Tumor Necrosis
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VCGP Introduction:

The purpose of the VCGP guidelines is to provide standardized methods to evaluate neoplasms in animals. These guidelines are aimed at pathologists of all experience levels – trainees to experts – to ensure foundational standardization. Although it seems obvious that the methods used to assess various tumor parameters need to be defined prior to implementation, this has not always happened (e.g., the area in which mitotic figures were counted was never standardized). The goal of these guidelines in standardizing tumor pathology is to allow for accrual of standardized data so that, over time, large data sets with comparable information can be evaluated and studies can be validated uniformly. Ultimately this will enable investigators to reach meaningful conclusions and provide accurate prognostic information that will improve patient care. New methods and modifications of tumor evaluation are encouraged, and importantly, these need to be described in such detail that others can replicate and validate results. The VCGP guidelines and protocols are “living” documents that will be modified as new information becomes available to authors. This guideline is intended to be applicable to all animal tumors.

Tumor Necrosis Introduction:

The first mention of necrosis as a diagnostic criterion in the grading of soft tissue sarcomas is in human pathology in a 1984 report by Trojani et al.[10] In this study of 155 human soft tissue sarcomas (STS), seven tumor features (tumor cellularity, tumor differentiation, nuclear atypia, presence of multinucleated cells, mitotic count [MC], vascular emboli, and presence of necrosis) were subjected to monofactorial and multivariate analysis in relation to survival, local recurrence, and metastasis. Of these seven features, only three (tumor differentiation, MC, and presence of necrosis) correlated with patient survival and tumor metastasis.

In canine soft tissue tumors/soft tissue sarcomas (STT/STS), an increased percent of tumor necrosis has been associated with increased risk of death due to tumor related causes.[5] Additionally, the amount or presence of tumor necrosis is included in multiple other published tumor grading systems in veterinary medicine including canine splenic hemangiosarcoma (assessed with thresholds of 0%,<25%, 25-50%, or >50%), feline injection site sarcoma (assessed with thresholds of 0%,<50%, or ≥50%), canine osteosarcoma (two published grading systems, with thresholds of 0%,<15%, 15-50%, or >50% or thresholds of <25%, 25-50%, and >50%), canine multilobular tumor of bone (assessed as present or absent), canine mast cell tumor (3-tier [1984] grading system assesses edema and necrosis, combined, as minimal, diffuse areas, or common, while the 2-tier [2011] grading system does not include necrosis as a criterion), and canine pulmonary carcinoma (assessed with thresholds of 0%,1-20%, 21-50%, or >50%).[1]

However, the means to assess and assign a numerical score or quantitate the percentage of tumor that is necrotic has not yet been clearly defined or standardized. For example, was the percent necrosis determined both grossly and microscopically or only microscopically? Was any gross estimate confirmed microscopically? How was the tumor sectioned (e.g. randomly, systematically, or biased to avoid grossly necrotic areas), and how many sections were examined grossly and/or microscopically compared to the size of the tumor? Was the amount of necrosis estimated as an average of all examined slides, or estimated based on the slide deemed most representative of the tumor, or based on the slide with the most necrosis present? Was the amount of necrosis estimated manually/visually by the pathologist or was computer assisted technology with whole slide imaging used? Currently, all of these questions may be answered differently by different pathologists even though each is using the same grading system; therefore, standardization in the methods to assess tumor necrosis is urgently needed. This Guideline provides recommendations to determine the percent necrosis of any tumor, as well as recommendations for future studies (see Future Considerations below).

Histologic Definition of Necrosis:

Although it may seem so basic as to be unnecessary, studies should include a definition of necrosis to aid in study reproducibility and validation. If simple details like this are not included in studies then methods may not be reproducible and studies may incorrectly conclude that a parameter is not prognostically useful, either independently or as part of a grading system.

DEFINITION: Tumor necrosis includes areas of coagulative and/or lytic necrosis. Coagulative necrosis is an area of clusters and/or sheets of cells with increased cytoplasmic eosinophilia and at least one of the following features: nuclear shrinkage (nuclear pyknosis), nuclear fragmentation (karyorrhexis), nuclear disappearance (karyolysis), and shadows or ghosts of tumor cells with retained visible cellular outlines (Figure 1). Lytic necrosis is an area with loss of cellular architecture and/or tissue architecture and some of the following features: basophilic nuclear debris, granular eosinophilic debris, amorphous eosinophilic debris, and tumor cell outlines are not visible (Figures 2A and 2B). Areas of necrosis do not need to be contiguous.

