JAVMA

Grade shifts in recurrent canine soft tissue sarcomas and mast cell tumors

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Received January 31, 2023
Accepted May 3, 2023

doi.org/10.2460/javma.23.01.0050

OBJECTIVE
To determine the incidence of histologic grade shift (alteration of grade relative to the original tumor) in recurrent canine soft tissue sarcoma (STS) and mast cell tumor (MCT), and to determine the level of agreement between blinded pathologist review and original histology interpretation of STS and MCT grades.

ANIMALS
15 dogs with recurrent cutaneous/subcutaneous STS and 5 dogs with recurrent cutaneous MCT. All included dogs underwent excision of both the primary and recurrent tumors and had tumor samples available for review.

PROCEDURES
The medical records and histology database from a single institution were reviewed, and data were recorded and analyzed. A single board-certified veterinary pathologist performed blinded evaluation of all excisional tumor samples, including both primary and recurrent disease, and these were evaluated independently and in conjunction with initial pathologic diagnoses.

RESULTS
Based on single pathologist review, 7 of 15 (46.7%) dogs with recurrent STS had grade shift characterized by a higher or lower recurrent tumor grade in 4 of 7 and 3 of 7 cases, respectively, and 1 of 5 dogs with recurrent MCT had grade shift characterized by an increased grade of the recurrent tumor. Variability in reported grade between original histology report and pathologist review occurred for 13 of 30 (43.3%) STS excisional biopsy samples and 0 of 10 MCT excisional biopsy samples.

CLINICAL RELEVANCE
Grade shift has been reported in multiple tumor types in people and has the potential to alter prognosis and treatment recommendations. This is the first study to document this phenomenon in dogs. Additional large-scale studies are needed to determine factors associated with grade shift as well as prognostic significance of grade shift for recurrent canine STS and MCT.

Soft tissue sarcomas (STS) and mast cell tumors (MCT) are two of the most common cutaneous and subcutaneous malignant tumors in dogs. An important prognostic factor for each of these tumor types is histologic grade, with higher grade tumors having greater incidence of both local recurrence and metastasis. For cutaneous and subcutaneous STS, a grade of 1 to 3 is assigned on the basis of cellular differentiation, mitotic count, and percentage of tumor necrosis **(Supplementary Table S1)**.[1,2] There is a movement to refer to STS as soft tissue tumors in veterinary medicine.[3,4] However, as this terminology has not been widely adapted yet and since all the cases in this study were diagnosed as STS and graded on the basis of this terminology, STS will be used throughout the manuscript to maintain consistency and clarity. For cutaneous MCT, the following 2 main grading schemes are used: 3-tier, which assigns a grade of 1 to 3 on the basis of disease extent, cellularity and cellular morphology, mitotic count, and stromal reaction, and 2-tier, which assigns a grade of low or high on the basis of mitotic count, multinucleated cells, bizarre nuclei, and karyomegaly **(Supplementary Tables S2 and S3)**.[5–7] Because of the important influence on prognosis and therapeutic decisions, histologic tumor grade should be evaluated following excision. Anecdotally in

veterinary medicine, tumors that recur locally following excision have the potential to be a different grade compared to the primary tumor, known as grade shift. To date, this has not been described in the veterinary literature, though limited reports of grade shift with recurrent disease exist in people with varying tumor types, including mammary neoplasia, oral squamous cell carcinoma, and urothelial neoplasia.[8–10] These human studies have documented both progression and regression of grade in recurrent tumors. Grade shift in recurrent neoplasia may occur due to loss or gain of genetic mutations in the remaining tumor cells with a resulting more aggressive or indolent phenotype. Because the grade of STS and MCT influences prognosis and treatment recommendations and recurrent disease is relatively common for these canine tumors, it is important to determine the incidence of grade shift with recurrent disease as well as any potential risk factors for and prognostic significance of grade shift.[11,12] This information may influence recommendations for both treatment and surveillance.

