Changes in the dimension and volume of feline injection-site sarcomas following formalin fixation as determined by use of the ellipsoid volume formula and three-dimensional computed tomography software

Jesse L. Terry DVM
Milan Milovancev DVM
Christiane V. Löhr DVM, PhD
Sarah Nemanic DVM, PhD

OBJECTIVE
To evaluate changes in the dimension and volume of feline injection-site sarcomas (FISSs) before (in vivo) and after surgical excision and formalin fixation (ex vivo) as determined by measurements obtained from 2-D and 3-D CT images.

SAMPLE
10 excised FISSs.

PROCEDURES
The maximum length, width, and depth of each FISS were measured on contrast-enhanced 2-D CT images of the tumor obtained in vivo and ex vivo. Those measurements were used to estimate tumor volume with the standard ellipsoid formula. Tumor volume was also calculated from 3-D CT images with software that used a volume-rendering algorithm. Student paired t tests were used for comparisons between the in vivo and ex vivo assessments.

RESULTS
Small decreases were detected in maximum tumor length, width, and depth between the in vivo and ex vivo assessments; however, tumor length was the only dimension that decreased significantly between the 2 assessments. Median tumor volume decreased significantly between the in vivo and ex vivo assessments regardless of the method used to estimate it. Tumor volume estimated by the ellipsoid formula was significantly lower than that estimated by the 3-D CT software at both assessments.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that shrinkage of FISSs following excision and formalin fixation was small and may be less than that of grossly normal tissue. Tumor volume estimated by the ellipsoid formula was consistently less than that estimated by 3-D CT software and should not be used when accuracy of tumor volume is of particular concern and advanced CT imaging is available. (Am J Vet Res 2016;77:620–626)
tumor tissue. Adherence to these recommendations has resulted in longer median survival times and lower recurrence rates, compared with more conservative surgical excision of FISSs.

It is of utmost importance that surgeons understand all factors that can affect histologic examination of tumor margins. Intuitively, those factors may include grossly normal surgical margin shrinkage following excision, tumor shrinkage, microscopic tumor infiltration beyond palpable diseased tissue, and additional tissue changes following formalin fixation and processing such as alcohol dehydration of sections obtained from paraffin-embedded tissue blocks. Unfortunately, the number of reports that describe tissue shrinkage following surgical excision in the veterinary literature are few and have involved only grossly normal tissue. In a study that involved grossly normal skin specimens from cats, significant tissue shrinkage was detected immediately after excision, with no additional shrinkage noted after the tissue underwent formalin fixation. Although the authors of that study did not report the mean percentage of tissue shrinkage, data provided in the tables of that report indicate that the tissue shrinkage was < 10% at all points measured. For grossly normal canine specimens, tissue shrinkage ranges from 15% to 32%. On the basis of unpublished data obtained by our laboratory group, the grossly normal-appearing lateral margins (as determined by the unenhanced eyesight of the surgeon and palpation) of excised FISSs shrink by a mean of 29.2% immediately after excision but do not undergo any significant additional shrinkage during formalin fixation. Neoplastic tissue differs from the tissue (primarily extracellular matrix components and muscle) that typically comprises the surgical margins of excised tumors; therefore, it is likely that ex vivo shrinkage of tissue that contains neoplastic cells will differ from that of tissue that contains healthy tissue. In a study of basal cell carcinoma tissue specimens extirpated from human patients, tissue shrinkage was nonuniform among specimens and was disproportionate high along the tumor-free margins; tumor-bearing skin shrank by a mean of 11%, whereas the adjacent tumor-free skin contracted by a mean of 19%. This discrepancy suggests that tumor-free margin estimates may be underestimated, if those margins were determined with the assumption that tissue contraction is uniform, and could have substantial implications on prognosis and postsurgical treatment recommendations for radiation therapy and chemotherapy. To our knowledge, data regarding changes in tumor size and volume immediately after excision and following formalin fixation are not currently available for any type of veterinary neoplasm.

