Use of histologic margin evaluation to predict recurrence of cutaneous malignant tumors in dogs and cats after surgical excision

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Objective—To assess the usefulness of histologic evaluation of surgical margins to predict local recurrence of cutaneous malignant tumors in dogs and cats treated by means of surgical excision.

Design—Prospective case series.

Animals—40 dogs and 20 cats.

Procedures—60 surgically excised tumors (20 soft tissue sarcomas [STSs], 20 mast cell tumors [MCTs], and 20 carcinomas) were examined histologically. Margins were classified as clean, close, or infiltrated; histologic grade was assessed in STSs and MCTs. Recurrence rates and recurrence-free intervals (RFIs) during a 24-month follow-up period were recorded, and method accuracy was calculated.

Results—Surgical margins were clean in 29 of 60 (48%) tumors, close in 11 (18%), and infiltrated in 20 (33%). Tumors recurred in 27 of 60 (45%) animals, with a mean ± SD RFI of 229 ± 173 days. Recurrence rates for animals that had tumors with infiltrated (16/20) or close (8/11) margins were significantly higher than recurrence rate for animals that had tumors with clean margins (3/29). Margin classification was a significant predictor of RFI. Accuracy of the method to predict recurrence was 94% for carcinomas, 87% for STSs, and 76% for MCTs.

Conclusions and Clinical Relevance—Histologic assessment of margin status was useful for predicting local recurrence of cutaneous malignant tumors in dogs and cats treated by means of excision alone. Method accuracy varied among tumor types and grades. Recurrence times suggested postsurgical follow-up should continue for ≥ 2 years. Results were similar for animals with infiltrated and close tumor margins, and careful postsurgical management is recommended for both. (*J Am Vet Med Assoc* 2012;240:1181–1187)

Surgery is the treatment of choice for primary cutaneous tumors located in areas amenable to excision, and it is generally accepted that complete resection greatly improves RFI, whereas incomplete resection increases the probability of local treatment failure.[1–4] However, because neoplastic cells often extend beyond the observable or palpable tumor mass, clinical assessment of tumor boundaries rarely corresponds to actual tumor extension and completeness of excision can only be determined by means of histologic examination.[5–8] Therefore, the microscopic assessment of surgical margins is an important prognostic factor for postsurgical monitoring and subsequent adjuvant treatment planning.[8]

During the past 5 decades, several studies[9–12] in the field of human pathology addressed the technical issue of detecting residual tumor cells at surgical margins, with the conclusion that there is not an optimal processing technique suitable for all sample types; instead, the technique is linked to tissue characteristics. In the veterinary literature, only a few studies[5–8,13] have focused on the histologic assessment of surgical margins, and methods and terminology, including the definitions of close margins and tumor recurrence, have not been standardized.

Abbreviations

MCT	Mast cell tumor
RFI	Recurrence-free interval
STS	Soft tissue sarcoma

The purpose of the study reported here was to assess the usefulness of histologic evaluation of surgical margins to predict local recurrence of cutaneous malignant tumors in a cohort of dogs and cats treated by means of surgical excision alone. We also sought to evaluate associations between tumor size, type, and grade and method accuracy.

Materials and Methods

Animals—Client-owned dogs and cats with surgically removed cutaneous STSs, MCTs, and carcinomas referred to the Pathology Division of the Department of Veterinary Medical Sciences, University of Bologna,

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Italy, between January 1, 2007, and December 31, 2009, for histologic evaluation of excised tumor margins were eligible for enrollment in the prospective study. Data collected included breed, sex, age at diagnosis, anatomic location of the tumor, number of surgeries at the same tumor site, and date of the most recent surgery. Patients were routinely monitored for 24 months after surgery via regular telephone contact with owners or referring clinicians. Study exclusion criteria included any additional treatment (ie, chemotherapy or radiotherapy) prior to or after surgery or a lack of adequate follow-up information (ie, date of local recurrence, if any). Dogs and cats were enrolled in the study until an equal number of animals (20 animals/tumor type) were assigned for the 3 tumor types of interest. Owner consent was obtained prior to enrollment of animals in the study. Because the study involved the use of excised tissues and patient records and did not influence therapeutic decisions, approval by the University of Bologna Ethical Committee for Animal Care was not required.