Of note, necrosis may result from causes that exempt these areas from consideration as part of the tumor necrosis assessment. Pathologists should exclude necrosis caused by any of the following from the necrosis assessment: fine needle aspiration, incisional biopsy, chemotherapeutic or radiation treatment, ulceration, and patient-induced trauma; pathologists should also exclude areas of mucinous or hyaline change and hemorrhage from the necrosis assessment.[9]

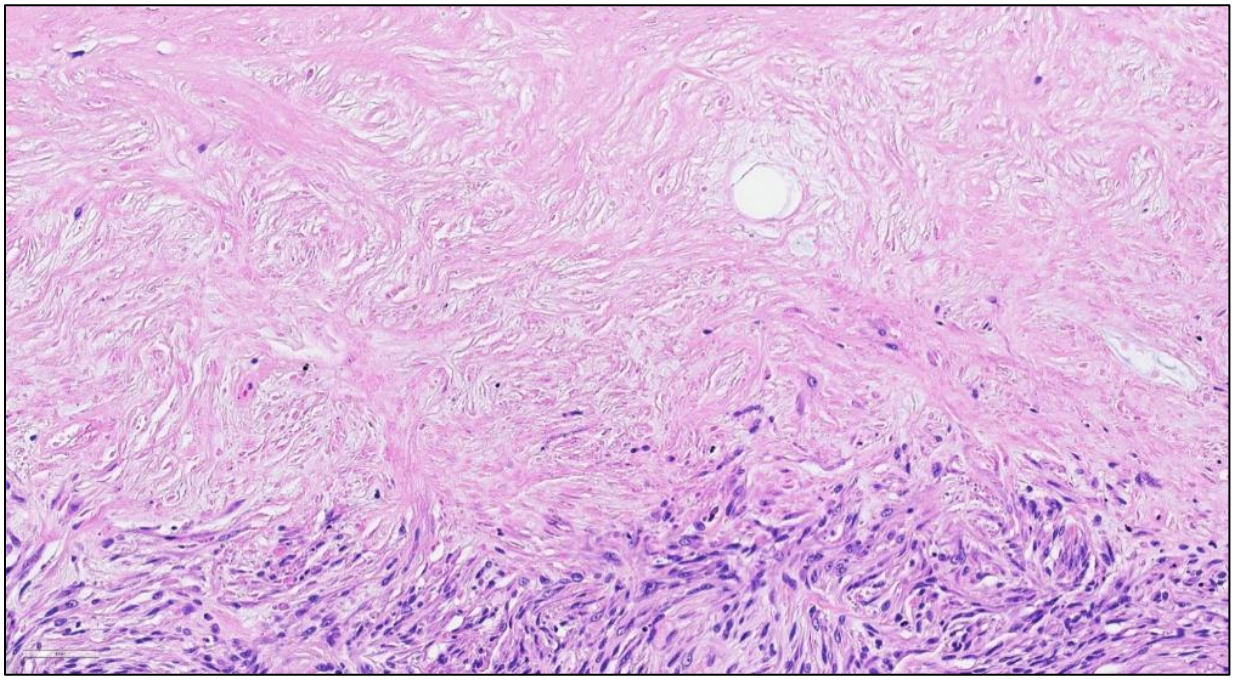


Figure 1. Canine subcutaneous soft tissue tumor/sarcoma (STT/STS). Viable tumor (bottom of image) abuts an extensive focus of coagulative necrosis characterized by sheets of cells with increased cytoplasmic eosinophilia, nuclear disappearance (karyolysis), and shadows or ghosts of tumor cells with retained visible cellular outlines.