Tumor volume has prognostic value and is an important component of staging for various tumors of dogs and cats. The importance of tumor volume for staging FISSs has been postulated but not definitively determined. For example, in 1 study, FISS volume was not predictive of whether tumor-free margins were attained during surgical excision. In another study, the tumor volumes of FISSs were determined but no attempt was made to correlate that volume with clinical outcome. The FISS tumor volume in both of those studies was estimated with the volume formula for an ellipsoid, in which volume is estimated on the basis of gross tumor measurements in 3 dimensions (length, width, and depth) as determined by the use of calipers or measurements obtained from preoperative CT or MRI images. Although this approach disregards projections or cavitations of the tumor, it is widely used to estimate tissue volume.

Three-dimensional volume-rendering software that allows the image of a tumor to be displayed and measured in 3 planes on multidetector CT images is becoming increasingly available. This technology allows clinicians to more accurately assess tumor size and location prior to surgery than ever before. Tumor volume measurements as determined by this 3-D CT software have been validated and are highly accurate. In 1 study, volume-rendered 3-D CT reconstructions of canine stifle joints following intra-articular injection of a 10% iopamidol (370 mg of I/mL) and saline (0.9% NaCl) solution were made, and the resultant joint volume estimated from those reconstructions was highly correlated ($R^2 = 0.90, P < 0.005$) with the original volume of fluid injected. To our knowledge, tumor volumes estimated by the traditional ellipsoid volume formula have not been compared with those determined by measurements obtained with 3-D CT software for any neoplasm of veterinary interest.

A detailed understanding of the 3-D shape of a tumor and how it may change after formalin fixation is important for planning surgical excision and accurate and detailed communication with pathologists regarding margins of concern. The purpose of the study reported here was to evaluate the extent of change in the dimension and volume of FISSs following wide surgical excision as determined by the use of the standard ellipsoid volume formula calculations derived from 2-D CT images, compared with that determined by use of validated 3-D volume-rendering CT software. Our hypotheses were that there would be small and insignificant decreases in tumor dimensions following formalin fixation, compared with preoperative dimension measurements, and that the estimated tumor volumes as determined by the ellipsoid formula would differ from those calculated by the 3-D CT software.

### Materials and Methods

#### Animals

Client-owned cats admitted to the Oregon State University Veterinary Teaching Hospital between March 2013 and March 2015 for wide surgical resection of a cytologically or histologically confirmed FISS were considered for study enrollment. Cats were excluded from the study if they had received preoperative neoadjuvant therapy or had undergone prior tumor excision. The cats were concurrently enrolled...
in another study, in which the association between histologic diagnosis and imaging-identified peritumoral FISS lesions was evaluated. That study was approved by the Oregon State University Institutional Animal Care and Use Committee, and owner consent was obtained in writing for each cat prior to study enrollment. Because the study reported here simply involved measurement of the tumors excised as part of that other study, a review of the study procedures by the institutional animal care and use committee was not necessary.

**CT**

While each cat was anesthetized for surgical excision of the tumor, a dual-phase CT angiogram was performed with a 64-detector helical CT scanner before and after administration of nonionic iodinated contrast medium (1 mL/kg, IV) with a power injector. A bolus of saline solution was not administered following injection of the contrast medium, and the acquisition timing was set to obtain postcontrast images in both the arterial and venous phases. Helical images were acquired as a volume with the following settings: voxels, 0.5 mm; reconstruction interval, 0.5 mm; rotation speed, 0.5 seconds; matrix, 512 × 512; kVp, 120; tube current, 150 to 350 mA; and pitch, 0.83. The volume data were reconstructed in bone and soft tissue algorithms and in isovolumetric transverse, sagittal, and dorsal image planes with a 2-mm slice thickness. Images were initially presented and measured with a window width of 120 and window level of 40 for soft tissue and a window width of 2,700 and window level of 350 for bone, but observers were allowed to adjust those settings. After completion of tumor excision, which ranged from 3 to 5 hours following the initial CT angiogram, specimens were placed in neutral-buffered 10% formalin for 48 to 96 hours as deemed necessary by a board-certified veterinary anatomic pathologist (CVL) to ensure complete fixation, which was dependent on specimen size and thickness. Following fixation, each specimen underwent CT imaging again with the following settings: voxels, 0.5; reconstruction interval, 0.5 mm; rotation speed, 0.5 seconds; matrix, 512 × 512; kVp, 100 to 120; tube current, 50 to 250 mA; and pitch, 0.83.

