Skin and Subcutaneous Soft Tissue Tumors – Canine
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Introduction

In veterinary medicine, proposed grading systems for soft tissue tumors (STT) formerly designated soft tissue sarcomas (STSs), have suffered from lack of scientific rigor due to small numbers of patient samples, conflation of a variety of tumor types in a single study, lack of standardized histological criteria, and incomplete, unstandardized outcome assessment. A survey of 250 STT/STS pathology reports revealed the histological criteria used to determine grade were included in only 1.2% of reports, preventing correlation between histopathological findings and outcome assessment.[22] The purpose of this protocol is to provide standards for accruing data so that, over time, large data sets with comparable information can be evaluated to enable meaningful conclusions and accurate prognostic information. This protocol is a “living” document which will be modified as new information becomes available. We intend this protocol to guide reviewers in assessing manuscripts for publication to ensure authors have included all required data. Investigators can use this protocol as a checklist to ensure complete data sets are included for study participants. As the histologic criteria that separate benign from malignant canine STT/STS have not been defined, we propose evaluating and grading these tumors using complete data sets as indicated in this protocol to help identify the subset of tumors which will behave aggressively. The tumor type should be diagnosed as specifically as possible, and the histological diagnosis and grade of each tumor, as well as the entire group, correlated with known clinical outcomes.

This protocol is intended for use with the following types of tumors: Perivascular wall tumors (PWT), nerve sheath tumors (NST), fibrosarcoma, myxosarcoma, leiomyosarcoma, liposarcoma, rhabdomyosarcoma or unclassified spindle cell tumor/sarcoma arising in the dermis or subcutis. The intent is to address data gathering from skin and subcutaneous STT/STS which are either difficult to definitively diagnose (such as PWT and NST) or tumors which lack well - defined prognostic criteria.

This protocol is not intended for the following tumor types: Neoplasms in tissues deep to the subcutaneous tissue, including tumors arising in deep skeletal muscle, tumors arising in spinal nerves, chondrosarcoma, osteosarcomas, hemangiosarcomas, histiocytic tumors, lymphoma or mast cell tumor. (Note A). Tumors which involve tissues deep to the subcutis should be excluded unless origin from the dermis or subcutis can be confirmed (ie, early onset restricted to dermis and subcutis or the bulk of the tumor involves dermis and subcutis). However, this protocol could serve as a template for the excluded tumor types and, as unique features of each tumor are published, they would need to be incorporated into the parameters catalogued.

A grading scheme that can be applied to any skin/subcutaneous STT simplifies tumor grading but may not be “best practice”. Future studies need to determine whether there is prognostic value in identifying and grading specific histological diagnoses or validate grading systems which group all STT tumors together. Perhaps identifying the tumor type has distinct prognostic information that may be lost when evaluated in the group as a whole. Moreover, criteria other than grade may better predict outcome or treatments. For example, tumor size and depth have greater prognostic value than histological grade for PWT.[1,30] Alternatively, a different grading system altogether may be preferable. Future studies need to evaluate a greater number of parameters than the present 3 histological features that are used. Canine studies accepted the 3 parameters chosen for human tumors yet in the original description by Trojani there were 4 additional parameters that might be predictive for canine tumors but were not evaluated.[32] Grading criteria should be readily available and reproducible so others can replicate and validate these studies.

Parameters to be included in STT/STS reports

Histological Type (Note A)

_____ PWT

_____ NST

_____ Fibrosarcoma

_____ Myxosarcoma

_____ Leiomyosarcoma

_____ Liposarcoma

_____ Rhabdomyosarcoma

_____ Other (indicate)

_____ Undetermined

Differentiation (Note L) (see future considerations)

_____ Score 1

_____ Score 2.

_____ Score 3.

Surgical Procedure

_____ Incisional biopsy (Note B)

- _____ Wedge
- _____ Needle
- _____ Punch

_____ Excision

_____ Amputation

_____ Re-excisional biopsy (Note C)

Mode of Tissue Assessment

____ Manual light microscopy with glass slide evaluation

____ Whole slide digital image assessment

Tumor Site Within Skin or Subcutis

_____ Extremities (indicate site)

____ Forelimb distal to elbow

____ Forelimb proximal to elbow

____ Hindlimb distal to hock

____ Hindlimb proximal to hock

____Origin from nerve or nerve root?

_____ Trunk (indicate site) _____

_____ Other (indicate site) _____

Tumor Size: (Indicate if gross or histologic assessment)