Tissue processing—All samples were processed by 1 pathologist (FS). Surgically excised tissues were measured with calipers, and tumor size was defined as the largest grossly measured diameter of the mass. Cut surfaces were marked with India ink and allowed to dry for 30 minutes, and specimens were fixed in neutral-buffered 10% formalin. Fixed tissues were trimmed into 2- to 3-mm-thick transverse and longitudinal vertical slices according to the breadloaf-cross method.[12] For larger specimens (tumor diameter, > 7 cm), peripheral vertical sections were also obtained.[12] After gross examination, a variable number of pieces were selected according to the size of the excised mass (1 to 4 pieces for tumors < 2.5 cm in diameter, 5 to 12 pieces for tumors 2.5 to 7 cm in diameter, and 13 to 25 pieces for tumors > 7 cm in diameter) and placed in labeled cassettes for routine histologic processing. Deep surgical margins were routinely identified and processed for all specimens. Sketch maps of the samples submitted for histologic processing, including the position and number of sections created, were drawn to assist subsequent histologic examinations. Sections were cut 5 µm thick and stained with H&E; for MCTs, additional sections were stained with toluidine blue to improve the detection of mast cells at surgical margins as needed.

Histologic examination and margin status—All tumor samples were examined by 1 pathologist (GB) and classified according to World Health Organization criteria.[14,15] The histologic grades of STSs and MCTs were assigned according to previously described grading systems.[1,16] Grading scales ranged from I to III for both tumor types. Samples were assessed with a light microscope[a] equipped with a camera,[b] and width of surgical margins was measured on a video screen with appropriate software tools.[c] Margins were classified as clean (> 2 mm of normal tissue between the tumor and the inked edges in all directions), close (tumor cells extending to within 2 mm of the inked edges in ≥ 1 section), or infiltrated (tumor cells extending to the inked edges in ≥ 1 section). Infiltrated margins were further classified on the basis of the amount of tumor cells detected as focally infiltrated (subjectively small foci of tumor cells in ≥ 1 margin) or diffusely infiltrated (large foci of tumor cells in ≥ 1 margin or involvement of an entire margin in the section).

Recurrence—Local recurrence was defined as the development of a tumor of the same type within 2 cm of the surgical site, confirmed by cytologic or histologic examination of a sample. The RFI was defined as the time (days) between surgical excision (day 0) and confirmation of local recurrence.

Statistical analysis—Normality of data distribution was evaluated with the Kolmogorov-Smirnov test; age and RFI were normally distributed, and tumor size was not. Curves for RFIs were generated according to the Kaplan-Meier product-limit method. Development of local tumor recurrence was considered a complete event. Patients were censored on the last day of the study if there was no evidence of local recurrence by the end of the study period. Patients that died before the end of the study period without developing any local recurrence were censored on the date of death. Differences among the RFI curves associated with anatomic location of the tumor (head and neck, trunk, or extremities), tumor size (smaller or larger than the median value), number of surgeries (1 [first surgery] or > 1 [re-excision]), tumor type (STS, MCT, or carcinoma), grade (I, II, or III), or margin status (clean, close, or infiltrated) were evaluated by means of univariate (log-rank test) and multivariate (Cox proportional hazards regression model) analysis. Tests were performed with commercially available software packages.[d,e]

Margin classification was related to the development of local recurrence (present or absent), and the sensitivity, specificity, positive and negative predictive values, and accuracy of histologic evaluation of margins to predict tumor recurrence were calculated as follows:

$$\text{Sensitivity (percentage of true-positive results)} = TP/(TP + FN) \times 100$$

$$\text{Specificity (percentage of true-negative results)} = TN/(TN + FP) \times 100$$

$$\text{Positive predictive value (percentage of recurring tumors among those with infiltrated margins)} = TP/(TP + FP) \times 100$$

$$\text{Negative predictive value (percentage of tumors not recurring among those with clean margins)} = TN/(TN + FN) \times 100$$

$$\text{Accuracy (percentage of cases in which the histologic assessment of margins correctly predicted recurrence or nonrecurrence)} = TP + TN/(TP + TN + FN + FP) \times 100$$

where TP, TN, FP, and FN represent true-positive, true-negative, false-positive, and false-negative results, respectively. These 4 statistical descriptors were also evaluated and compared among tumors of different sizes, types, and grades. The effect of close margin classification on sensitivity, specificity, positive and negative predictive values, and accuracy was assessed by excluding these data from the analyses and alternately reclassifying close margins as clean or infiltrated for analyses. To determine recommendations for postsurgical follow-up duration, overall accuracy of histologic evaluation of surgical margins was calculated at 6, 12, and 24 months

after excision. Values of $P < 0.05$ were considered significant for all tests.

