A Retrospective Study of 87 Cases of Canine Soft Tissue Sarcomas, 1986–2001

David A. Heller, DVM[*]
Martha E. Stebbins, DVM, PhD[†]
Taylor L. Reynolds, DVM[†]
Marlene L. Hauck, DVM, PhD[*]

[*]*Department of Clinical Sciences*
[†]*Department of Population Health and Pathobiology*
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

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ABSTRACT

This study was undertaken to determine if a previously published variable for soft tissue sarcomas (STS), histologic grade, had relevance in a clinical setting, as well as to identify other potential prognostic variables and to determine the metastatic rate of canine STS for future studies. Medical records of dogs presented to the North Carolina State University Teaching Hospital for evaluation of histologically confirmed soft tissue sarcomas were reviewed retrospectively. Data collected included information on signalment, tumor characteristics (histologic grade, mitotic index, and tumor volume), treatments administered, local recurrence, and the development of metastases. Using multivariate analysis, the only prognostic variable for development of local recurrence was total radiation dose. In the metastasis model, prior tumor surgery, hyperthermia treatment, and non-intact state regarding spaying and castration were identified as prognostic variables. This information will assist in the prospective evaluation of adjuvant modalities on the outcome for dogs with STS.

Dr. Heller is currently with the College of Veterinary Medicine, University of Illinois, Urbana IL.

INTRODUCTION

Soft tissue sarcomas (STS) are a relatively common neoplasm in the dog, comprising between 15% and 20% of all cutaneous and subcutaneous tumors.[1] These tumors develop from a wide variety of mesenchymal tissues, including fibrous and adipose tissue, skeletal and smooth muscle, endothelial tissue and its associated structures, tenosynovial tissue, and others. STS are pseudoencapsulated, locally invasive tumors. Fibrosarcomas, myxosarcomas, liposarcomas, hemangiopericytomas, neurofibrosarcomas, and undifferentiated sarcomas are classified as STS based on their similar biologic behavior as relates to local invasiveness and metastatic potential.[2] Surgery is often the initial treatment option in the management of STS, with wide surgical margins considered essential for the management of local disease.[3,4] Completeness of surgical margins has been previously shown to be a positive prognos-

tic indicator for the control of local disease.[4] Radiation is effective at managing microscopic disease.[5,6] The role of chemotherapy in the treatment of canine STS has not been clearly elucidated.

The metastatic potential of STS has been previously associated with higher histologic grade and mitotic count.[3,4] Tumors metastasize most commonly to the lungs, while regional lymph nodes are a relatively rare site of metastases.[5] Prior reports have demonstrated metastatic rates between 8% and 17%.[4,6] A study by Bostock and Dye followed 187 dogs with histologically confirmed STS postoperatively for 3 years or until death during that time period.[3] This study demonstrated local recurrence rates of 62% for high-grade tumors and 25% for low-grade tumors, along with metastatic rates of 15% for high-grade STS and 1.67% for low-grade tumors, these differences being statistically significant. Tumor margins were not evaluated in this study. Higher histologic grade was also shown to be a negative prognostic factor for survival, with number of mitotic figures/10 high performance fields (HPF) as the standard classification scheme.[3] In a separate study evaluating the influence of tumor grade on local recurrence, metastasis, and overall survival, dogs with tumors that had >19 mitotic figures/10 HPF survived significantly less time (median survival, 236 days) than dogs with tumors that had 10–19 mitotic figures/10 HPF (median survival, 532 days) and < 10 mitotic figures/10 HPF (median survival, 1,444 days).[4]

The role of hyperthermia in the treatment of STS has been extensively investigated. Numerous canine studies have examined the role of both local hyperthermia (LH) and whole-body hyperthermia (WBH) in combination with radiation therapy in the management of STS.[7–9] WBH in conjunction with LH has previously shown no significant advantage in duration of local control. In addition, dogs that received WBH in conjunction with LH have been shown to have higher rates of tumor metastases.[8] However, the role of LH and radiation therapy in the treatment of canine STS remains to be more clearly elucidated. A previous phase II study by Gillette et al. demonstrated increased local control in patients receiving LH combined with radiation compared with patients receiving radiation alone.[7] There were no significant differences in complications or metastatic rates between the 2 groups.