Although tumor grade is one of the most important prognostic factors for canine STS and MCT, several challenges exist in considering the accuracy of grading. For canine MCT, substantial variation in grading between board-certified veterinary pathologists has been documented with 3-tier grading schemes. In one study, agreement between pathologists was only 62.1% with uniform use of 3-tier classifications for canine cutaneous MCT.[13] The 2-tier grading system was subsequently proposed in an effort to more accurately predict biologic behavior, with reported interobserver agreement as high as 96.8% with this system, though evidence is lacking regarding direct comparison of interobserver agreement for the 2- and 3-tier grading systems.[7,14] Similarly, pathologist agreement on grading for canine STS is limited, and a recent study reported an interobserver agreement of only 47.0% in assigning grade for canine STS.[15] Therefore, evaluation of pathology reports performed by different board-certified veterinary pathologists with regard to incidence of grade shift for recurrent tumors should be performed with caution, as it is possible that differences in grading among recurrent tumors may actually be attributed to differences in pathologist interpretation rather than true differences in tumor morphology. Thus, one way to remove the confounding factor of interobserver variability in determining the incidence of grade shift for recurrent canine STS and MCT is via a single board-certified pathologist review of all tumor samples. Importantly, and in support of this proposed method, the same canine STS grading study that reported high interobserver variability in grading also reported strong intraobserver agreement with intraclass correlation coefficients ranging from 0.78 to 1.0 (perfect agreement).[15] On the basis of this study, histologic assessment of STS appears to be highly reproducible for an individual pathologist.

The primary objectives of this study were to characterize grade shift in recurrent canine STS and MCT and to assess the incidence of grade shift in this population. A secondary objective was to determine the level of agreement between blinded single veterinary pathologist review of the primary and recurrent canine STS and MCT tissues as compared to the initial histology report.

Materials and Methods

The medical record database of the Colorado State University James L. Voss Veterinary Teaching Hospital was retrospectively searched to identify dogs that had recurrent cutaneous/subcutaneous STS or cutaneous MCT with formalin-fixed paraffin-embedded tumor blocks and slides of both the primary and recurrent tumors available for pathologist review. Initial pathology diagnostic interpretation was performed at Colorado State University Veterinary Diagnostic Laboratory for all samples. All included cases had excisional biopsies of both primary and recurrent tumors for review, and incisional biopsy samples were excluded. All included cases had recurrent disease on the basis of development of the same tumor type (STS or MCT) at the location of the scar following excision of the primary tumor; cases with tumors that arose anatomically distant from the surgical scar were excluded. Subcutaneous MCT were excluded due to differences in reported prognosis and lack of studies with direct comparison of cutaneous and subcutaneous tumors using the cutaneous grading system.[16]

Information obtained from the medical records, when available, included signalment, staging at the time of primary tumor excision and recurrent tumor excision(s), neoadjuvant and adjuvant treatments, tumor type, tumor size, tumor location, dates of tumor recurrence, dates of primary and recurrent tumor excisions, surgical margins and intent of excision, original histopathology results (tumor type and subtype, grade, tumor-free histologic margin evaluation, lymphovascular invasion, tumor differentiation, mitotic count [number of mitotic figures/2.37 mm^2 or the equivalent of 10 hpf], percent tumor necrosis [microscopic estimation], multinucleation, bizarre nuclei, karyomegaly, cytoplasmic granules, anisokaryosis, anisocytosis, and nucleus-to-cytoplasm ratio), patient outcomes, date of death or last follow-up, and cause of death.

A single board-certified veterinary pathologist (KH) subsequently evaluated all primary and recurrent tumor samples and recorded the date of review, tumor type, and tumor grade based on individual and cumulative score of tumor differentiation, mitotic count, and percent tumor necrosis for STS and mitotic count, extent of tumor involvement, multinucleation, bizarre nuclei, karyomegaly, cellularity and cellular morphology, and stromal reaction for MCT (Supplementary Tables S1–S3).[2,6,7] The pathologist was blinded to all prior pathology results and reports. The original slides were reviewed and when unavailable, recuts at a depth of 5 μm from all formalin-fixed paraffin-embedded tumor blocks were performed; cases in which slide recuts were used were documented.