Greatest dimension: _____

Additional dimensions: _____

Number of histological sections examined: _____

Deepest Tissue Layer Infiltrated: (Indicate if histological assessment vs assessment by imaging/CT scan, etc)

_____ Dermis

_____ Subcutis

_____ Fascial Planes (describe)

_____ Muscular layer

_____ Other

Other Diagnostic Tools

____ IHC (Note D)

____ Molecular (Note E)

____ Imaging studies (CT scan with measurements is preferred imaging technique to estimate depth of invasion)

Mitotic Count (per 2.37 mm^2)

Record number of mitotic figures counted on the appropriate line. See Guidelines 1 and 2

_____ 0 -9

_____ 10 – 19

_____ ≥ 20

Necrosis

(See Guideline 5, Note F and below) *(Estimated percent of tumor which is necrotic.)*

_____ 0 – 10%

_____ 11 – 50%

_____ > 50%

_____ Necrosis estimated by microscopic assessment only

_____ Necrosis estimated by gross and microscopic assessment

_____ Necrosis estimated by imaging, state mode

Margins

(See Guideline 3)

Histologic tumor free distance (HTFD) is the shortest distance between tumor and the inked margin. Measure margins in mm (no decimals) as accurately as possible. Consider reporting focal if only a few foci of tumor cells are present at the margin and diffuse if large numbers of tumor cells are at the margin. (Note G; Guideline 3). Indicate if imaging or other technology was used to determine tumor infiltration of surrounding tissues.

Method of margin assessment

(See Guideline 3)

___radial

___tangential

___combination (specify)

___ number of sections examined

___ parallel (breadloaf)

___width between sections

Lateral (Peripheral) Margin

(See Guideline 3)

_____ number of sections examined

_____ no tumor at margin; HTFD (mm tumor to margins)

_____ tumor is at margin

____ focal

____ diffuse

___tangential (since HTFD cannot be assessed in tangentially sectioned margins), indicate:

___ tumor cells present at margin

____ Number of sections with tumor cells

___ tumor cells not present at margin

____ Were margins inked at the time of surgery?

_____Yes

_____No

_____ if no, were margins inked by lab personnel? Y N

_____ Margins not assessed (Explain)

Deep Margin (See Guideline 3)

____ number of sections examined

____ no tumor at margin; HTFD (mm tumor to margins)

_____ tumor is at margin (Note G)

____ focal

____ diffuse

___tangential (since HTFD cannot be assessed in tangentially sectioned margins), indicate:

___ tumor cells present at margin

____ Number of sections with tumor cells

___ tumor cells not present at margin

____ Were margins inked at the time of surgery?

_____Yes

_____No

_____ if no, were margins inked by lab personnel? Y N

_____ Margins not assessed (Explain)

_____ Fascial plane below tumor?

Lymphovascular Invasion

(See Guideline 4)

Lymphovascular Invasion (report format below)

_____Not identified

_____ Equivocal

____ Present

Criteria used to determine lymphovascular invasion

____Thrombus adherent to intravascular tumor

_____Tumor cells invading through a vessel wall and endothelium

_____Neoplastic cells within a space lined by lymphatic or blood vascular endothelium

_____Neoplastic cells in a structure that has been confirmed to be a lymphatic or blood vessel using immunohistochemistry

Number of LVI foci (within a minimum of one representative section of tumor and peritumoral tissue. Report the number of foci of LVI within all sections examined.)

_____ Few (< 5 foci)

_____ Moderate (5 – 10 foci)

_____ Many (> 10 foci)

Type of vessels invaded

_____ Muscular wall evident

_____ No muscular wall evident

Site of lymphovascular invasion

_____ Intratumoral (number of LVI foci)

_____ Peritumoral (number of LVI foci)

Metastasis (Note J)

Indicate the means to confirm metastatic tumor (i.e., histopathology from biopsy/autopsy tissue; fine needle aspirate cytology, etc).