**Tumor measurements on 2-D CT images**

All CT images were stored in Digital Imaging and Communications in Medicine format. For each tumor, the length, width, and depth were measured on the preoperative postcontrast venous phase (in vivo) CT images and on the postfixation (ex vivo) CT images with the linear measurement tool of commercially available software. All measurements were acquired by the same observer (JLT) and represented the maximum dimension of the tumor in a given plane in millimeters. Width was defined as the dimension of the tumor in a plane parallel to the cat’s vertebral column, and length was defined as the dimension of the tumor perpendicular to its width. Depth was defined as the dimension of the tumor in a plane from superficial to deep. Tumor attenuation (in HUs) was measured for the venous phase of all in vivo CT angiograms and on the ex vivo CT images with the software region of interest tool. For each tumor, attenuation was measured for each of 3 separate, nonoverlapping 0.3-cm² regions that were confined to within the visible tumor boundaries. The mean was calculated for those measurements and used for analysis.

**Tumor measurements on 3-D CT images**

For each tumor, 3-D reconstructions were made from the in vivo preoperative postcontrast venous CT images as well as the ex vivo CT images by use of commercially available software. The window width and window level were adjusted as described until the tumor was easily discernable from the surrounding tissues. This created an attenuation difference that allowed 3-D trimming of all nontumor tissue from the reconstructions. The software volume-rendering tool was used to measure the volume of the tumor on the final reconstructed image.

**Statistical analysis**

To account for the large range of tumor sizes in the study, ex vivo measurements (length, width, depth, ellipsoid volume, and 3-D CT volume) were normalized by expressing each as a percentage of its corresponding in vivo measurement. The mean for each normalized ex vivo measurement was calculated to provide the overall percentage of tumor shrinkage in each dimension or volume. For each tumor, the ellipsoid volume was calculated as (length × width × depth) / (π/6).

A Student paired t test was used to compare normalized ex vivo measurements with the corresponding in vivo measurements. All analyses were performed with commercially available statistical software, and values of P ≤ 0.05 were considered significant. Unless otherwise indicated, data were presented as the mean ± SEM with the corresponding 95% confidence intervals when appropriate.

**Results**

Ten cats met the criteria for study enrollment. Four cats underwent wide excision of the musculature of the lateral thigh region. Three cats underwent hind limb amputation via hip joint disarticulation. Two cats underwent wide excision of the epaxial muscles in the dorsal neck region, and the remaining cat underwent a forequarter amputation (ie, removal of the forelimb and scapula).

**Tumor dimensions and volume**

The reference range for clinically normal, noncontrast-enhanced soft tissue attenuation is 40 to 60 HUs. The mean ± SEM tumor attenuation was 74.1 ± 7.7 HUs for the postcontrast in vivo CT images.
and did not differ significantly \((P = 0.61)\) from that for the ex vivo CT images \((70.2 \pm 3.8 \text{ HUs})\), which indicated that FISSs had persistent contrast enhance-

Discussion

Results of the present study indicated that the length, width, and depth of the FISSs evaluated shrank by a mean of 4.8%, 2.2%, and 6.1%, respectively, following fixation in neutral-buffered 10% formalin. However, length was the only one of those measurements that decreased significantly between the in vivo and ex vivo assessments. Conversely, tumor volume decreased significantly between the in vivo and ex vivo assessments regardless of whether it was calculated with the ellipsoid formula or by 3-D CT software. Also, the median tumor volume determined by the 3-D CT software was significantly greater than that determined by the ellipsoid formula. Collectively, those findings supported our hypotheses that the dimensions of the FISSs would undergo significant shrinkage during formalin fixation, and that the tumor volume estimated by the ellipsoid formula would differ significantly from that calculated by the 3-D CT software.