Surgical dose, relative to the gross tumor in situ, was defined as marginal if the tumor was excised

immediately beyond any visible capsule or pseudocapsule, narrow if < 2 cm grossly normal tissue peripherally and 1 fascial plane deep were excised, and wide if ≥ 2 cm grossly normal tissue peripherally and 1 fascial plane deep were excised or, for MCT < 2 cm diameter only, if proportional peripheral margins (relative to the maximal gross tumor diameter) and 1 fascial plane deep were excised.[17] The residual tumor classification scheme was used with R0 representing a complete histologic excision with histologic tumor-free margin > 0 mm, R1 representing an incomplete histologic excision without detectable residual gross disease, and R2 representing an intralesional resection with residual gross disease.[18]

Following data collection, the incidence of grade shift between primary and recurrent tumors was determined for both STS and MCT on the basis of the single pathologist review. In addition, the incidence of discrepancy between the single pathologist review relative to the original histology report was determined for both canine STS and MCT. Descriptive statistics were calculated relative to grade shift and outcome variables.

Results

Twenty dogs met the inclusion criteria. Eleven dogs were male castrated and 9 were female spayed. The following breeds were represented: mixed (n = 7), Labrador Retriever (3), Golden Retriever (2), Boxer (1), Husky (1), Pharaoh Hound (1), Bernese Mountain Dog (1), English Springer Spaniel (1), Vizsla (1), Jack Russell Terrier (1), and Border Collie (1). Dates of primary tumor excision occurred between June 30, 2014, and June 3, 2020. The median age of dogs at the time of primary tumor excision was 10.2 years (range, 3.1 to 14.8 years). Of the included dogs, 15 had STS and 5 had MCT. Original histology reports were provided by 14 board-certified pathologists. All grading data is provided **(Table 1)**.

Soft tissue sarcomas

For dogs with STS and local recurrence, tumors were located on the thoracic limb (n = 4), thorax (3), axillary region (3), pelvic limb (3), and ear base/pinna (2). The median maximal dimension of the primary STS at the time of excision was 4 cm (range, 0.75 to 20 cm). One dog received metronomic chemotherapy (16 weeks duration, protocol not reported) following incomplete excision of the STS by its referral veterinarian prior to excision of the STS; no other dogs received neoadjuvant treatments. On preoperative staging, 1 dog had a mass in the thoracic cavity, but fine-needle aspiration and cytology was nondiagnostic; no other dogs with STS had evidence of metastatic disease prior to primary tumor excision. Surgical dose for excision of the primary STS was marginal in 11 dogs, narrow in 1 dog, and wide in 3 dogs. Upon original histologic margin evaluation, 9 primary STS were characterized as R1 and 6 were characterized as R0. Two tumor subtypes were classified as peripheral nerve sheath tumors. In the original pathology report of the primary excised STS, 2 tumors were grade 1, 12 tumors were grade 2, and 1 tumor was grade 3. Upon blinded pathologist review, 8 tumors were grade 1, 5 tumors were grade 2, and 2 tumors were grade 3. Of the 15 primary STS excisional samples, tumor block recuts were made during single pathologist review in 4 cases. The original histology report and single pathologist review differed in grading for 7 of 15 (46.7%) primary STS excisional biopsies; for all of these cases,

Table 1—Grade data for each dog's excisional biopsy samples (primary tumor and recurrent tumor), relative to both the original histology report and single pathologist review. Grade shift data provided are relative to single pathologist review.