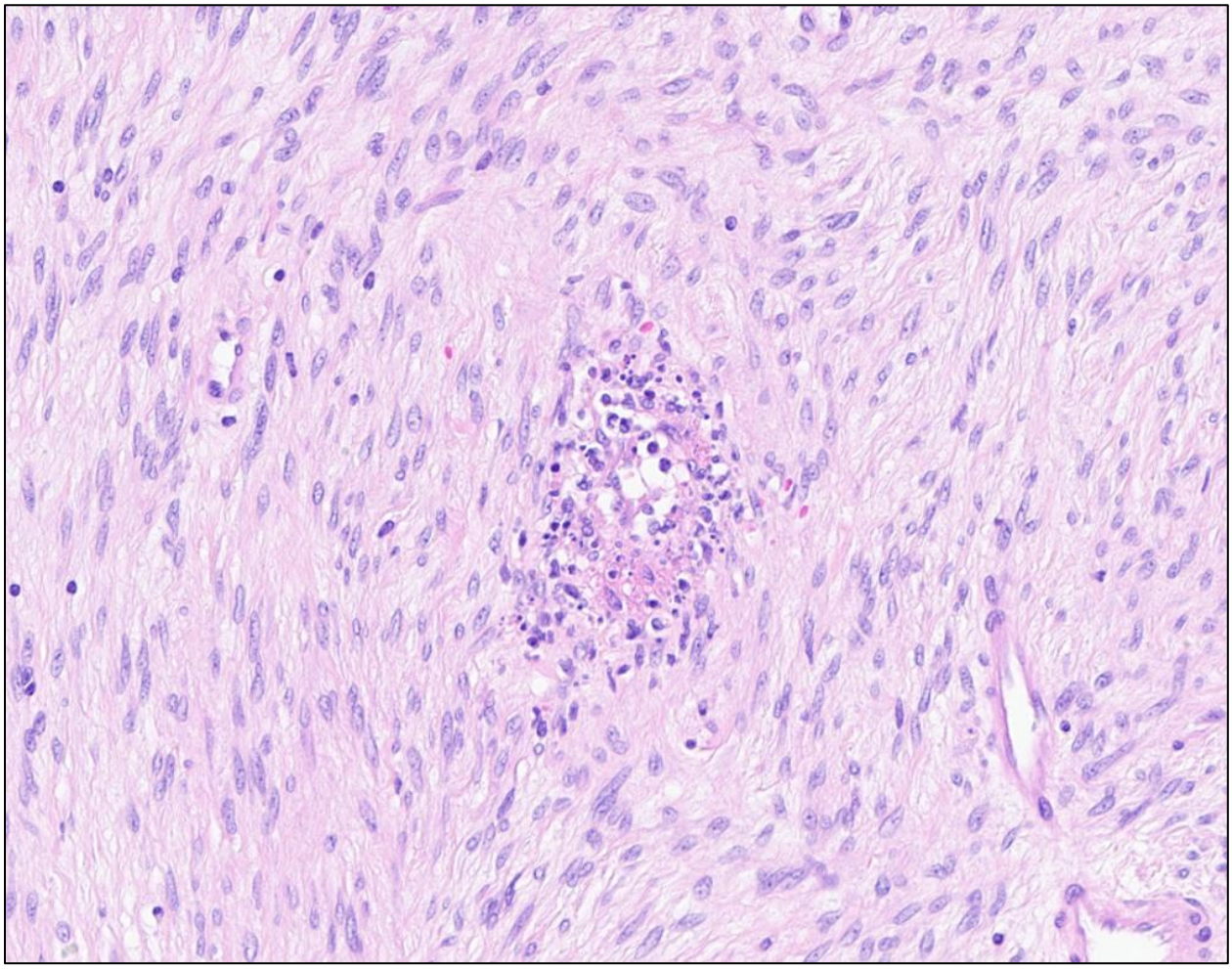


Figure 2A: Canine subcutaneous soft tissue tumor/sarcoma. A small focus of lytic necrosis (center of image) is surrounded by viable tumor. This focus has loss of cellular and tissue architecture with replacement by basophilic nuclear debris, granular eosinophilic debris, and amorphous eosinophilic debris, and tumor cell outlines are not visible.