Results

Animals—Forty dogs (17 males and 23 females) and 20 cats (13 males and 7 females) were enrolled in the study. Mean ± SD age at the time of referral was 8.2 ± 3.5 years for dogs and 10.4 ± 3.1 years for cats. Breeds most commonly represented among dogs were Boxer (n = 7), Labrador Retriever (4), and Beagle (2). The cats included 17 domestic shorthairs and 3 Chartreux. Five dogs had STSs, 20 (including all 7 Boxers) had MCTs, and 15 had carcinomas. Fifteen cats (including all 3 Chartreux) had STSs, and 5 had carcinomas.

Eight of 60 (13%) tumors were located on the head and neck (1 STS, 3 MCTs, and 4 carcinomas), 37 (62%) were on the trunk (15 STSs, 8 MCTs, and 14 carcinomas), and 15 (25%) were on the extremities (4 STSs, 9 MCTs, and 2 carcinomas). Nine of 60 (15%) tumors (3 carcinomas and 6 STSs) were reported as local recurrences of previously excised tumors. Median size was 2 cm (range, 0.5 to 12 cm) for all tumors; ranges were 0.5 to 10 cm for STSs, 0.5 to 12 cm for MCTs, and 0.5 to 3 cm for carcinomas.

Histologic examination and classification of margins—Tumor types included in the study were STSs (n = 20), MCTs (20), and carcinomas (20; **Table 1**). Soft tissue sarcomas included fibrosarcomas (n = 9), injection-site sarcomas (4), giant cell tumors of soft tissue (4), malignant peripheral nerve sheath tumors (2), and hemangiopericytoma (1). Carcinomas included perianal gland carcinomas (8), squamous cell carcinomas (6), apocrine carcinomas (3), and basosquamous carcinoma, sebaceous carcinoma, and anal sac carcinoma (1 each).

Six STSs were classified as grade I, 8 as grade II, and 6 as grade III. Among MCTs, 8 were classified as grade I, 8 as grade II, and 4 as grade III.

Margins were clean in 29 of 60 (48%) tumors, including 9 STSs, 11 MCTs, and 9 carcinomas, and were close in 11 (18%) tumors, including 5 STSs, 3 MCTs, and 3 carcinomas. Of 20 (33%) tumors (6 STSs, 6 MCTs, and 8 carcinomas) with infiltrated margins, infiltration was focal in 5 (4 MCTs and 1 carcinoma) and diffuse in 15 (7 carcinomas, 6 STSs, and 2 MCTs). Because of the small numbers of tumors, grade II and III tumors were combined to improve statistical power for analysis of this variable.

Outcomes—Overall, 27 of 60 (45%) tumors recurred locally during the 24-month follow-up period (Table 1). Recurrence was confirmed by means of biopsy and histologic examination of samples from 12 patients and by means of cytologic evaluation of fine-needle aspirate samples from 15 patients.

Overall, 16 of 20 tumors with infiltrated margins, 8 of 11 tumors with close margins, and 3 of 29 tumors with clean margins recurred locally ($P < 0.001$ for infiltrated and close margins vs clean margins). Recurrence rate among animals that had tumors with diffusely infiltrated margins was 14 of 15, whereas that of animals that had tumors with focally infiltrated margins was 2 of 5 ($P = 0.032$). Local recurrence of 12 STSs (6/6 with infiltrated margins, 4/5 with close margins, and 2/9 with clean margins), 6 MCTs (3/6 with infiltrated margins, 2/3 with close margins, and 1/11 with clean margins), and 9 carcinomas (7/8 with infiltrated margins and 2/3 with close margins) was detected. Recurrence rate at 6 months (14/60 [23%]) was significantly ($P = 0.002$) lower than that observed at the end of the 24-month follow-up period (27/60 [45%]).