The present study looks at naturally occurring STS in dogs that were treated with a variety of modalities excluding chemotherapy. The treatment modalities that were part of this retrospective analysis included surgery combined with postoperative radiotherapy, preoperative radiotherapy and subsequent surgery, or LH combined with radiotherapy. While these tumors can theoretically arise from any surface in the body that contains mesenchymal cells, for the purposes of the present study tumors were restricted to those that were measurable externally. The purpose of the present study is to retrospectively examine the influence of tumor volume and histologic characteristics on the control of local disease and the development of metastases in a population of cases of canine STS that were not treated with chemotherapy. Additionally, the clinical utility of a histologic grading scheme will be evaluated. Ultimately, this information was collected to facilitate the design of prospective trials evaluating the role of chemotherapy in the treatment of canine STS.

Criteria for Selection of Cases

Selection of cases for inclusion into the study was limited to those deemed likely to have adequate follow-up, based on records contained within the North Carolina State University database. Patients that were enrolled in clinical trials involving radiation and hyperthermia were deemed to have adequate follow-up data and were included in the study. Patients enrolled in definitive radiation therapy protocols were also eligible for inclusion, but patients receiving palliative radiation were excluded. Patients

treated with curative surgical resection alone were excluded, due to the inconsistent follow-up data. Patients that received chemotherapy were excluded due to the unknown effects of chemotherapeutics on the development of metastases.

Procedures

Records of 87 dogs referred to the North Carolina State University Veterinary Teaching Hospital (NCSU-VTH) for the management of histologically confirmed STS were examined retrospectively. Patients were initially diagnosed with STS from 1986 to 2001. Data collected included information on patient age at the time of diagnosis, sex, breed, tumor type and anatomic location, histologic grade as determined by a pathologist, mitotic count, treatment modalities used, local recurrence, time to local recurrence, development of metastases, time to the development of metastases, sites of metastases, and tumor volume. Mitotic count was determined by counting the number of mitotic figures in 10 400X HPF using light microscopy. Tumors were graded based on a classification scheme using number of mitotic figures per 10 HPF, then assigned a grade based on that number. Tumors with 9 or fewer mitotic figures in 10 HPF were classified as low grade, and those with 10 or more mitotic figures per 10 HPF were classified as high grade. Tumor volume was defined as the product of the 3 measurable tumor dimensions multiplied by 3.14 (approximating π) and divided by 6. Time to first event was defined as either local recurrence, detectable metastases, or death, and all first event and survival analyses were calculated as the number of days from initial diagnosis. Local recurrence was defined as a biopsy-confirmed tumor at the site of original surgical resection. For patients receiving hyperthermia and radiation to gross disease, local recurrence was defined as a biopsy-confirmed increase in tumor volume of 25% or greater. Detection of metastasis was determined with thoracic radiography, aspiration of regional lymph nodes, or abdominal imaging as indicated. Survival analysis was based on the time from first confirmed histologic diagnosis to the time of death. All causes of death were included in the final survival analysis. This is due to the fact that extrapolation of cause of death as tumor-related or not was impossible in numerous instances.

Slide reviews were performed by a veterinary pathologist (T.L.R.). Cases were selected for review based on inadequate data in the original pathology report. If the original report made no mention of either mitotic count per 10 HPF or mitotic grade, the slides were completely reviewed. If a grade was given, but no mitotic count was present, the slides were reviewed to determine a mitotic number per 10 HPF. Tumor grades were changed as necessary according to a revised mitotic count. If the original report mentioned a mitotic number but did not assign a grade, a grade was assigned using the aforementioned classification scheme, and these slides were not reviewed. The decision to not review all slides was made in order to assess the usefulness of the grading scheme in clinical practice.