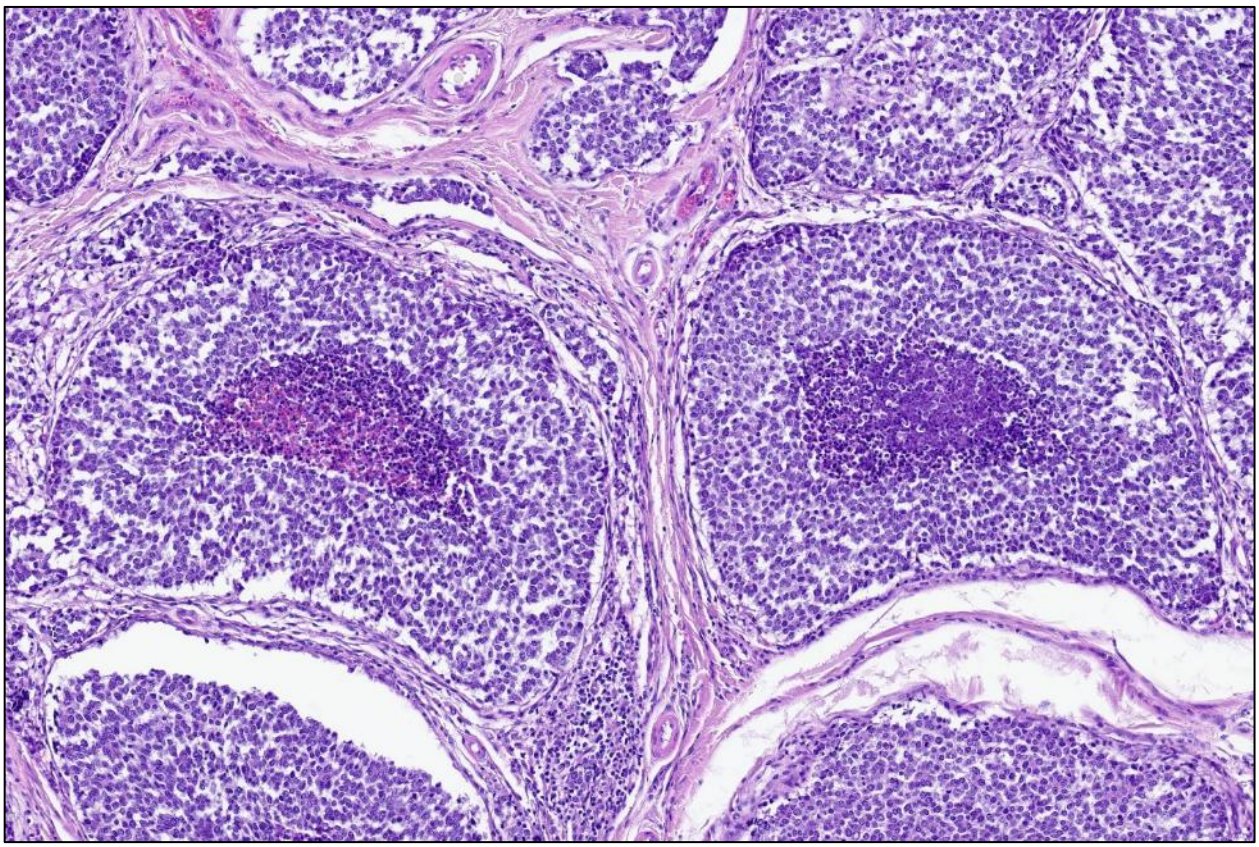


Figure 2B: Canine mammary comedocarcinoma. Two foci of lytic necrosis in the center of neoplastic nodules. These foci have loss of cellular and tissue architecture with replacement by basophilic nuclear debris, granular eosinophilic debris, and amorphous eosinophilic debris, and tumor cell outlines are not visible.

Practical Issues with Assessing Tumor Necrosis

Gross vs. Microscopic Assessment of Necrosis

In human oncological studies, percent necrosis has been determined by estimating the amount of necrosis identified grossly (macroscopically) and histologically (microscopically).[3,6,10,11] In the Trojani et. al. 1984 study of human STS, the authors developed a scoring system for grossly and histologically assessed necrosis with scores of 0-2: 0 points were assigned when necrosis was absent, 1 point for tumor necrosis comprising less than 50% of the examined sections, and 2 points for necrosis comprising over half the sample. In this scoring system, a necrosis grade of 2 could also be assigned for any STS with a gross appearance described as “mainly necrotic” by a

surgeon or pathologist even if no necrosis was seen histologically within the examined sections.[3,10] This last gross-only criterion for a grade of 2 was later removed from accepted human STS grading systems which now require microscopic confirmation of macroscopic evidence suggesting necrosis.[3,6] Similarly, the authors of this VCGP Guideline also recommend against using only gross appearance of necrosis in any tumor grading system because **the presence of necrosis can only be *estimated* on gross examination**. Even for an experienced pathologist, the macroscopic diagnosis of necrosis may be problematic. For example, areas of edema or exudate may appear grossly necrotic, and areas of hemorrhage, which are often associated with necrosis, may far exceed the boundaries of actual necrotic tissue. Furthermore, certain heterogeneous tumors such as sarcomas may have areas of myxomatous change and formation of cystic spaces, both of which may grossly appear necrotic, in addition to edema, hemorrhage, and exudate. For these reasons, **gross/macroscopic assessment of necrosis requires histologic confirmation**. As a consideration, however, in large tumors, histologic confirmation of all areas grossly interpreted as necrotic may not be practical in veterinary medicine compared to human medicine, because comparatively smaller numbers of histologic sections are examined relative to the overall tumor size, whereas our human pathology counterparts are less constrained to few histologic sections.

Published veterinary studies have not indicated whether gross estimates of necrosis were used in combination with microscopic assessment when developing tumor grading systems, or if only microscopic assessments were evaluated.[4,5,7] We presume only microscopic assessment was used, but this is only an assumption. Future studies must include in the methods whether gross and microscopic or only microscopic assessment was used to determine the amount of necrosis. Future studies using standardized, systematic methods are needed to determine the value of gross necrosis assessment in veterinary medicine.