Mean RFI was 229 ± 173 days for all animals in which local tumor recurrence was detected during the 24-month follow-up period (n = 27; Table 1). Among these, mean RFIs for primary and recurrent tumors were 256 ± 186 days and 151 ± 103 days, respectively. Concerning tumor type, mean RFI was 294 ± 202 days for STSs, 167 ± 136 days for MCTs, and 184 ± 135 days for carcinomas. Four of 60 patients (7%; all with carcinomas) died before the end of the 24-month follow-up period; none of the deaths were attributed to tumor-

Table 1—Mean ± SD RFIs and local recurrence rates of tumors (n = 60) grouped according to histologic classification of surgical margins in 60 animals (40 dogs and 20 cats) with cutaneous malignant tumors (STSs, MCTs, or carcinomas) treated by means of surgical excision.

		Recurrence rate*						
					Infiltrated margins			
Tumor type	No. of tumors	All margins	Clean margins	Close margins	All	Focal	Diffuse	RFI (d)†
STS	20	12/20	2/9	4/5	6/6	0/0	6/6	294 ± 202
Grade I	6	2/6	0/3	1/2	1/1	0/0	1/1	334 ± 269
Grades II and III	14	10/14	2/6	3/3	5/5	0/0	5/5	286 ± 203
MCT	20	6/20	1/11	2/3	3/6	2/4	1/2	167 ± 136
Grade I	8	0/8	0/5	0/0	0/3	0/2	0/1	NA
Grades II and III	12	6/12	1/6	2/3	3/3	2/2	1/1	167 ± 136
Carcinoma	20	9/20	0/9	2/3	7/8	0/1	7/7	184 ± 135
All	60	27/60	3/29	8/11	16/20	2/5	14/15	229 ± 173

Tumors were grouped according to type and World Health Organization tumor grade for analysis. Margins were classified histologically as clean (> 2 mm of normal tissue between the tumor and marked edges in all directions), close (tumor cells extending to within 2 mm of marked edges in ≥ 1 section), or infiltrated (tumor cells extending to the marked edges in ≥ 1 section).

*Number of recurrences/total number evaluated in each category. †Data mean ± SD for RFI are reported for 27 animals in which local recurrence was detected.

NA = Not applicable.

related causes, and none of these animals had evidence of local tumor recurrence as reported at the time of last follow-up.

Analysis of prognostic factors—Prognostic factors for local recurrence were evaluated via univariate analysis (**Table 2**). Local tumor recurrence during the 24-month follow-up period was associated with tumor size > 2 cm (log-rank test, $P = 0.01$) and with tumor grades ≥ grade II (log-rank test, $P = 0.004$). Local recurrence was associated with > 1 surgery (ie, previous surgery at the same location for the same tumor type [log-rank test, $P = 0.018$]), but was not associated with tumor type or anatomic location.

The RFIs were also significantly (log-rank test, $P < 0.001$ for all tumor types) shorter for patients that had tumors with infiltrated or close margins than for those with clean margins, whereas no significant difference was observed between patients that had tumors with close and infiltrated margins. The relationship between RFI and margin status (clean vs close and infiltrated) was stronger for carcinomas (log-rank test, $P < 0.001$) and STSs (log-rank test, $P = 0.004$) than for MCTs (log-rank test, $P = 0.032$; **Figures 1–4**). On multivariate analysis, the histologic classification of margin status

Table 2—Univariate (log-rank test) analysis of variables potentially associated with RFIs throughout a 24-month postsurgical follow-up period in 60 animals (40 dogs and 20 cats) with cutaneous malignant tumors (STSs, MCTs, or carcinomas) treated by means of surgical excision.

Variable	No. of tumors	RFI (d)*	*P* value
Anatomic location of tumor			0.174
Head and neck	8	403 ± 275	
Trunk	37	506 ± 271	
Extremities	15	554 ± 266	
Number of surgeries			0.018
1	51	543 ± 258	
> 1	9	280 ± 255	
Tumor size (largest diameter)			0.01
≤ 2 cm	40	561 ± 262	
> 2 cm	20	389 ± 260	
Tumor type			0.318
STS	20	561 ± 267	
MCT	20	468 ± 261	
Carcinoma	20	484 ± 285	
Tumor grade (MCT and STS)			0.004
Grade I	14	674 ± 156	
Grades II and III	26	429 ± 276	
Margin status			< 0.001
Clean margins	29	677 ± 161	
Close and infiltrated margins	31	342 ± 258	
STS			0.004
Clean margins	9	633 ± 182	
Close and infiltrated margins	11	333 ± 237	
MCT			0.032
Clean margins	11	669 ± 193	
Close and infiltrated margins	9	429 ± 285	
Carcinoma			< 0.001
Clean margins	9	NA	
Close and infiltrated margins	11	283 ± 240	

*Data are reported for all 60 animals at the end of the follow-up period.