_____Not present

_____Suspected based on image studies, but not confirmed (Note J); Indicate if presence of metastasis was based upon imaging studies with no confirmatory tissue sampling.

_____ State mode of imaging

_____Present (indicate sites)

_____ Lymph nodes (Indicate sites)

_____ Lungs

_____ Other (Indicate)

_____ Not determined

Grade

(Optional) Indicate system used [1-5] (Note K)

_____ 1

_____ 2

_____ 3

Discussion:

The term STT/STS encompasses a wide range of tumor types in humans and in animals and, although the veterinary terminology and various grading schemes have, in many instances, been borrowed from the human literature, the types of neoplasms which commonly comprise soft tissue tumors in humans are very different from the tumor types typically encountered in animals.[7,10,11,14,20,24,28,32] This is exemplified by liposarcomas, which are common in humans and rare in dogs, and PWTs that are very common in dogs

but rare in humans. The common denominator between species is an origin in non-epithelial soft tissues. In the case of canine tumors, STT is recommended in place of the designation soft tissue sarcoma (STS) because the biological behavior of the most common forms of these tumors (PWT and NST) in dogs is predominantly indolent and does not warrant the diagnosis of sarcoma. The term sarcoma suggests the group of neoplasms are malignant, however present outcome assessment data indicates that the majority do not recur and metastases are infrequent.[7,20,24,28]

The effect of grouping disparate tumors within the same grading scheme needs to be compared to grading tumors segmented into specific histological diagnoses so that important predictive parameters may be determined. Canine STT/STS studies initially grouped these tumor types together due to their morphological similarities and the assumption that biological behavior was also similar. In recent years, knowledge advances have allowed us to better differentiate types of STT/STS, leading to an improved understanding of their behavior. It can now be argued that the inclusion of PWTs, which metastasize infrequently (4-10%), in the same category with malignant nerve sheath tumor, fibrosarcoma and undifferentiated sarcoma is inappropriate.[1,2] Moreover, the collective grouping of various tumor types under the umbrella term STT/STS complicates the overall prognosis for STT/STS because the inclusion of rare tumors, which may behave much differently, clearly biases statistical analysis of outcomes. Hypothetically, statistical analysis performed on a group of 75 PWT and 2 liposarcomas, it seems obvious the *statistical behavior* predicted for the 3 rare tumors is biased by the 75 tumors, which are of different cell origin and with no obvious relationship to the PWTs, other than mesodermal heritage. Alternatively, grading systems which appropriately categorize low, intermediate and high - grade neoplasms, regardless of the type can provide appropriate prognostic information, if each included tumor type is adequately represented to allow meaningful statistical analysis. Although this protocol uses the term STT/STS, the authors recommend that each of the specific tumor types be studied individually in addition to collective assessment. Exceptions to this may be studies of tumors with undifferentiated histological features or features intermediate between NST and PWT where a specific diagnosis may not readily be made without ancillary studies (IHC, molecular studies, etc).

Histological features characteristic of PWT and NST have been described, but there is overlap of many histological patterns found in these two tumor types[1-3,6,19,23,28,31,33] which can complicate definitive diagnosis in routinely stained sections. Whorls, streams, fascicles, storiform arrangements and Antoni A and Antoni B patterns are described in both NST and PWT. Verocay bodies are a distinctive feature of benign nerve sheath tumors and consist of regions of palisading nuclei forming compact rows separated by fibrillary cell processes.[17] Regions reminiscent of Verocay bodies have been described in diverse, cutaneous, non-neural tumors in humans[5] and have been described in canine PWT.[1,3] Recognition of this pattern may aid in the prevention of misdiagnosis of neural origin in tumors of diverse histogenesis. Future studies need to clarify how important this histologic feature is to differentiate tumors that look similar.