Future studies should investigate the utility of advanced imaging modalities to estimate the amount of tumor necrosis in veterinary medicine. In human oncological studies, the extent of tumor necrosis has been determined using various advanced

clinical imaging technologies such as contrast-enhanced magnetic resonance imaging.[2,3,6,8,10,11] These methods have not yet been introduced into grading systems in veterinary oncology. This may be because advanced clinical imaging in veterinary medicine is not yet as widely available as it is in human medicine, which makes this method of estimating necrosis less broadly applicable in our field. This is an area for future development.

Tumor Sectioning Methods

Sectioning areas of tumor that appear grossly necrotic is not typically performed in most veterinary laboratories. Purposely avoiding areas of necrosis in tissue selection will bias any determination of percent necrosis in histologic sections. Furthermore, because we presume only histology was used to develop tumor grading systems in veterinary pathology, and because of this common practice of biasing histologic sections, it may be assumed that the microscopic samples used to develop these grading systems likely were not representative of overall tumor necrosis. The bias introduced by this commonplace practice of only sampling viable tissue for histological examination undermines the utility of tumor necrosis as a parameter in tumor grading systems by thwarting accurate and repeatable results. *Tumor necrosis may be the only histologic parameter that we purposefully bias by avoiding the parameter we are trying to measure*!

Another important component to minimizing sampling bias in tumor sectioning for assessing tumor necrosis is to document how many sections are evaluated per tumor, grossly and microscopically, in comparison to the size of the tumor. A recent publication suggested to prepare 1 tissue block for each 2 cm diameter of soft tissue tumor.[9] Since no formulae for number of blocks/slides per tumor have been described in published grading systems in veterinary medicine, this seems like a good starting point, but future studies should evaluate this metric for utility.

Furthermore, some pathologists estimate the average amount of necrosis present across all examined sections of the tumor, whereas others select a single representative section or a single section with the most necrosis. Therefore, to

standardize the data that is collected between different pathologists, the number of sections used to estimate necrosis must be reported.

Estimating the Amount of Necrosis

Microscopically estimating the amount of necrosis can be performed manually/visually with either glass slides or digital histologic whole slide images (WSI), or by using image annotation software using WSI. WSI with use of computer assisted technology has been used to evaluate necrosis in human tumors.[11] If WSI has drawing software simply outline the entire tumor circumference (X) as well as the areas of necrosis (Y, confirmed at high magnification), followed by calculation of (Y/X) x 100 = % necrosis in one section (Figures 3A and 3B). In the absence of image annotation software or if using a microscope with glass slides, then visually estimate the amount of necrosis using varying magnifications (using high magnification to confirm the areas are indeed necrotic). Future studies could easily compare manual/visual estimates of the amount of necrosis with computer assisted measurements with a goal to assess reproducibility, accuracy, and usefulness of the different methods. However, the reproducibility and therefore utility of necrosis estimate requires standardized tissue sectioning (see Tumor Sectioning Methods section above).

The percent necrosis in histologic sections may be easy to estimate if values of greater than or less than 50% are used as thresholds, even if manually/visually estimating the amount of necrosis. However, implementing more narrow targets may prove problematic, such as a 10% level of tumor necrosis (a 10% threshold was reported as prognostically significant in one study, see note A).[5]