Tissue shrinkage following excision has been well described in human medicine\(^{20,27}\) and documented in the veterinary medicine.\(^{16–19}\) In 1 study,\(^{18}\) the mean lateral dimensional shrinkage ranged from 21.1% to 32.0% for resected canine skin specimens, and the ex vivo dimensional changes were dependent on tissue

**Figure 1**—Representative 2-D contrast-enhanced CT images of an FISS obtained in the transverse plane in vivo (A) and ex vivo (B) following excision and fixation in neutral-buffered 10% formalin. The maximum tumor length \((L)\) and depth \((D)\) are indicated in both panels; maximum tumor width was measured in the sagittal plane (not shown).

**Figure 2**—Representative 3-D contrast-enhanced CT images of an FISS in vivo (A) and ex vivo (B) following excision and fixation in neutral-buffered 10% formalin. Use of 3-D CT computer software enabled reconstruction of the tumor following removal of all non–contrast-enhanced tissue (B) so that the volume of the tumor could be calculated. Notice the multiple irregularities and projections of the tumor that were readily apparent in the reconstructed ex vivo image in panel B.
compartmentalization of normal tissues during ex vivo surgical margins. In our study, the shrunken volume of FISSs was determined by use of the ellipsoid volume formula and the 3-D CT software calculated tumor volume by use of a volume-rendering algorithm instead of a surface-rendering algorithm. We chose to use the volume-rendering algorithm because results of another study suggest that it is accurate for estimation of tissue volume. The disparity between the tumor volumes estimated by the ellipsoid formula and the 3-D CT software was likely associated with the 3-D complexity and irregularity of the FISSs, which could not be accounted for in the ellipsoid formula. The ellipsoid formula is an accurate and simple method to estimate the volume of relatively uniform structures such as grossly normal testicles and renal masses, but consistently underestimates the kidney and prostate volumes in humans. The findings of the present study emphasized the potential limitations of the ellipsoid formula for estimations of irregularly shaped structures and may discourage researchers from using that method when volume measurements are of particular importance (such as for patient staging) and more advanced and validated software for volume estimation is available.

Limitations of the present study include a fairly small sample size (n = 10) and the fact that the measurements were performed by 1 observer. Review of currently available veterinary literature indicates that other reports of tissue dimension changes following surgical excision involved sample sizes that were similar to that of the present study. We have no reason to believe that the FISSs evaluated in the present study were not representative of FISSs that occur in the general cat population. On the basis of the sample size and SDs for the variables assessed, the present study had adequate power (80% with a 1-sided α = 0.05) to detect a ≥ 4.9% shrinkage in length, ≥ 10.7% shrinkage in width, ≥ 10.9% shrinkage in depth, ≥ 12.3% shrinkage in tumor volume as estimated by the ellipsoid formula, and ≥ 13.2% shrinkage in tumor volume as estimated by the 3-D CT software. Thus, we may have been unable to detect shrinkages less than those calculated by previous studies, the median volume of FISSs as determined by use of the ellipsoid volume formula ranges from 7.57 to 23 cm$^3$. For both the in vivo and ex vivo assessments of the present study, the ellipsoid formula consistently yielded a significantly lower tumor volume, compared with that calculated by the 3-D CT software. It is important to note that, in the present study, the 3-D CT software calculated tumor volume by use of a volume-rendering algorithm instead of a surface-rendering algorithm. We chose to use the volume-rendering algorithm because results of another study indicate that it is accurate for estimation of tissue volume. The disparity between the tumor volumes estimated by the ellipsoid formula and the 3-D CT software was likely associated with the 3-D complexity and irregularity of the FISSs, which could not be accounted for in the ellipsoid formula. The ellipsoid formula is an accurate and simple method to estimate the volume of relatively uniform structures such as grossly normal testicles and renal masses, but consistently underestimates the kidney and prostate volumes in humans. The findings of the present study emphasized the potential limitations of the ellipsoid formula for volume estimation of irregularly shaped structures and may discourage researchers from using that method when volume measurements are of particular importance (such as for patient staging) and more advanced and validated software for volume estimation is available.