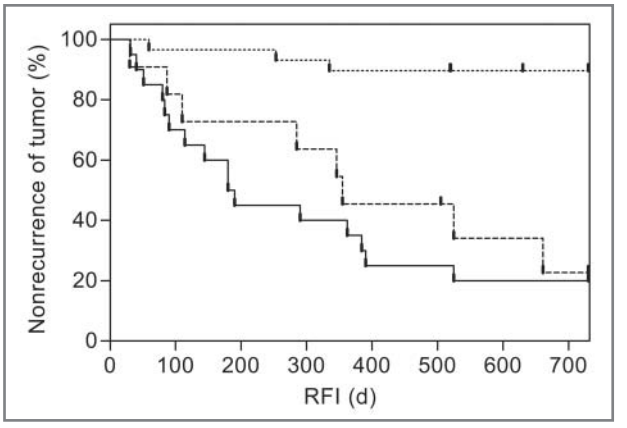

Figure 1—Kaplan-Meier plot depicting RFI in 60 animals (40 dogs and 20 cats) with cutaneous malignant tumors (STSs, MCTs, or carcinomas) treated by means of surgical excision and grouped according to histologic classification of surgical margins as clean (dotted line), close (dashed line), or infiltrated (solid line). Values were significantly (log-rank test, $P < 0.001$) different among patients that had tumors with clean (mean RFI, 677 ± 161 days) versus close and infiltrated surgical margins (mean RFI, 342 ± 258 days).

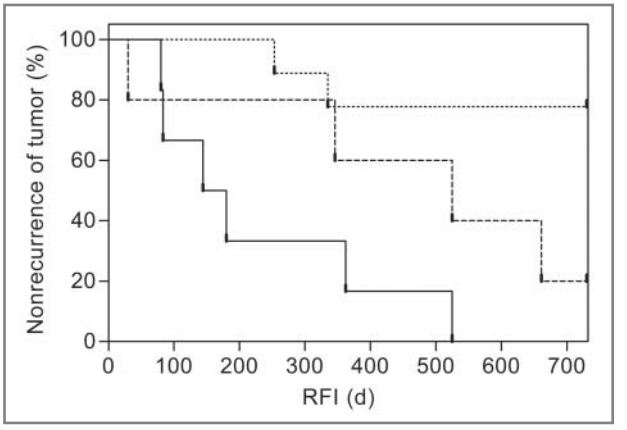

Figure 2—Kaplan-Meier plot depicting RFI in 20 animals (5 dogs and 15 cats) with cutaneous STSs treated by means of surgical excision and grouped according to histologic classification of surgical margins. Values were significantly (log-rank test, $P = 0.004$) different among patients that had tumors with clean (mean RFI, 633 ± 182 days) versus close and infiltrated surgical margins (mean RFI, 333 ± 237 days). *See* Figure 1 for remainder of key.

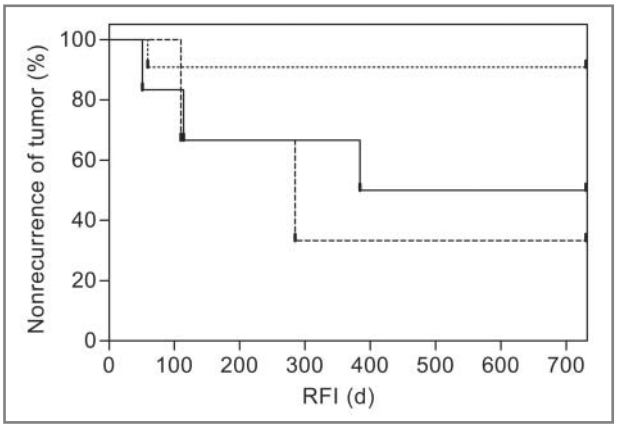

Figure 3—Kaplan-Meier plot depicting RFI in 20 dogs with cutaneous MCTs treated by means of surgical excision and grouped according to histologic classification of surgical margins. Values were significantly (log-rank test, $P = 0.032$) different among patients that had tumors with clean (mean RFI, 669 ± 193 days) versus close and infiltrated surgical margins (mean RFI, 429 ± 285 days). *See* Figure 1 for remainder of key.

emerged as the best predictive factor for RFI ($P < 0.001$; OR, 10.9; 95% confidence interval, 3.1 to 38.0).