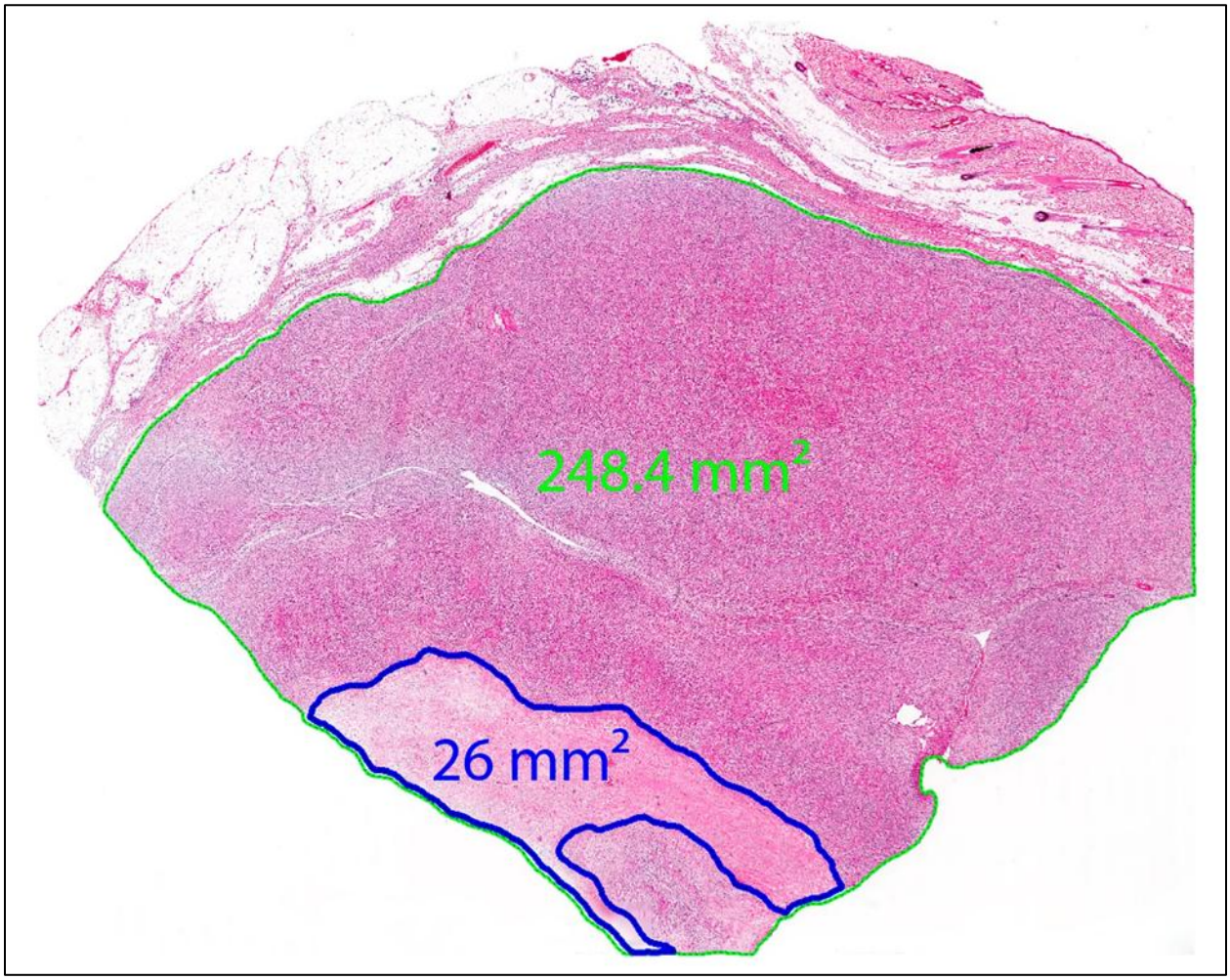


Figure 3A. Computerized measurement of necrosis area (blue line) and tumor area (green line) in a whole slide image (0.5 x magnification) of a canine, subcutaneous soft tissue tumor/sarcoma. The two regions were demarcated manually using a “pen tool” from the viewing software Aperio ImageScope (Leica Biosystems, Wetzlar, Germany) and the software accurately calculates the demarcated areas. In this case the necrosis area (26 mm²) is 10.5% of the tumor area (248.4 mm²) in this evaluated histological section. This technique accurately denotes the percent necrosis within an individual histologic section, but requires additional information to know whether this section is representative of the whole tumor.