Cases were treated with a combination of surgery plus radiation or LH plus radiation. Therapeutic regimens were categorized as presurgical radiation [16 fractions (Fx), 48 gray (Gy) total dose] followed by surgery, surgery followed by radiation (19 Fx, 57 Gy), LH concurrent with radiation therapy (25 Fx, 56.25 Gy), and other protocols (surgery with different radiation therapy protocols, such as 12 Fx, 48 Gy). Cases that presented to the NCSU-VTH oncology service exclusively for radiation therapy to an incompletely resected tumor were all treated with 19 fractions, 3 Gy per fraction, for a total dose of 57 Gy. All radiation was performed using megavoltage ^{60}Co radiation at the NCSU facility.

Univariate analysis was performed to determine predictors of local recurrence and metastases. For the final model, the decision to pick candidate covariates was based on the results of univariate analysis. If an odds ratio (OR) < 0.5 or > 1.5, or a P-value < 0.10 was detected in the univariate analysis, the variables were included in the multivari-

ate model testing. Variables included in the model testing were mitotic count or mitotic grade, sex, intact/altered status, tumor type, prior tumor surgery, age at the time of diagnosis, radiation dose, hyperthermia treatment, development of metastases, tumor volume, and site of metastases. Since mitotic count and mitotic grade are colinear, they were tested independently of one another. Univariate analysis was done using Analyse-It version 1.63 (Analyse-It Software, Leeds, England). SAS 8.0 (SAS Corp., Cary, NC, USA) was used for both the multivariate model building and for the survival analysis.

RESULTS

A total of 87 records were included in the final analysis. Forty-two cases were female and 45 were male. Thirty-seven of the females were spayed females (SF) and the other 5 were intact females (F). Twenty-eight males were castrated (MC) and 17 (M) were intact. Twenty-nine different breeds were represented, with mixed-breed dogs (n = 21) the most common, followed by Golden Retrievers (n = 18) and Labrador Retrievers (n = 14). Seven other breeds were represented more than once. The most common tumor types seen were fibrosarcomas (FSAs; n = 50), followed by spindle-cell sarcomas (SSAs; n = 8), hemangiopericytomas (HPCs; n = 5), myxosarcomas (MSAs; n = 3), and other tumor types (including anaplastic sarcomas and liposarcomas; n = 21). The location of the tumors was highly variable, with tumors located on the head, limbs, and trunk.

Tumors were classified by grade according to the mitotic index. A total of 61 tumors were low grade and 26 tumors were high grade. The mean number of mitotic figures per 10 HPF was 2.9 for the low-grade tumors (range, 0–9) and 22.3 for the high-grade tumors (range, 10–50). Mitotic figures per 10 HPF were not available for 6 of the low-grade tumors and 2 of the high-grade tumors. These 8 cases were not available for review, and consequently they were included in the analysis of the grade variable but excluded from the mitotic count variable. Volume measurements were available for 45 of the tumors. The mean tumor volume was 131.7 cm^3, with a range of 1.3 cm^3 to 943.2 cm^3. In the remaining 41 cases volume measurements were inconsistently recorded or the dogs initially presented with microscopic disease and the referral information did not contain specific volume measurements.

A total of 23 patients received LH as part of an experimental protocol. Of the 23 patients that received hyperthermia, the majority (n = 17) were concurrently treated with a standardized radiation therapy protocol (56.25 Gy in 2.25-Gy fractions). One patient received this radiation therapy protocol without hyperthermia, as it was determined after radiation therapy was begun that the tumor could not be heated. A total of 6 patients received hyperthermia in combination with various other radiation therapy protocols. Treatment outcome of these 23 patients has been previously published.[8] Of the 64 patients that did not receive hyperthermia, 30 received postsurgical radiation at the NCSU standard protocol (57 Gy in 3-Gy fractions). Eighteen patients received presurgical radiation followed by surgical excision of the tumor (48 Gy in 3-Gy fractions). A total of 16 patients received various other pre- and postsurgical radiation therapy protocols.