Tumor type	Primary tumor–original pathology grade	Primary tumor–single pathologist review grade	Recurrent tumor–original pathology grade	Recurrent tumor–single pathologist review grade	Grade shift (no, increased, or decreased)
STS	2†	1*†	2	2*	Increased
STS	2	2	3†	2†	No
STS	2†	1*†	2	2*	Increased
STS	2†	1†	2†	1†	No
STS	2	2*	2†	1*†	Decreased
STS	2	2	2	2	No
STS	2†	1*†	2	2*	Increased
STS	2†	1†	2†	1†	No
STS	1	1*	2	2*	Increased
STS	1	1	1	1	No
STS	3	3	3	3	No
STS	2†	1†	1	1	No
STS	2	2	Not graded	2	No
STS	2	2*	3†	1*†	Decreased
STS	2†	3*†	2	2*	Decreased
MCT	High/3	High/3	High/3	High/3	No
MCT	High/3	High/3	High/3	High/3	No
MCT	Low/2	Low/2	Low/2	Low/2	No
MCT	Low/2	Low/2	Low/2	Low/2	No
MCT	Low/2	Low/2*	High/2	High/2*	Increased

*Cases with grade shift (relative to single pathologist review). †Cases with interobserver (original histology report vs single pathologist review) variability.

the grade differed by a single point between pathologists. For the primary STS cases in which tumor block recuts were made, the original histology report and pathologist review differed in grading for 1 of 4 biopsies. Following primary STS excision, definitive fractionated radiation therapy was performed in 2 dogs, and 1 dog received 5 doses of adjuvant doxorubicin.

The median time to tumor recurrence following primary STS excision was 216 days (range, 58 to 708 days). The median time from primary STS excision to recurrent STS excision was 216 days (range, 61 to 847 days). The median maximal dimension of the recurrent STS at the time of excision was 2.2 cm (range, 1 to 7 cm). No neoadjuvant treatments were performed prior to recurrent STS excision. No dogs with STS had evidence of metastatic disease prior to recurrent tumor excision. Surgical dose for excision of the recurrent STS was marginal in 9 dogs, narrow in 1 dog, and wide in 5 dogs. Upon original histologic margin evaluation, 9 recurrent STS were characterized as R0 and 6 were characterized as R1. In the original pathology report of the recurrent excised STS, 2 tumors were grade 1, 9 tumors were grade 2, 3 tumors were grade 3, and 1 tumor was not graded but features were consistent with a grade 2 to 3. Upon single pathologist review, 6 tumors were grade 1, 8 tumors were grade 2, and 1 tumor was grade 3. Of the 15 recurrent STS excisional samples, tumor block recuts were made during single pathologist review in 3 cases. The original histology report and single pathologist review differed in grading for 6 of 15 (40.0%) recurrent STS excisional biopsies; for 5 of these cases, the grade differed by a single point between pathologists, and for 1 case, the grade differed by 2 points between pathologists. For the recurrent STS cases in which tumor block recuts were made, the original histology report and pathologist review differed in grading for 1 of 3 biopsies. Based on the single pathologist reviews, grade shift occurred between the primary and recurrent STS in 7 of 15 (46.7%) dogs; in 4 dogs the grade increased and in 3 dogs the grade decreased. Alternatively, based on the original histology reports, grade shift occurred between primary and recurrent STS in 4 of 15 (26.7%) dogs; in 3 dogs the grade increased and in 1 dog the grade decreased. Following recurrent STS excision, definitive fractionated radiation therapy was performed in 3 dogs, and no dogs received adjuvant chemotherapy.