Studies relating histological features to patient outcome in animals suffer from limited sample size and lack of standardization of data collection and outcome assessment. Greater than 95% of reported STT/STS did not recur when margins were free of tumor.[20,24] We have studied these neoplasms for 40 years and know that if they are graded 90% will be grade 1 or 2; a small percentage are grade 3 and aggressive. As the histologic criteria that separate benign from malignant STT/STS have not been defined, we propose evaluating and grading these tumors using complete data sets as indicated in this protocol to help identify the subset of tumors which will behave aggressively. Molecular profiles are now used in human pathology to help predict tumor-host behavior, and this is needed for canine STT/STS.

The existing grading scheme, adapted from the French system in humans,[7,11,15,20] needs validation with methods described in such detail that others can duplicate them. Some criteria, such as determination of the percentage of necrosis via gross and/or histological criteria, are poorly defined in the human literature and were not clarified in the veterinary manuscripts. Percent necrosis for human tumors was determined by estimating the amount seen grossly and histologically. There are a number of distinctions between how the grading systems are used for human tumors and how they are applied to canine tumors, and these have not been addressed in the veterinary studies,[7,11,20,24,32] in particular, the need to determine histological tumor type and confirmation of the diagnosis

of sarcoma *prior* to applying the human grading systems. That is, the grading systems for human tumors are not intended to distinguish benign from malignant neoplasms but are used to grade malignant neoplasms.[11,15,29,32] In many instances in dogs, the grading system is applied to tumors before the determination of malignancy is made and, in some instances, without designation of specific tumor type beyond STT/STS. The distinctions between human and canine STT/STS and how the grading systems are applied warrants broadening the number of parameters evaluated in dog tumors to assess if any of the initial criteria evaluated in humans could be useful in grading dog STT/STS despite the apparent lack of utility in assessing human tumors. The histological features not investigated in dogs are tumor cellularity (Score 1: tumor cells < 50%; ie stroma = more than half of the tumor; Score 2: tumor cells representing > 50% examined surface), atypical nuclei (Score 1: atypical nuclei in < 50% of tumor surface examined; Score 2: atypical nuclei in > 50% of tumor surface examined), malignant giant cells (Score 0: no malignant giant cells; Score 1: malignant giant cells < 50% of tumor cell surface; Score 2 malignant giant cells > 50% of tumor surface) and vascular emboli (Score 0: none; Score 1: present).[32] In humans, and likely in animals, the histological subtype can define the behavior, which could make tumor grade redundant. Research clarifying the differential behavior of STT/STS types with both univariate and multivariate analysis of histological features is expected to lead to improved application of grading or new means to evaluate these tumors.

For any proposed veterinary tumor grading system, the tumor type should be designated as precisely as possible and the criteria used to designate that diagnosis be provided (H&E, IHC etc). Each graded element must be clearly defined. For instance, the means to assess percent necrosis (gross, histology, both; see Note F) must be clarified if this is an element of a grading system and others are expected to duplicate the method.[20,25] Grading systems should compare the utility of evaluating tumor collections of uniform histological type versus assessing collections comprising tumors of diverse histological type.

Many grading systems and case series reviews focus on the MC as a key prognostic indicator. However, the literature on canine STT/STS has reported different MC cutoffs of: $\underline{\geq}$ 9, ranges of 0-5, 6-9, >9 and ranges of 0-9, 10-19 and >19.[7,20,24] None of these referenced papers

accurately reported the total area in which mitotic figures were counted. Grading criteria should be readily available and detailed so others can replicate and validate these studies. Results of published studies in which material and methods are inadequately described lack credibility and cannot be validated by other investigators. Ten years ago a call was made for improved design and reporting of canine STT/STS prognostic studies,[14] yet some of the most critical knowledge gaps are yet to be addressed and even recent studies fail to meet described standards. This is exemplified in a recent publication reporting outcomes of dogs with STS treated with radiation therapy in which STS grades were assigned by various systems, mitotic figures were assessed by outdated methods, various treatment modalities were included and data was obtained through phone surveys of owners. Tumor grades were obtained through medical record review but criteria for determining grades was not indicated. Criteria for confirming tumor recurrence or presence of metastatic lesions were not described.[13] How can any scientifically useful information be obtained from heterogeneous sources of information? Reviewers of manuscripts are important gatekeepers of sound, reproducible scientific data. Editors and reviewers of canine STT/STS prognostic studies must vigilantly enforce the standards outlined in 2011 and updated in this document if we are to improve prognostication and clinical management.