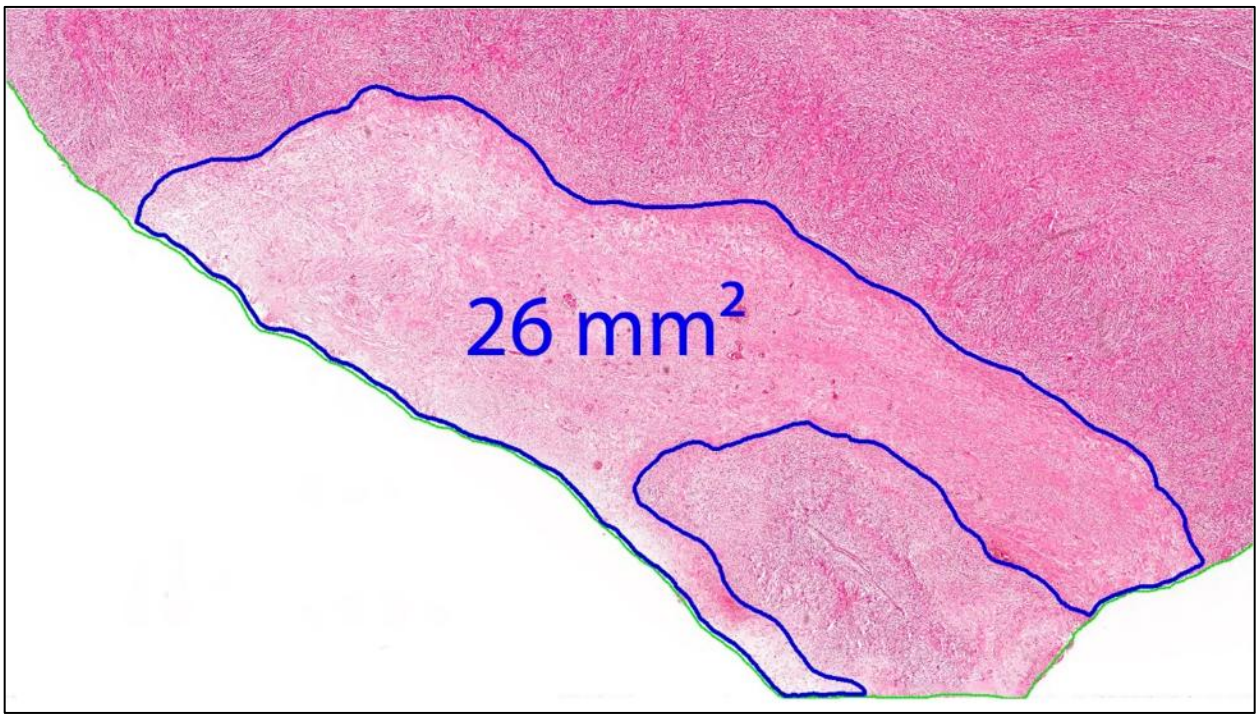


Figure 3B. Higher magnification of Figure 3.

Discussion and Recommendations:

There is currently a highly concerning lack of standardization in the methods of assessment of tumor necrosis. New studies are urgently needed to determine if assessment of necrosis can be documented in a sufficiently standardized fashion to be repeatable. Only when (or if) studies determine that necrosis can be assessed in a sufficiently repeatable manner will it be worthwhile to determine if this parameter correlates with patient outcome. This Guideline proposes a detailed set of methods to enable accrual of standardized data so that future studies may determine the repeatability of necrosis assessment (see Standardized Reporting Format below).

Importantly, for necrosis to be objectively assessed, sampling (i.e., tumor sectioning) bias must be minimized. For this to be accomplished, grossing personnel must sample tumors systematically rather than selecting viable areas only. Grossing personnel must not purposefully avoid areas of tumor that appear necrotic, hemorrhagic, or edematous. Most veterinary pathologists only have microscopic sections available to estimate necrosis. These microscopic sections likely represent only a small proportion of the entire tumor, and if sampling is biased against grossly

necrotic appearing areas, the sections assessed microscopically may be extremely unrepresentative of the tumor. Furthermore, in many cases, the gross description of necrosis is inadequate unless grossing personnel are trained to recognize and report the percent of the entire tumor that appears grossly necrotic. If studies lack sufficient cases with adequate gross assessment, gross assessment of necrosis should be abandoned as a criterion.

With so much variability in tumor sectioning methods, it is difficult to propose standard tumor necrosis thresholds to report. A cutoff of greater or less than 50% tumor necrosis seems like a reasonable starting point, although even this seems to be too subjective to be reliable or reproducible, much less the 10% threshold reported by some.[5] Unless future studies can clearly define and objectively assess this 10% threshold, findings must be considered tentative.

Future studies should consider using morphometry and/or computational pathology (see VCGP Guideline on Computational Pathology [CPATH]) of histological sections (Figures 3A and 3B) and compare this to estimated (visual/manual) assessment of percent necrosis. There are reports that visual estimates of necrosis were comparable to computer assisted evaluations; this was at a cutoff of 10% necrosis.[11] Veterinary studies have also never determined how many sections of a tumor are sufficient to adequately determine necrosis, margins, or mitotic count.

As with all other parameters, the utility of tumor necrosis as a paramater must be compared to accurate patient outcomes.

The methods used to assess tumor necrosis must be described in such detail that others can replicate and validate results. Importantly, the size of the tumor, method of sectioning, number of cut surfaces examined grossly and/or histologically, and the method of estimating amount of necrosis (i.e., WSI with computer assisted technology, or visually with glass slides or WSI) must be documented.

Standardized Reporting Format:

Location on body:

Tumor Size:

(Indicate if gross and/or histologic assessment)

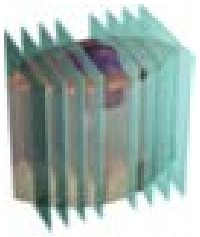
Greatest dimension: _____

Additional dimensions: _____

Method of sectioning:

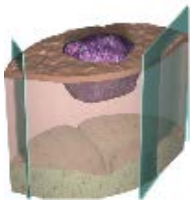
____Radial (cross and longitudinal sections; points of compass) - two perpendicular cuts