Sensitivity, specificity, positive and negative predictive values, and accuracy of the histologic evaluation of surgical margins for prediction of local tumor recurrence were calculated (**Table 3**). Accuracy to predict recurrence of carcinomas, STSs, and MCTs after excision was 94%, 87%, and 76%, respectively. For MCTs, accuracy was 62% for grade I tumors, compared with 89% for grade II and III tumors, whereas for STSs, accuracy was 100% for grade I tumors and 82% for grade II and III tumors. Method accuracy was apparently increased by considering close margins as infiltrated (83%) rather than clean (75%), particularly in STSs (85% vs 70%); accuracy was also apparently increased when the duration of postsurgical follow-up was increased from 6 to 12 (78% to 80%) or 24 (86%) months. Because of the small sample size, statistical power was too low to conclude whether the observed differences in accuracy were significant.

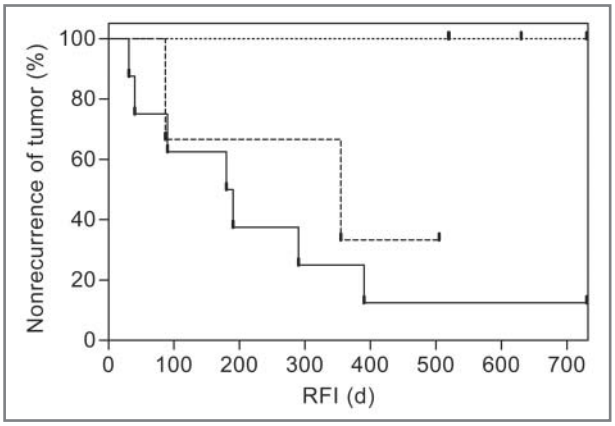

Figure 4—Kaplan-Meier plot depicting RFI in 15 dogs and 5 cats with cutaneous carcinomas treated by means of surgical excision and grouped according to histologic classification of surgical margins. Values were significantly (log-rank test, $P < 0.001$) different among patients that had tumors with clean (no recurrence) versus close and infiltrated surgical margins (mean RFI, 283 ± 240 days). *See* Figure 1 for remainder of key.

Discussion

Various clinical and histopathologic prognostic factors have been identified for predicting postsurgical recurrence of cutaneous tumors in small animals, including the interval between diagnosis and first treatment, extent and number of surgeries, degree of necrosis detected from histologic examination, and mitotic rate of tumor cells[1,3,17–19]; however, status of the surgical margins appears to be the most important factor among these.[1,5,13,20] In the present study, the surgical margins of 60 cutaneous tumors from dogs and cats were histologically evaluated and the influence of several clinicopathologic variables on the usefulness of the method to predict tumor recurrence was assessed. Overall, there was a very strong association between histologic evidence of residual disease at the margins of the resected tumor and onset of local recurrence, with an overall recurrence rate of 80% (16/20) among excised tumors that had infiltrated margins ($P < 0.001$). The accuracy of histologic evaluation of surgical margins to predict tumor recurrence varied among tumor types and grades but was not consistently affected by tumor size. Accuracy was apparently affected by categorization of close margins (as clean or infiltrated) and by the duration of postsurgical follow-up, although the statistical sig-

Table 3—Sensitivity, specificity, positive and negative predictive values, and accuracy of histologic evaluation of surgical margins to predict local recurrence of surgically excised malignant skin tumors (n = 60) of various sizes, types, and grades in 40 dogs and 20 cats throughout a 24-month postsurgical follow-up period.