These studies will require accurate, standardized diagnoses and parameter assessments by pathologists correlated with accurate standardized outcome assessments on as many cases as possible. Proposed grading systems should give detailed descriptions and examples of the scored parameters and interobserver variation should be assessed among a group of pathologists. Presently, outcome assessments are the weakest link and without these any grading schemes lack clinical utility. Published manuscripts of canine STT/STS have too few cases designated as *high grade* and correlated with outcomes to be reliable.[7,20,24] As an example, in one study only 9 of 139 graded tumors were high grade and only 4 of these had outcome assessments.[24] Conclusions based on such limited outcome assessment may be flawed, and are certainly weak.

Histologic classification of some types of STT/STS is difficult, and final classification may require IHC or other ancillary tests. In veterinary medicine, the costs for these tests are incurred by owners. If the tests are declined, it is unreasonable to expect a precise

classification of some of these tumors. A grading scheme that can be applied to any tumor within the parent group is therefore desirable. However, our existing scheme needs to be broadened to determine if parameters originally rejected for human soft tissue sarcomas may, in fact, be predictive in dogs. Furthermore, the methods described to assign scores for necrosis, MC and differentiation for canine tumors are not detailed enough that others can replicate them, and the number of dogs reported with high grade STT/STS that have outcome assessments is too small to be reliable. These studies need to be repeated with additional parameters described such that others can replicate them and with greater numbers of dogs with accurate standardized outcome assessments if we wish to improve patient care.

Notes:

A. This protocol is intended to address the extent of data that should be gathered on skin and subcutaneous STTs which have been designated in the prior literature as soft tissue sarcoma and consist of neoplasms originating in the skin and subcutis and variably identified as nerve sheath tumors, perivascular wall tumors, fibrosarcomas, myxosarcomas, leiomyosarcomas, rhabdomyosarcoma, liposarcomas or poorly differentiated spindle cell tumors or sarcomas. Primary tumors of muscle origin or tumors arising in tissues other than the skin or subcutis should be considered separately from STT/STS in this protocol. Nerve sheath tumors can arise from spinal nerves or nerve roots and should be considered separately from NST arising in the dermis or subcutis. If NST is documented to arise from spinal nerves/nerve roots, this should be documented in tumor location. The data gathered for STT/STS may prove useful for other tumor types but should not be grouped together with STT/STS. Studies should evaluate the prognostic utility of assessing specific tumor types separately (if the tumor type can be identified) to determine if the specific histological diagnosis is predictive of tumor behavior. Additional statistical evaluation of the entire group of STT/STS can be compared to that of individual tumor types to determine if outcome is equally associated with histological diagnosis versus a general grading system applicable to any STT/STS.

B. It may not be possible to assess all parameters in biopsy specimens. A preliminary study comparing presurgical biopsy and excisional surgical specimens showed low sensitivity for detecting high-grade lesions but high specificity for identifying high-grade neoplasms.[27] Excisional biopsies should be the goal for inclusion in this protocol.

C. If the specimen is from a re-excisional biopsy procedure as described by Bacon et. Al.,[4] the original diagnosis and tumor grade should be reported together with diagnosis and grade of the re-excised tumor. Finding residual neoplastic cells in a re-excision specimen from dogs in which tumor may be at the margin poses a problem in interpretation; confounders are how extensively the specimen is sampled and that standardized protocols are not validated.[8,9] Presence or absence of tumor in re-excision specimens did not accurately predict recurrence in one study of canine STT/STS. In this study, 39/41 dogs had tumor at the original excision margin and 32 (82%) dogs did not have tumor seen in the re-excision. Nine of 41 dog (22%) had tumor in the primary re-excision specimen whereas 32/41 (78%) had no tumor in the re-excision specimen. Six tumors recurred, 4 of which had no tumor seen in re-excision specimen and 2 of which had tumor seen in the er-excision specimen.[4] Attempts to improve trimming of surgical margins have been made with well defined and reproducible sampling protocols.[1] Surgical margin assessment in human STS demonstrates the need for more detailed assessment of the relationship of the tumor cells to critical anatomic structures including perineural and perivascular tissue, bone and tissue barriers.[10] In addition to searching for neoplastic cells in re-excision specimens, determination if the re-excision biopsy extends to normal tissue should be reported, indicating the entire lesion was removed.