Following the initial recurrent STS excision, additional recurrence was documented in 3 dogs at 71, 283, and 285 days following excision of the initial recurrent tumor and 225, 499, and 346 days following excision of the primary tumor, respectively. None of these dogs had grade shift between the primary and initial recurrent tumors on the basis of single pathologist review (1 dog had grade 1 STS, and 2 dogs had grade 2 STS). No additional treatment was elected for 1 dog, 1 dog was euthanized, and the tumor was cytoreduced with residual gross disease remaining (R2) in 1 dog (no histology result available). At study completion, 7 dogs with STS were lost to follow-up and 8 dogs with STS were dead (euthanasia in 7 of 8, and unknown etiology in 1 of 8). For dogs lost to follow-up, the median duration of follow-up after the recurrent STS excision was 149 days (range, 12 to 612 days). For dogs that were dead, the median survival time after the recurrent STS excision was 626 days (range, 123 to 988 days). Of the 8 dogs with recurrent STS that did not survive, causes of death included suspected pulmonary metastasis and aspiration pneumonia (based on thoracic radiographs) in 1 dog that had tumor grade shift (grade 1 to grade 2) on single pathologist review, a progressively enlarging pulmonary nodule/mass (unknown diagnosis) in 1 dog that had grade 1 recurrent STS with no evidence of grade shift, declining mobility in 1 dog, pulmonary carcinoma with metastasis (based on necropsy) in 1 dog, disease recurrence in 1 dog with recurrent grade 1 STS without evidence of grade shift, suspected progressive disease (unknown local vs systemic) in 1 dog with grade 2 STS and no evidence of grade shift, and unknown cause in 2 dogs.

Mast cell tumors

For dogs with MCT and local recurrence, tumors were located on the pelvic limb (n = 2), inguinal region (1), perianal region (1), and thoracic limb (1). The median maximal dimension of the primary MCT at the time of excision was 3 cm (range, 3 to 8 cm). No neoadjuvant treatments were performed prior to primary MCT excision. No dogs with MCT had evidence of metastatic disease prior to primary tumor excision. Surgical dose for excision of the primary MCT was marginal in 4 dogs and wide in 1 dog. Upon original histologic margin evaluation, 4 primary MCT were characterized as R1 and 1 was characterized as R0. In the original pathology report of the primary excised MCT, 3 tumors were low grade/grade 2 and 2 tumors were high grade/grade 3. Upon single pathologist review, 3 tumors were low grade/grade 2 and 2 tumors were high grade/grade 3. Of the 5 primary MCT excisional samples, tumor block recuts and new slides were made during single pathologist review in 1 case. The grade determined by single pathologist review was consistent with that of the original histology report for all 5 of the primary MCT excisional biopsies. Following primary MCT excision, definitive fractionated radiation therapy was performed in 1 dog and 2 dogs received adjuvant chemotherapy (lomustine and vinblastine in 1 and vinblastine alone in 1).

The median time to tumor recurrence following primary MCT excision was 380 days (range, 40 to 1,303 days). The median time from primary MCT excision to recurrent MCT excision was 385 days (range, 41 to 1,355 days). The median maximal dimension of the recurrent MCT at the time of excision was 1.2 cm (range, 0.7 to 3 cm). No neoadjuvant treatments were performed prior to recurrent MCT excision. No dogs with MCT had evidence of metastatic disease prior to recurrent tumor excision. Surgical dose for excision of the recurrent MCT was marginal in 3 dogs and wide in 2 dogs. Upon original histologic margin evaluation, 2 recurrent MCT were characterized as R1 and 3 were characterized as R0. In the original

pathology report of the recurrent excised MCT, 2 tumors were low grade/grade 2, 1 tumor was high grade/grade 2, and 2 tumors were high grade/grade 3. Upon single pathologist review, 2 tumors were low grade/grade 2, 1 tumor was high grade/grade 2, and 2 tumors were high grade/grade 3. Of the 5 recurrent MCT excisional samples, tumor block recuts were made during single pathologist review in 1 case. The grade determined by single pathologist review was consistent with that of the original histology report for all 5 of the recurrent MCT excisional biopsies. Based on both the single pathologist reviews and original histology reports, grade shift occurred between the primary and recurrent MCT in 1 of 5 dogs; in this case, the 2-tier grade increased (low to high) but the 3-tier grade (2) did not change. Following recurrent MCT excision, 2 dogs received adjuvant chemotherapy (vinblastine in 1 and not documented in 1) and no dogs received adjuvant radiation therapy.