Prior to initial evaluation at the NCSU-VTH, 51 dogs had surgical excision of their tumor by their local veterinarian. The number of prior surgical excisions was variable and inconsistently recorded, and consequently this potential variable was excluded from analysis. Initial histopathology was used to determine tumor grade. If the initial histopathology report did not indicate a specific grade but did contain a mitotic index, tumors were classified as either low grade (9 or fewer mitotic figures/10 HPF) or high grade (10 or more mitotic figures/10 HPF). A total of 24 cases were reviewed. Grade changes were made based on mitotic count in 2 of the 24 reviews. In both of these cases the grade was changed from low to high. In the final analysis, 61 tumors were

Table 1. Univariate Analysis of Variables for the Local Recurrence Model

Variable	Odds Ratio	*P* Value	Test	Include in Model?
Male/Female	1.093	1.0	Fisher	No
Intact status	0.747	0.77	Fisher	No
Prior surgery	1.591*	0.4329	Fisher	Yes
Hyperthermia	2.400*	0.1276	Fisher	Yes
Metastasis	2.004*	0.2181	Fisher	Yes
Sex (M/F)		0.2764	Chi-Square	No
Tumor type		0.5502	Chi-Square	No
Mitotic count		0.1456	Krukal-Wallis	Yes
Mitotic grade		0.2393	Krukal-Wallis	No
Age		0.3124	Krukal-Wallis	No
Radiation dose		0.007*	Krukal-Wallis	Yes
Tumor volume		0.1905	Krukal-Wallis	No

*Statistically significant for model inclusion. An odds ratio < 0.5 or > 1.5, or a *P*-value < 0.10 in the univariate analysis meant the variables were included in the multivariate model testing.

classified as low grade and 26 were classified as high grade. The local recurrence rate for all cases was 32/87 (36.8%) and the overall metastatic rate was 27/87 (31.0%). Of the 61 low-grade tumors, 20 (32.8%) had local recurrence, and of the 26 high-grade tumors, 12 (46.2%) had local recurrence. The median time to local recurrence was 411.5 days (range, 30–1,553 days). In comparing tumor grade with the development of metastases, 18/61 (29.5%) low-grade tumors and 9/26 (34.6%) high-grade tumors went on to metastasize. The median time to the development of metastases was 229.5 days (range, 0–1,188 days). In the subset of dogs receiving hyperthermia as part of their treatment protocol and who went on to develop metastasis, the median time to the development of metastasis was 300 days.

Univariate analysis in the local recurrence model allowed for the following variables to be included in the model: mitotic count, prior tumor surgery, radiation therapy dose, hyperthermia treatment, and metastases. Table 1 shows the results of the analysis of specific variables versus local recurrence in the univariate analysis. The only variable that remained in the final model was radiation dose. Specifically, the comparison of treatment group 1 (treated with 57 Gy in 3-Gy fractions) to those in treatment group 4 (treated with a variable protocol) was statistically significant using the Wald chi-square test, with $P = 0.0086$. No other variable remained in the model.

Univariate analysis allowed for the inclusion of the following variables in the metastasis model: sex (M/F), intact/altered status (F/FS/M/MC), prior tumor surgery, age at first tumor diagnosis, hyperthermia treatment, and tumor volume. For the results of the analysis of specific variables versus metastasis in the univariate analysis, see Table 2. Statistical modeling using logistic regression identified the following prognostic variables: prior tumor surgery, hyperthermia treatment, intact status. These variables were indicative of a worse prognosis and an increased likelihood of developing metastatic disease. For both the local recurrence and metastasis models, mitotic count and mitotic grade failed to be of prognostic significance.

DISCUSSION

Soft tissue sarcomas are often considered as a single entity in terms of biologic behavior. The STS behave differently from other, more highly metastatic sarcomas, such as osteosarcoma, rhabdomyosarcoma, hemangiosarcoma, and synovial cell sarcoma.[10] In that group

Table 2. Univariate Analysis of Variables for the Metastatic Model

Variable	Odds Ratio	*P* Value	Test	Include in Model?
Male/Female	0.420*	0.1705	Fisher	Yes
Intact status	5.262*	0.0031*	Fisher	Yes
Prior surgery	0.214*	0.0029*	Fisher	Yes
Hyperthermia	8.125*	0.0002*	Fisher	Yes
Metastasis	2.004*	0.2181	Fisher	Yes
Sex (M/F)		0.0001*	Chi-Square	Yes
Tumor type		0.9610	Chi-Square	No
Mitotic number		0.5393	Krukal-Wallis	No
Mitotic grade		0.6393	Krukal-Wallis	No
Age		0.0668*	Krukal-Wallis	Yes
Radiation dose		0.1811	Krukal-Wallis	No
Tumor volume		0.2801	Krukal-Wallis	No