table 1
descriptive statistics for dimensions and volume of FISSs excised from 10 client-owned cats as measured from preoperative contrast-enhanced (in vivo) and postexcision (ex vivo) CT images.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>In vivo</th>
<th>Ex vivo</th>
<th>Median (95% confidence interval)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median ellipsoid volume (cm$^3$)</td>
<td>5.04</td>
<td>3.99</td>
<td>–12.8 (–24 to –1.65)</td>
<td>0.029</td>
</tr>
<tr>
<td>Median 3-D CT volume (cm$^3$)</td>
<td>5.40</td>
<td>4.08</td>
<td>–12.7 (–24.7 to –0.7)</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM unless otherwise indicated. Ex vivo measurements were obtained after the tumors had been fixed in neutral-buffered 10% formalin. For each tumor, the ellipsoid volume was calculated as (length X width X depth) X (π/6), and the 3-D CT volume was automatically calculated by commercially available 3-D volume rendering CT software.

*Value differs significantly (P ≤ 0.05) from that for the median ellipsoid volume.
the power calculations, which would represent a type II error. We chose to have only 1 observer perform tumor measurements because results of another study indicate high inter- and intraobserver agreement for 3-D CT volumetric measurements. Contrast medium administration was not standardized in the present study in regard to catheter size and catheter limb laterality in relation to the primary tumor, and it is unknown how those factors may have affected tumor measurements. Finally, all measurements in this study were obtained on the basis of contrast enhancement of the suspected tumor. Although strong contrast medium enhancement has been described for FISSs, it is possible that not all tumor boundaries were equally enhanced by the contrast medium, which could have resulted in inaccurate dimension and volume measurements. To our knowledge, data regarding the effect of surgical excision, hemorrhage, and postoperative manipulation on the contrast attenuation of FISSs on ex vivo CT images are unknown. It is possible that contrast-enhanced tissue at the edge of an FISS (ie, the reactive zone), which was included in the tumor volume calculations, was not actually neoplastic tissue.

In the present study, there were significant small decreases in the FISS length and tumor volume following surgical excision and formalin fixation. On a percentage basis, the decrease in tumor volume was similar between the volumes estimated by the ellipsoid formula and the 3-D CT software; however, the tumor volume estimated by the ellipsoid formula was consistently and significantly lower than that estimated by the 3-D CT software. These findings may help elucidate the various factors that contribute to the disparity often observed between the grossly normal surgical margins of a tumor excised en bloc and the histologic tumor-free margins subsequently reported by a pathologist and could influence interpretation of CT and histologic margins and protocols for tissue volume measurement and patient staging. On the basis of the results of the present study, we discourage clinicians from using the ellipsoid formula to estimate tumor volume when accuracy of that estimate is of particular concern and advanced imaging with a multidetector CT scanner is available. Additional studies to evaluate the shrinkage of clinically normal and neoplastic feline tissue following excision and fixation and to refine planning of the surgical margins of FISSs are warranted.

Acknowledgments

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Footnotes


c. Toshiba Aquilion, Tochigi, Japan.

d. Isovue/Iopamidol (300 mg of I/mL), Bracco Diagnostics Inc, Princeton, NJ.

e. eFilm, Merge HealthCare, Milwaukee, Wis.


g. GraphPad Prism, version 6.02 for Windows, GraphPad Software, San Diego, Calif.

References