D. A number of IHC markers have been described in NST and PWT.[2,3,23,31] Recommended IHC markers for PWT are smooth muscle actin (clone 1A4) or muscle actin (clone HHF35) and calponin. Calponin is regarded as the most sensitive for PWT identification. Desmin IHC is an optional marker for PWT. For

confirmation of malignant NST, IHC for nerve growth factor receptor and for Olig 2 are recommended.[31]

E. Indicate type of molecular test performed, method and results. Molecular profiling can aid in the diagnosis and characterization of tumors and has been shown to correlate with therapeutic responsiveness and prognosis in humans.[12,26] Molecular studies have not been extensively employed in veterinary oncology although molecular profiling has been performed in canine thyroid tumors and lymphoma.[16,18]

F. The percent tumor necrosis is included in this protocol because this parameter has been utilized in published tumor grading schemes in humans and animals[7,11,20,24,25,29,32] The means of assessing the percent of necrotic tumor has not been fully defined and remains subjective. Original methods used for human tumors included gross and histologic assessment of necrosis. Studies on dogs did not indicate if gross observations were used in combination with histological assessment, as in humans, or if only histologic assessments were evaluated.[20,24] The grading scheme in one canine study assigned scores for necrosis using a 50% threshold similar to the French system but changed the assigned scores used in the human scoring system: Score 1 = none; score 2 = < 50% and score 3 = > 50% to grade the tumors. In the results section of the paper a different threshold for necrosis was addressed (ie, 10% threshold) indicating that dogs with tumors with > 10% necrosis were 2.7 times more likely to die of tumor related causes. The data relating to the 10% necrosis threshold was not reported.[20] Other studies have not referenced the 10% necrosis threshold and we are not aware of pathologists reporting or oncologists requesting an estimate of 10% necrosis. For necrosis to be objectively assessed as a parameter for future grading schemes, new studies must determine if gross assessment of necrosis can be documented in a standardized fashion and if this parameter correlates with outcome assessment. This requires documentation of systematic gross sampling of both necrotic and viable tissue and confirmation of necrosis by histological evaluation. For this to be accomplished, grossing personnel must include sectioning of tumor sites which appear necrotic,

hemorrhagic, or edematous, regions typically avoided in most grossing procedures. Most pathologists will only have microscopic sections to estimate necrosis and these sections are likely to be a small percentage of the entire tumor. Furthermore, in many cases, the gross description will be inadequate unless grossers are instructed to search and report the percent of the entire tumor that appears necrotic. How pathologists who used this parameter determined when greater or lesser than 50% of the tumor was necrotic seems too subjective to be reliable or reproducible. Unless future studies can clearly define and objectively assess this parameter, findings must be considered tentative. Also, if studies lack sufficient cases with adequate gross assessment, gross percent of necrosis should be abandoned as a criterion. Future studies should consider using morphometry of histological sections and compare this to subjective assessment of the percent necrosis. Veterinary studies have also never determined how many sections of a tumor adequately estimates parameters such as percent necrosis, margins or MC. As with other parameters, the utility of necrosis as a feature of tumor behavior must be compared to patient outcomes.

G. The histologic tumor free distance (HTFD) should be reported as accurately as possible; in mm with no decimals; report lateral and deep margins. The HTFD is the shortest distance from the tumor to the inked margin. If ink is not present when tissue is received indicate if lab personnel inked what they interpreted as deep and lateral margins. When tumor cells are seen at a margin consider indicating *focal* (only a few foci of tumor cells are present) or *diffuse* (large number of tumor cells present at the margin). There are inherent inaccuracies in margin assessment as a result of post-surgical tissue shrinkage, shifting of tissue planes following surgery, marking ink dissection along fascial planes, function of cut and formalin fixation tissue shrinkage.[21] Alternate methods of reporting surgical margins are topics of current discussion, including a system modified from human systems designed to address breast cancer.[21] (see Guideline 3)

H. It can be difficult to distinguish true vascular invasion from pseudovascular invasion. The more stringent criteria for defining vascular invasion are demonstration of

invasion of vascular wall by neoplastic cells and presence of a neoplastic embolus/thrombus attached to a vascular wall. In the absence of these findings, the presence of intravascular neoplastic cells could represent either true lymphovascular invasion or pseudovascular invasion. (see Guideline 4).

