs were classified as well differentiated (n = 87), intermediately differentiated (163), and poorly differentiated (51); however, any relationship between tumor type and adequacy of margins was not reported.

A recent study6 evaluating the use of modified proportional margins for surgical resection found
that for 40 dogs with 47 cutaneous MCTs, 40 tumors were completely excised, and 7 had incomplete margins. Twenty-one MCTs were classified as grade I, 18 MCTs were classified as grade II, and 2 MCTs were classified as grade III. In that study, 1 dog with a grade III cutaneous MCT and clear lateral (≥ 1-mm margin free of neoplastic cells) margins had recurrence. Of 7 incompletely excised tumors, 3 patients received no further treatment, and the authors reported no evidence of local recurrence at 120, 300, and 330 days. This could suggest that tumor grade may be a more important variable affecting the risk of local tumor recurrence than the completeness of surgical excision. However, it is currently common practice to perform a scar revision in any incompletely excised MCT if sufficient tissue remains laterally and in the deep plane for clean wide margins to be obtained.

In patients with MCTs, planning the size of the margins on the basis of the tumor grade would be ideal. However, in a clinical setting, currently the diagnosis of an MCT in dogs is often made only following cytologic examination of fine-needle aspirates, with no incisional or excisional biopsies performed prior to definitive surgery. Knowledge of the magnitude of the difference between preoperative surgically planned and postfixation lateral margins could allow more accurate surgical planning based on the desired tumor-free margins (ie, extrapolating from a 4- to 3-mm fixed margin toward the surgical planned margin). This may affect the surgical decision, particularly for patients with tumors located in regions where less mobile skin can affect the ability to obtain a wider lateral margin in surgery.

It is not known whether presurgical planned margins truly reflect tumor-free skin. In the report of a study evaluating tumor-free margins in 42 human patients with basal cell carcinoma (44 tumors), the authors proposed making marker incisions with a scalpel blade circumferentially at the visible tumor margins prior to resection to allow comparison with histologic margins. The visible tumor margin was accurately identified (within 1 mm) in 106 of 139 histologically assessed margins. The tumor margin was overestimated by 1 mm in 47 margins and underestimated in 6 margins. This additional marking technique may have value to further identify the true tumor margins in dogs with cutaneous MCTs, and we are currently enrolling patients in a prospective study to evaluate this.

On the basis of results of the present study, histologic measurement may significantly underestimate the tumor-free margins in dogs undergoing surgical resection of cutaneous and subcutaneous MCTs. Further study is indicated to evaluate the relationship between the change in surgical margins with routine histologic processing and patient outcome.

References

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From this month’s AJVR

Effects of oxymorphone hydrochloride or hydromorphone hydrochloride on minimal alveolar concentration of desflurane in sheep

Rebecca S. Sayre et al

Objective—To establish the minimum alveolar concentration (MAC) of desflurane and evaluate the effects of 2 opioids on MAC in sheep.

Animals—8 adult nulliparous mixed-breed sheep.

Procedures—A randomized crossover design was used. Each sheep was evaluated individually on 2 occasions (to allow assessment of the effects of each of 2 opioids), separated by a minimum of 10 days. On each occasion, sheep were anesthetized with desflurane in 100% oxygen, MAC of desflurane was determined, oxymorphone (0.05 mg/kg) or hydromorphone (0.10 mg/kg) was administered IV, and MAC was redetermined. Physiologic variables and arterial blood gas and electrolyte concentrations were measured at baseline (before MAC determination, with end-tidal desflurane concentration maintained at 10%) and each time MAC was determined. Timing of various stages of anesthesia was recorded for both occasions.

Results—Mean ± SEM MAC of desflurane was 8.6 ± 0.2%. Oxymorphone or hydromorphone administration resulted in significantly lower MAC (7.6 ± 0.4% and 7.9 ± 0.2%, respectively). Cardiac output at MAC determination for desflurane alone and for desflurane with opioid administration was higher than that at baseline. No difference was identified among hematologic values at any point. Effects of oxymorphone and hydromorphone on durations of various stages of anesthesia did not differ significantly.

Conclusions and Clinical Relevance—MAC of desflurane in nulliparous adult sheep was established. Intravenous administration of oxymorphone or hydromorphone led to a decrease in MAC, however, the clinical importance of that decrease was minor relative to the effect in other species. (Am J Vet Res 2015;76:583–590)