Variable	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
Tumor size (largest diameter)					
≤ 2 cm	100	84	69	100	88
> 2 cm	75	100	100	71	85
Tumor type					
STS	75	100	100	78	87
Grade I	100	100	100	100	100
Grades II and III	71	100	100	67	82
MCT	75	77	50	91	76
Grade I	NA	62	0	100	62
Grades II and III	75	100	100	83	89
Carcinoma	100	90	88	100	94
Close margin classification*					
Clean	59	88	80	72	75
Infiltrated	89	79	77	90	83
Postsurgical follow-up period					
6 mo	91	74	50	96	78
12 mo	81	79	65	90	80
24 mo	84	87	80	90	86

*Effect of close margin classification was assessed by alternatively regarding these as clean or infiltrated for analysis.
See Table 1 for remainder of key.

nificance of these differences could not be evaluated because of the small number of cases.

Veterinary oncology studies on the evaluation of surgical margins have typically focused on 1 tumor type or groups of tumors with similar histologic features, generally MCTs or STSs. Contrary to studies[21,22] in human medicine, carcinomas have been less commonly evaluated for margin status in small animals, although local recurrence is relatively common in dogs and cats with cutaneous cancer, including perianal or squamous cell carcinomas.[23]

In the present study, we sought to test the usefulness of routine histologic examination of surgical margins to predict tumor recurrence by collecting a heterogeneous sample of surgically removed cutaneous neoplasms that commonly recur in small animals, including carcinomas. To assess the influence of tumor type on prognostic value of the method, dogs and cats were enrolled in the study until equal numbers of animals were assigned to the 3 tumor types of interest (STSs, MCTs, and carcinomas), with up to 20 animals for each type. To generate uniformity, all samples were processed and evaluated with standard procedures by the same individuals. The main limitation of the present study was the small sample size, which was attributable to the fact that most patients with close or infiltrated margins underwent reexcision or radiation treatment and were thereby excluded from the analysis.

When evaluating tumors grouped according to type, identification of infiltrated margins appeared more useful to predict local recurrence in carcinomas (accuracy, 94%) than in STSs (accuracy, 87%) and MCTs (accuracy, 76%), although statistical differences could not be evaluated. More specifically, accuracy was affected by false-negative results in the evaluation of STSs and by false-positive results in the evaluation of MCTs. Two grade III STSs in cats with margins classified as clean recurred locally (an injection-site sarcoma that recurred after 335 days and a giant cell tumor of soft tissue that recurred 253 days after surgery). This type of error can be explained by the tendency of STSs to asymmetrically grow within fascial planes and to form thin tentacle-like projections that may create satellite lesions in surrounding tissues even at a considerable distance from the primary mass.[8,13] The extremely long RFIs of STSs (mean, 294 ± 202 days) may also raise the question of whether these tumors were recurrences or de novo–developed tumors. Microscopic detection of a tumor of the same type within 2 cm of the surgical site was identified as recurrence, even if it could not be distinguished from a subsequently developed de novo tumor.

Conversely, 3 of 6 MCTs with margins classified as infiltrated via histologic evaluation did not recur locally. This finding is consistent with previous studies[5–7] in which false-positive results were reported in the assessment of margin status after excision of MCTs and may have several explanations. Indeed, the main concern in evaluating MCT margins resides in the interpretation of whether clustered, well-differentiated mast cells not in direct connection with the main mass are neoplastic mast cells, normal resident mast cells, or part of an inflammatory response.[5] Because standard light microscopy alone cannot be used to determine the neoplastic potential of such cells, the use of ancillary techniques, such as immunohistochemical analysis for CD117 (also called v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog or KIT) or morphometry, has been suggested.[7,24] Nevertheless, according to the authors' experience, this possibility is limited by the difficulty in assessing CD117 patterns in a small number of cells and by the lack of consistency of immunohistochemical labeling at tissue section edges. This issue may be particularly challenging in well-differentiated tumors: in the present study, all false-positive identifications were made in grade I MCTs (ie, 0/3 tumors with margins classified as infiltrated recurred), whereas all determinations of grade III MCTs identified as having infiltrated margins were true-positive identifications (ie, 3/3 recurred). Additional explanations may include a lesser malignancy of low-grade MCTs with a corresponding lack of recurrence or longer recurrence times, compared with high-grade MCTs.[6,16,19]