I. Tissue retraction can form a space which may surround tumor cells mimicking tumor in a lymphovascular space. Lack of demonstrable endothelial lining distinguishes retraction artifact from intravascular neoplasia. Retraction artifact is common in circumanal gland neoplasms. Pseudovascular invasion is artifactual displacement of neoplastic cells into a vascular space. In this instance, a true lymphovascular space (i.e., demonstrable endothelial lining in routine or immunohistochemical-stained sections) surrounds neoplastic cells. (see Guideline 4).

J. Metastatic sites should be confirmed by histological evaluation. Imaging results suggestive of metastasis but not confirmed histologically should be reported as suspected metastases and mode of imaging stated: radiographs, CT, MRI, etc (see Guideline 10). If lymph nodes are evaluated, method of evaluation should be included (e.g. FNA vs incisional biopsy).

K. There is one basic grading system, developed originally by Trojani,[32] in which 7 parameters were evaluated for use with human tumors and three were selected: MC, differentiation and necrosis. These three were subsequently validated by others.[11,15,29] However, the literature on canine STT/STS have reported different MC thresholds as parameters to predict prognosis ($\geq$ 9),[7] ranges of 0-5, 6-9, >9[24] and ranges of 0-9, 10-19 and >19,[20] none of which accurately reported the total area in which mitotic figures were counted). Two of these graded the canine tumors via the French system; however, differentiation and necrosis were not described in sufficient detail for others to replicate the M&M. Both studies lacked robust numbers of high-grade tumors with follow up data (e.g., one study graded 139 tumors, only 9 were high grade and only 4 of these had outcome assessments).[24] Grading criteria should be readily available and reproducible with detailed, objective, well-illustrated cut

points defined so others, including novices, can replicate and validate these studies. Furthermore, no studies compared the different MC thresholds to know which one is the most predictive. Some current studies still use incorrect terminology and methods described so poorly that results cannot be compared or validated.[13]

L. Histological differentiation is one of the original criteria evaluated by Trojani, modified by Coindre and currently utilized to evaluate soft tissue sarcomas in humans.[11,29,32] One of the early systems proposed for dog STT/STS grading includes a differentiation score based upon the scores used for human tumor grading.[20] Multiple studies indicated that histologic differentiation is one of the most problematic components of the human grading system[29] and poses a similar problem for dogs. A recent manuscript assessed intra- and inter-observer agreement in histological assessment of canine soft tissue sarcoma. In this manuscript, the specific type of soft tissue sarcoma was not reported, and all tumors were evaluated as a group. Although intra-observer differentiation assessment showed concordance, there was extensive interobserver disagreement in differentiation scoring.[34] No description or explanation of how the human soft tissue tumor differentiation score should be applied to the canine tumors was given in the proposed canine STT/STS grading system, and the human system needs to be modified for dogs.[20] The three differentiation scores are described as Score 1: Resembling normal, adult mesenchymal tissue, Score 2: Specific histologic type and Score 3: undifferentiated. The criteria for scoring was not further defined and no examples of the various differentiation scores were provided.[20] Differentiation score 1 poses particular problems in assessing canine STT/STS. Few STT/STS display differentiation that could be classified as adult mesenchymal tissue, with the possible exception of well-differentiated fibrosarcoma or liposarcoma, neither of which is a common skin nor subcutaneous neoplasm. On the other hand, PWT and NST form the majority of STT/STS in most studies, but do not resemble any *normal adult mesenchymal tissue* type. Some tumors can be reliably classified as PWT but others can be difficult to distinguish from NSTs or other tumor types, requiring IHC to differentiate.[3] To be effective, differentiation scoring systems must provide clear

criteria for determining the classification of these common tumor types. To address these issues we propose a new scoring system should be developed (see future considerations): 1. Histological features consistent with a specific diagnosis (ie PWT, NST); 2. Less definitive histological pattern suggestive of but not diagnostic of specific tumor type. Additional studies, such as IHC may be needed to make a definitive diagnosis; 3. Undifferentiated sarcoma/sarcoma of unknown type.

