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, tongue mucosa, and the liver as well as for porcine intestinal specimens as reported in a recent experimental study). A 2005 study 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 defined by means of the Patnaik scale were recorded.

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.

All specimens were trimmed following a similar protocol for skin masses, according to the recommendations of Kamstock et al. 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 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 cranioventrally 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 ± 6.9 mm; ventral: mean, 12.7 ± 6.9 mm; cranial: mean, 12.6 ± 6.9 mm; caudal: mean, 12.6 ± 6.9 mm).

A 2-tailed Student’s t 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.
SMALL ANIMALS

Dogs with a normal BCS (all truncal specimens, and for all truncal specimens for each lateral margin, for all specimens combined, for dogs with a normal BCS between pre-excised and fixed specimens. No significant difference was found in any 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 ± 4.11 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

<table>
<thead>
<tr>
<th>Margin</th>
<th>Surgical margin (mm)</th>
<th>n</th>
<th>Histologic margin (mm)</th>
<th>n</th>
<th>Difference (mm)</th>
<th>n</th>
<th>Decrease (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal</td>
<td>21.9 ± 7.2 (20.0 [20–30])</td>
<td>51</td>
<td>12.6 ± 6.6 (12.0 [7–19])</td>
<td>50</td>
<td>8.5 (5–13)</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Ventral</td>
<td>21.9 ± 7.2 (20.0 [20–30])</td>
<td>51</td>
<td>12.4 ± 7.2 (12.0 [7–16])</td>
<td>51</td>
<td>9.0 (5–12)</td>
<td>51</td>
<td>40</td>
</tr>
<tr>
<td>Cranial</td>
<td>21.4 ± 6.9 (20.0 [20–30])</td>
<td>51</td>
<td>12.3 ± 6.9 (10.5 [7–16])</td>
<td>50</td>
<td>8.5 (5–13)</td>
<td>50</td>
<td>42</td>
</tr>
<tr>
<td>Caudal</td>
<td>21.6 ± 6.7 (20.0 [20–30])</td>
<td>51</td>
<td>13.3 ± 8.9 (13.0 [9–17])</td>
<td>50</td>
<td>7.0 (5–12)</td>
<td>50</td>
<td>36</td>
</tr>
</tbody>
</table>

Values are mean ± SD (median [range]).

*Percentage decrease in mean histologic margin versus surgically measured margin; a 2-tailed t-test for paired samples was performed to evaluate pairwise directional differences. P value = significantly (P < 0.001) different from value for surgical margin.

n = Sample size.

<table>
<thead>
<tr>
<th>Margin</th>
<th>Difference (mm)</th>
<th>Shrinkage (%)</th>
<th>BCS 4–6</th>
<th>Shrinkage (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal</td>
<td>9.5 ± 7.3 (8.0 [5–10])</td>
<td>41</td>
<td>11.7 ± 6.9 (10 [5–19])</td>
<td>43</td>
<td>0.28</td>
</tr>
<tr>
<td>Ventral</td>
<td>8.8 ± 4.3 (8.0 [5–10])</td>
<td>41</td>
<td>11.9 ± 7.0 (10 [5–19])</td>
<td>47</td>
<td>0.15</td>
</tr>
<tr>
<td>Cranial</td>
<td>5.6 ± 8.0 (7.0 [5–11.5])</td>
<td>40</td>
<td>12.6 ± 8.6 (12 [8–19])</td>
<td>44</td>
<td>0.09</td>
</tr>
<tr>
<td>Caudal</td>
<td>7.7 ± 6.4 (7.0 [5–10.5])</td>
<td>37</td>
<td>13 ± 8.2 (12 [5–21])</td>
<td>49</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Values are mean ± SD (median [range]). P values listed are for raw differences, not percentages. A non-parametric Wilcoxon test was performed to test for significant influence of BCS on margin difference.

*Scale of 1 to 9.

<table>
<thead>
<tr>
<th>Margin</th>
<th>Difference (mm)</th>
<th>Shrinkage (%)</th>
<th>Truncal</th>
<th>Shrinkage (%)</th>
<th>Appendicular</th>
<th>Shrinkage (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal</td>
<td>10.8 ± 7.2 (10.0 [5–13])</td>
<td>42</td>
<td>4.3 ± 2.3 (4.0 [4–6])</td>
<td>32</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventral</td>
<td>10.4 ± 6.1 (10.0 [5–12])</td>
<td>44</td>
<td>8.5 ± 6.1 (5.0 [3–8])</td>
<td>64</td>
<td>0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cranial</td>
<td>9.6 ± 6.6 (10.0 [5–15])</td>
<td>41</td>
<td>6.8 ± 3.3 (7.0 [3–5])</td>
<td>51</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudal</td>
<td>9.1 ± 7.5 (7.5 [5–12.5])</td>
<td>39</td>
<td>14.2 ± 4.9 (3.0 [9–9])</td>
<td>50</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD (median [range]). Pairwise difference in specimen shrinkage for the 4 lateral margins of truncal versus appendicular MCTs was evaluated by means of a nonparametric Wilcoxon rank sum test.
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, Irwin 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 study 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 study of human patients found that the mean closest free margin of specimens decreased 34% after formalin fixation. Tissue shrinkage is not unique to skin. Various amounts of tissue shrinkage ranging from 19% to 57% depending on the fixative technique used, buccal mucosa (21.2%), and tongue (23.9%).

One limitation of the present study was that the data were collected retrospectively. However, the only patients included were those with a complete record including a detailed surgery report prepared by the assisting surgeon, read and approved by the primary surgeon performing the surgery, as well as a complete histopathology report with all 4 lateral margins reported. Additionally, all specimens were processed according to the same standard laboratory protocol, and all measurements were with a clear transparent ruler, graded in millimeters, recording the distance from the palpable outer margin of the neoplasm as confirmed by means of light microscopy, with the inked margin of the specimen. Margins data were collected only from areas of specimens 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 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, only the Patnaik et al grade is reported.

In this study, the deep margin size difference 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. 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 skin as well as for truncal versus head and neck skin. 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, 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 study 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. In another retrospective study 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 group 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). 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. Contrary to those reports, Michels et al 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 study 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), intermediate differentiated (163), and poorly differentiated (51); however, any relationship between tumor type and adequacy of margins was not reported.

A recent study 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 1, 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

23. Dauendorffer JN, Bastuji-Garin S, Guéro S, et al. Shrinkage of...

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 re-determined. 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)

See the midmonth issues of JAVMA for the expanded table of contents for the AJVR or log on to avmajournals.avma.org for access to all the abstracts.