Assessment of the extent of margin infiltration by tumor cells may aid correct margin classification in low-grade MCTs, considering that in 2 of 3 grade I MCTs with false-positive results for margin evaluation in the present study, margins were only focally infiltrated, whereas in all recurrent MCTs, margins were diffusely infiltrated. The extent of margin infiltration was also associated with recurrence in other tumor types; when the entire study sample was considered, the local recurrence rate among animals that had tumors with diffusely infiltrated margins was higher (14/15) than that of animals that had tumors with focally infiltrated margins (2/5; $P = 0.032$). This finding suggests the benefit of systematically recording the infiltration extent of margins. In human oncology, patients who have tumors with extensively infiltrated margins identified via histologic evaluation after excision are considered at higher risk for local tumor recurrence than are patients with focally positive margins.[21,22]

Because it is not technically possible to examine the entire surface of all margins of an excised tumor, it might be anticipated that accuracy of histologic evaluations would be lower for larger tumors. However, in the present study, although tumor size ($<$ or $>$ 2 cm) was prognostic for local recurrence, the effect of size on accuracy was minimal. A possible explanation may reside in the observation that larger tumor size is generally associated with a greater likelihood of failure in terms of obtaining clean margins, but our method of increasing the number of sections with tumor size may have helped to reduce the influence of tumor size on the accuracy of margin evaluation.

In previous studies[1,2,6–8,13,20,25] evaluating margins of surgically excised tumors, a minimum follow-up period was often neglected or set at 6 or 12 months. Nevertheless, when gathering follow-up data in the present study, it was noted that local recurrence could develop after long intervals, prompting extension of the postsurgical follow-up period to 24 months. This dramatically affected detected recurrence rates, which were approximately doubled at 24 months, compared with the rate at 6 months (27/60 vs 14/60; $P = 0.002$). Therefore, we strongly recommend long-term monitoring (≥ 24 months) of dogs and cats following surgical excision of malignant skin tumors. Mean RFI was significantly ($P = 0.018$) shorter for tumors of the present study that were classified as recurrences of previous tumors than the mean RFI for those considered primary tumors (280 vs 543 days, respectively). Repeated excisions may possibly select an aggressive population of neoplastic cells with increased invasion capability, which underscores

the importance of obtaining adequate margins during the initial surgery.[3,20]

It is generally agreed that patients require additional surgery or adjuvant radiation treatment when surgical margins of excised malignant tumors are determined to be infiltrated; however, there is more controversy regarding treatment of patients with close margins. The evidence of tumor cells near to but not in strict contact with the surgical margin raises interpretative doubts. This distance has been empirically set at 1 to 3 mm from the surgical margin,[6,13,25,26] and such samples are also commonly referred to as having clean but close margins or narrow margins. Investigators of various studies[3,5,7,8,27–29] have considered close margins to be dubious, equivalent to infiltrated margins, or strictly clean. To address this issue in the present study, statistical descriptors of method effectiveness were evaluated in which close margins were alternatively regarded as clean or infiltrated. Combining data for tumors with close margins together with those for tumors with infiltrated margins did increase method sensitivity (from 59% to 89%; $P = 0.028$), but it also reduced specificity (from 88% to 79%). Nevertheless, overall method accuracy was apparently increased (from 77% to 85%) by considering close margins as infiltrated rather than clean. This was shown most clearly in results of STS analysis (in which accuracy values changed from 70% to 85%) because there was a high rate of local recurrence for tumors (4/5) with close margins. An explanation for these results is that if the tumor is very close to the margin, it is likely that it may have extended beyond the surgical margin at ≥ 1 undetected point. Although the use of a large number of sections may have helped to reveal whether margins were infiltrated in samples evaluated in the present study, differences in recurrence rates and RFIs between tumors removed with close or infiltrated margins were negligible. Therefore, we advocate that there should be no distinction in the clinical approach to management of canine and feline patients with close or infiltrated margins in excised malignant skin tumors.

Standardization of processing methods and terminology and evaluation of differences among pathologists in the assessment of surgical margins are warranted for a more effective use of this relevant prognostic indicator. The investigation of a larger number of cases will be necessary to establish definitive conclusions regarding the influence of tumor type and grade on method accuracy.

a. Nikon Eclipse 55i microscope, Nikon, Tokyo, Japan.
b. Nikon DS-Fi1 digital camera, Nikon, Tokyo, Japan.
c. Nikon DS-L2 control unit, Nikon, Tokyo, Japan.
d. GraphPad Prism, version 5.0, GraphPad Software Inc, San Diego, Calif.
e. STATISTICA, StatSoft Inc, Tulsa, Okla.

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