Following the initial recurrent MCT excision, additional recurrence was documented in 3 dogs at 74, 157, and 405 days following excision of the initial recurrent tumor and 155, 1,512, and 776 days following excision of the primary tumor, respectively. One of these dogs (the dog with recurrence at 157 days following initial recurrent tumor excision) had grade shift between the primary (low grade/grade 2) and initial recurrent (high grade/grade 2) tumors on the basis of single pathologist review; the other 2 dogs did not have evidence of grade shift between primary and initial recurrent MCT (high grade/grade 3 for both dogs). One dog received prednisone followed by palliative radiation therapy, 1 dog received palliative radiation therapy and vinblastine, and 1 dog received palliative radiation therapy alone. At study completion, 1 dog with MCT was lost to follow-up 527 days after the recurrent MCT excision, 1 dog with MCT was alive 1,330 days after the recurrent MCT excision, and 3 dogs with MCT were not alive (all euthanized). For dogs that were dead, the median survival time after the recurrent MCT excision was 272 days (range, 261 to 1,154 days). Of the 3 dogs with recurrent MCT that did not survive, causes of death included progressive local recurrent disease in 1 dog with high grade/grade 3 MCT without evidence of grade shift, disseminated MCT (including skeletal muscle, spleen, and heart involvement) in 1 dog, and unknown cause in 1 dog. The dog with disseminated MCT also had recurrence of local MCT disease, and this was the dog with MCT that had evidence of grade shift with increased tumor grade of the recurrent tumor relative to the primary tumor.

Discussion

This is the first known report in veterinary medicine to identify grade shift between primary and recurrent tumors and document its incidence within a cohort. Because of the potential for variability in grading between pathologists, a single pathologist performed blinded assessments and grading of all primary and recurrent tumors that were included in this study, such that interobserver variability was removed as a potential confounding factor in determination of grade shift relative to the single pathologist review. In addition, only cases with excisional biopsy samples (both primary tumor and recurrent disease) were included due to the potential for inaccuracy of incisional biopsy samples compared to excisional biopsy samples owing to tumor heterogeneity.[19] Ultimately, in this study and using these inclusion criteria, 7 of 15 (46.7%) STS recurred as a different grade than the primary tumor, and 1 of 5 MCT recurred as a different grade (2-tier) than the primary tumor. These findings importantly highlight the potential for grade shift in recurrent STS and MCT in dogs, even when possible confounding factors, such as interobserver variability and inaccuracy of incisional samples relative to excisional samples, are removed. This represents a potentially high proportion of dogs that can be expected to have a different grade, relative to the primary tumor grade, upon potential tumor recurrence. Interestingly, of the dogs with STS grade shift, 4 of 7 recurred with a higher tumor grade (by a single grade point) and 3 of 7 recurred with a lower tumor grade (by a single grade point), such that overall 4 of 15 (26.7%) dogs with recurrent STS had progression of tumor grade and 3 of 15 (20.0%) dogs with recurrent STS had regression of tumor grade. The only dog that had MCT grade shift had tumor recurrence with a higher 2-tier grade and no change in the 3-tier grade. These findings emphasize that if grade shift occurs, either progression or regression of tumor grade is possible, and not every case develops greater characteristics of malignancy upon recurrent disease if grade shift occurs. This finding is consistent with human literature on grade shift, in which both grade progression and regression have been documented with recurrence for multiple tumor types.[8–10] This information should be taken into account when considering prognosis in the setting of tumor recurrence as well as potential treatment options both following primary tumor excisional biopsy results as well as in the face of recurrent disease. In summary, in this patient population, nearly half of dogs with recurrent STS had a different grade compared to the primary tumor, and of those, slightly more than half had an increase in tumor grade. Although the recurrent tumor grade shifted by only 1 point for these cases, this difference may be clinically relevant with regard to tumor behavior, as grade-dependent propensity for local recurrence and metastasis has been well documented for primary STS in dogs, though this data is limited in the setting of recurrent disease.[12] The 1 case of grade shift for recurrent MCT in this study demonstrates that grade shift is also possible for recurrent MCT cases. However, the small sample size should be considered with regard to assessment of incidence of grade shift for these diseases.