Future Considerations:

1. Present grading system should be followed, but the methods described in detail such that others can duplicate and attempt to validate results using the present 3 characteristics of degree of differentiation, percent necrosis and MC. The scoring systems need to be defined in detail (see earlier in this guideline). Additional histological features beyond these three should be selected and evaluated in like detail.

2. Consider assigning a weighted score to differentiation or mitotic count, such that these parameters have greater influence in tabulating the grade. Determine significance with outcome assessment.

3. Apply grading systems for STT/STS as follows: First, apply grading systems to all tumor types and assess outcomes, then assess prognostic utility of the grading system when applied to each specific histological type of tumor within the broad category of STT/STS. Determine if the specific histologic subtype is predictive of outcome regardless of grade.

4. Develop and assess utility of a new scoring system for differentiation. Scoring criteria could include categories such as:
    a. Histological features consistent with a specific diagnosis (ie, PWT, NST)
    b. Less definitive histological pattern suggestive of but not diagnostic of specific tumor type. Additional studies, such as IHC may be needed to make a definitive diagnosis.

c. Undifferentiated sarcoma/sarcoma of unknown type

d. What percentage of a tumor or the sections evaluated needs to show a pattern in order for the neoplasm to be considered a certain diagnosis?

5. Evaluate utility of necrosis in grading STT/STS
    a. Determine means to assess necrosis (i.e., gross versus microscopic assessment) and develop standard protocol.
    b. Evaluate the 10% necrosis versus 50% threshold of necrosis.
    c. See Guideline 5 to help develop a scoring system.

6. Compare new grading systems to previous grading systems.

7. Incorporate additional histologic parameters into new grading system to evaluate utility. Include
    a. Tumor cellularity
    b. Atypical nuclei /nuclear pleomorphism
    c. Multinucleated giant cells
    d. Lymphovascular invasion: Use criteria of LVI in guideline 4 to define LVI and develop a scoring system based on guideline 4.

8. Perform univariable and multivariable analysis using all histological parameters in relation to outcome assessment.

9. Perform studies to further assess the distinction between PWT and NST
    a. Establish “gold standard” reference method for definitive diagnosis (ie, IHC, electron microscopy, etc).
    b. Assemble a large collection of both PWT and NST tumors (100 – 200 of each tumor type submitted from institutions around the world) which have been diagnosed definitively using the reference method. Collection should include both “classical” examples of the tumor type as well as tumors in which the histological pattern is less specific.

c. Post digital images of only H&E sections from the PWT/NST tumor collection for pathologists from a wide variety of institutions to make a diagnosis based upon routinely stained sections. All participants should be blind to definitive diagnoses and submissions of case material should come from a multitude of institutions.
d. Determine the accuracy of diagnosis of NST/PWT on routinely stained sections by comparing diagnoses with the results of the definitive assay. Assess interobserver variation in diagnosis of NST/PWT in routinely stained histological sections.
e. If NST/PWT cannot be reliably distinguished on the basis of routinely stained sections, a grading scheme which can be applied to a group of tumors may be more practical than requiring specific diagnosis before proceeding with applying a tumor grade.

10. Ensure use of defined area in mm^2 for enumeration of histological features used in grading systems.

11. Develop standards for assessment of re-excision biopsy specimens should be developed and results correlated with outcome assessment.

12. Explore use of computational pathology in assessment and grading of STT/STS – much of the above recommends developing scoring systems consider using types of technology that will objectively quantitate a histologic feature.

13. Investigate utility of molecular profiles in tumor grading/prognosis. These are used extensively in human STS to aid identification of type (subtype) and to estimate aggressiveness.

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