*Statistically significant for model inclusion. An odds ratio < 0.5 or > 1.5, or a *P*-value < 0.10 in the univariate analysis meant the variables were included in the multivariate model testing.

of tumors, systemic therapy is necessary in order to achieve long-term control of the more aggressive sarcomas.[5,10] In contrast to this, STS are often managed with treatments aimed at local control. Wide surgical margins, radiation of incompletely resected tumors, and preoperative irradiation of large tumors in which complete excision is less likely are the cornerstones of therapy for canine STS. However, little is known about the likelihood of local recurrence and metastatic potential of STS based on their histologic characteristics. The purpose of the work presented here was to evaluate the clinical utility of tumor mitotic rate and histologic grade as predictors of biologic behavior. In addition, this work retrospectively examined the potential prognostic factors that may predict local recurrence and the development of metastases in canine patients treated with both conventional and experimental modalities.

At both the univariate and multivariate levels, this study identified only radiation dose as prognostic for the prevention of local recurrence. Specifically, patients that received the NCSU standard protocol for incompletely resected STS (57 Gy in 3-Gy fractions) were more likely to have local control compared with patients who received other protocols. This finding is consistent with prior studies, where long-term local control was achieved with definitive postoperative radiation therapy (798 days and 1,082 days, respectively).[5,6] While the optimal dose and fractionation scheme has yet to be determined for the prevention of local recurrence in incompletely excised canine STS, it appears that doses of 42–63 Gy prevented local recurrence.[5,11]

No correlation was found in this study between tumor grade or mitotic count and the development of local recurrence. This is in contrast to a prior study by Bostock and Dye, in which tumor mitotic rate was prognostic for the control of local disease.[3] Tumor margins were not evaluated in either of these studies. Based on findings by Kuntz et al, where surgical margins were the only prognostic variable identified in the control of local disease, this variable should be considered as more significant than tumor mitotic rate or grade in determining whether local recurrence is likely or not.[4] Future studies should consider tumor margins when determining the likelihood of tumors recurring locally.

The metastatic rate identified in this study is higher than in previously reported studies.[3,4,6] This may be attributable, in part, to the extensive follow-up, including necropsy, required of patients enrolled in the various research protocols. The higher metastatic rate has implications in long-term survivors of

STS (> 3 years) and regular staging is indicated in these patients. Alternatively, the existence of residual gross tumors over a long period of time in patients treated with radiation and hyperthermia may have increased the potential for metastasis in this group. Mitotic count and mitotic grade failed to be of prognostic significance for the development of metastases in this study. This differs from findings in a prior study, where mitotic rate was prognostic for the development of metastases.[4] The lack of prognostic value of the Bostock and Dye grading scheme for STS suggests there may be a need for more complex tumor grading schemes or more consistent application of existing methods between pathologists to better predict the development of metastases. As used in a clinical setting on the cases in this retrospective study, tumor grading failed to be of prognostic value.

Canine STS may have a higher metastatic rate than previously appreciated. The presence of gross tumor may also predispose a patient to the development of metastatic disease. Tumor volume was evaluated as a potential prognostic variable in this study. Research on human sarcoma patients identified tumor volume as prognostic for the development of local recurrence and metastasis.[12,13] However, our findings suggest that tumor volume is not prognostic for either of these variables in canine STS. The role of intact status is difficult to interpret in a pathological fashion, and this finding may be a statistical anomaly due to the low number of intact animals in this study and the high number of statistical comparisons performed. Alternatively, sex hormones may play a permissive role in the development of metastasis. As a retrospective study, this work can only suggest directions of further investigation; however, this information on the incidence of metastasis and the prognostic application of mitotic index can be utilized to more accurately design prospective studies on prognostic variables and the role of adjuvant therapies in the treatment of STS in canine patients. Further study is necessary to more clearly define the role of adjuvant therapy in the management of canine STS.

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