An additional important finding of this study is the high incidence of discrepancy in tumor grading between pathologists (original histology report compared to single blinded pathologist review). Interestingly, differences in tumor grading relative to the original histology interpretation and blinded

pathologist review occurred in 13 of 30 (43.3%) STS excisional biopsy samples and in no MCT excisional biopsy samples. Interobserver variability in MCT grading, particularly the 3-tier grading scheme, has previously been reported as high, and the relatively low number of MCT excisional biopsy samples in our study (n = 10) should be taken into account; with a larger sample size, it is possible that greater interobserver variability would have been detected.[13] Our reported interobserver agreement for STS (17 of 30 [56.7%]) was relatively similar to that previously reported (47.0%).[15] With regard to the difference in pathologist interpretations, we considered the tumor block recuts and potential for evaluation of slightly different portions of the tumor between the blinded single pathologist and initial histology interpretations, as tumor heterogeneity could potentially alter grading. However, recuts of the formalin-fixed paraffin-embedded tumor blocks and assessment of new slides were only performed for 7 of 30 (23.3%) STS excisional biopsy samples and 2 of 10 MCT excisional biopsy samples, and variability in the reported grades occurred in the minority of cases with recuts (2 of 7 STS recut cases and 0 of 2 MCT recut cases). Additionally, since these were excisional biopsies, even a slight tumor difference that could occur with a 5-μm deeper cut was considered unlikely by the authors to be substantial enough to change the grade of a large representative sample of the tumor. However, for the recurrent STS excisional sample that differed by 2 grading points between pathologists, a recut of the tumor block was used for this specimen, such that heterogeneity between evaluated slides cannot be excluded as a potential cause for the large variation in pathologist grading of this case. Furthermore, for the majority of cases with altered grading between pathologists (11 of 13 [84.6%] STS), the same slides were reviewed without recuts between pathologists.

Regardless of the cause of interobserver variability, this highlights an inherent potential for differences in tumor grading between pathologists on the basis of the currently used STS grading scheme. Prior studies in humans with sarcoma have demonstrated the importance of histologic peer review, and a recent study reported that second pathologist review significantly altered management decisions in 25% of cases.[20,21] It is becoming routine practice in human oncology to require histology review by an in-house pathologist who is considered an expert in the field prior to making treatment decisions. The findings of our study support a need to consider pathology review in veterinary medicine, particularly patients with STS, as the potential for alteration in grade is high with second pathologist review, and tumor grade is an important prognostic indicator that strongly influences adjuvant treatment and surveillance recommendations. Currently, this practice is not readily available in veterinary medicine, such that this study was more realistic to standard diagnostic practices. A large-scale study with multiple pathologists and consensus diagnosis or, alternatively, a more refined and less subjective grading system may be able to more accurately predict prognosis in canine STS. Although multiple pathologist review and consensus diagnosis should be considered and further evaluated, optimization of accuracy and consistency of tumor grading by a single board-certified pathologist is important in veterinary medicine due to diagnostic and clinical limitations to routine multiple pathologist review for clinical cases. This highlights a potential need for refinement of the canine STS grading scheme in an effort to reduce the high rate of interobserver variability demonstrated by Yap et al and the current study results.[15] Additionally, there is potential for significant tumor heterogeneity and there are not clear guidelines in veterinary medicine on determining the appropriate or standard area of sections to ensure accurate grading and tumor representation.

Another finding of interest in this study is the long-term outcomes of many dogs with recurrent STS and MCT, and only 3 dogs of this study were known to have died of local disease progression (including 1 dog with concurrent disseminated MCT). Prior studies have documented increased risk of tumor-related death and reduced survival times associated with local recurrence of canine STS and MCT.[22–24] Importantly, however, one study demonstrated a difference between outcomes of dogs that were euthanized because of their locally recurrent disease (median postrecurrence survival time, 256 days) as opposed to with their locally recurrent disease (median postrecurrence survival time, 945 days).[23] All dogs in the current study underwent treatment via excision of the recurrent tumors, which is important in considering these results. Therefore, with surgical treatment (excision) and potentially additional adjuvant treatments, dogs with recurrent STS and MCT have potential for long-term survival and often succumb to unrelated disease processes.

This study had several limitations. Due to the retrospective nature of the study with incomplete clinical or original histologic report information and loss to follow-up for multiple patients, there is potential for under- or over-interpretation of findings such as long-term outcomes. Importantly, because of the relatively small sample size of dogs in this study (and smaller sample sizes for the subsets of dogs with STS and MCT), descriptive statistics alone were calculated given the risk for error with statistical analysis.[25] Therefore, it was not possible to determine factors associated with grade shift, effect of adjuvant/neoadjuvant therapies on grade shift or outcomes, or prognostic significance of grade shift for dogs with recurrent STS or MCT. However, the sample size was relatively limited due to the strict inclusion criteria of the study, characterized by definitive local recurrence, excisional tumor samples, and acquisition of all tumor blocks/slides for pathologist review, in an effort to reduce confounding factors and strengthen the study results. In addition, a single pathologist review was chosen to remove interobserver variability. Intraobserver variability remains possible in this study. Though strong intraobserver agreement has previously been demonstrated for canine STS histologic review, this has not been well described for canine MCT.[15] A larger sample size with multiple pathologists reviewing the cases and coming to consensus diagnosis could be a more comprehensive way to determine the

true incidence of tumor grade shift. However, individual pathologist interpretation is likely to remain clinically relevant in veterinary medicine and the current techniques for grading canine cutaneous tumors, particularly STS, should be critically evaluated and potentially refined to reduce the high interobserver variability rate, similar to the approach that has previously been taken with development of the 2-tier MCT grading system.

A final important consideration is that although our study methods were designed to minimize the effect of tumor heterogeneity on our results, it may still be a factor in the difference in grade between primary and recurrent tumors as opposed to a true grade shift. When assigning the grade of a tumor, representative regions of the entire tumor are evaluated histologically. Information is lacking with regard to what proportion of the tumor or how many representative slides need to be examined histologically for accurate tumor assessment and grading. Mitotic activity and necrosis contribute significantly to grade and can be variable throughout the tumor. Representative sections of the tumor may affect the grade depending on the heterogeneity within the tumor. It is possible therefore that when the primary tumor and the recurrent tumor were evaluated, even if they were biologically of the same grade, the heterogeneity of the tumor resulted in the assignment of a different grade. This has been demonstrated in studies comparing grade between incisional and excisional biopsies of the same tumors, where grade has been reported to be different in 41% of canine STS and up to 10% of canine MCT.[19,26]

In conclusion, this study represents the first to date in veterinary medicine to document the potential for grade shift with local recurrence of cutaneous/subcutaneous STS and cutaneous MCT in dogs. Based on this data, alteration in tumor grade appears to be common in recurrent canine STS. In the setting of grade shift, both increased and decreased tumor grades are possible with local recurrence. Variability between pathologists in STS grading is common and should be considered as a potential source of alteration in tumor grades when comparing primary and recurrent tumors. As such, as is becoming standard practice in human oncology referral centers, additional pathologist review should be considered prior to implementing treatment decisions. Strategies to refine canine tumor grading protocols should also be investigated to improve accuracy and reduce variability in grading between pathologists. Validated grading schemes that minimize the potential role of intra- and inter-observer variability and account for tumor heterogeneity are needed in companion animals. Additional large-scale studies with multiple pathologists are needed to determine factors that may be associated with progression and regression of tumor grade in recurrent disease as well as prognostic significance of grade shift for recurrent canine STS and MCT.

Acknowledgments

The authors received no grant funding in association with the cases described in this report.

The authors have nothing to declare.

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Supplementary Materials

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