____Number of cuts



_____Parallel (bread loaf; bologna) series of parallel cuts

_____Number of cuts



_____Tangential

_____Number of cuts

____Combinations of methods (list):

Necrosis, Gross

_____Assessment performed by: technician, pathologist, other

_____Number of cuts (surfaces) examined

Amount of necrosis (estimated percent of tumor which is necrotic) (Note A, B)

_____ None observed

_____ <50%

_____ >50%

_____Not assessed

Necrosis, Clinical Imaging

_____Amount of necrosis estimated by imaging, state mode and qualifications of person assessing necrosis

Necrosis, Histologic

Method of assessment:

____ Manual (visual); light microscopy with glass slide evaluation or unassisted whole slide imaging

____ Computer assisted whole slide imaging; was morphometry or other objective means used to quantitate? Describe

____ Number of sections included in the necrosis estimate (average of all sections, or representative slide?)

Amount of necrosis (estimated percent of tumor which is necrotic) (Note B, C)

_____ 0 – none seen in any histological section

_____ 1 – <10%; small foci of necrosis (See Note A)

_____ 10 – <50%

_____ > 50%

_____Not assessed

Notes:

A. The percent of necrosis within a tumor is a parameter used in grading systems in humans and animals.[3-5,7,10] The principle is that the greater the amount of necrosis presumably the more aggressive the tumor, but data on this for tumors in animals is not definitive or the techniques to assess are not described. The grading system in one canine study of STS/STT assigned scores for necrosis using a 50% threshold similar to the human oncology STS grading (i.e., French, or Trojani, et. al.) system, but changed the assigned scores: no necrosis changed from 0 to 1, <50% necrosis from 1 to 2, and ≥50% necrosis from 2 to 3; additionally, the results described a 10% threshold for necrosis, indicating that dogs with tumors with greater than 10% necrosis were 2.7 times more likely to die of tumor related causes.[5] The data relating to the 10% necrosis threshold was not reported.[5] Other studies have not referenced a 10% necrosis threshold and we are not aware of pathologists reporting or

oncologists requesting an estimate of 10% necrosis. Future studies should determine the prognostic significance of a 10% threshold for canine STT/STS, or other tumors.

B. Grossing procedures should include a description of the tumor sampling technique (e.g., Were areas of necrosis avoided for processing for microscopic examination? How many sections were assessed grossly?), and should include an estimated percentage of gross tumor necrosis. We recommend at least one section identified with necrosis grossly be submitted for microscopic confirmation. Furthermore, for tumor grading system development, the gross necrosis data should be recorded and reported (consider photographs) for study validation and replicability. Pathologists should report the extent of necrosis in histological sections examined and correlate gross and histological observations. Anyone that has a Standard Operating Protocol (SOP) or similar which addresses how to assess necrosis grossly is encouraged to send their protocol to the authors of this Guideline. If future studies determine that gross necrosis cannot be sufficiently standardized to make this parameter useful, the parameter should be excluded.

C. Currently there is no established method for determining percent of necrosis in a histologic slide. Consider methods below:

a. WSI with drawing software: outline the entire tumor circumference (X) outline the areas of necrosis (Y), calculate % necrosis = (Y/X) x 100 for that one examined section (Figures 3A and 3B). This may be assessed on a single slide (e.g., the slide with the most necrosis, or the estimated most representative section), or may be repeated on multiple slides and averaged; this method should be reported.

b. Microscope or WSI without drawing software: visually estimate how much necrosis is seen, like “a” above. The method of determining which slide(s) to assess should be reported.

c. Necrosis reporting thresholds: The range of less than or greater than 50% seems like a wide target and therefore simply estimating as in “b” may be sufficient. The idea of narrowing the target to a 10% range seems more difficult; however, there are reports on human tumors that were assessed using 10% thresholds.[11] Other reports have simply assessed necrosis as present or absent. If necrosis is to be used as an independent variable or as part of a grading system, the method needs to be reported and must be repeatable.

Future Considerations:

1. Determine if gross and/or histological assessment of necrosis can be documented in a standardized fashion, and if so, how this parameter correlates with patient outcomes.
    a. Develop a SOP that instructs grossing technicians how to determine the extent of gross necrosis at trimming.
    b. Establish a standard method for number of samples submitted for histological evaluation. One recommended starting point is to assess the utility of 1 sample for each 2 cm diameter in the tumor’s longest dimension.

2. Compare manual/visual estimates vs. computer measurements of necrosis: evaluate the use of morphometry and/or computational pathology (CPATH) to quantitate necrosis and compare to pathologist’s subjective estimate. Data could be tabulated as actual % necrosis as well as grouped by less than or greater than 50% or other cutoffs (e.g. 10%), or as present vs. absent. Then conduct a statistical analysis (e.g. agreement measured as a continuous variable [Pearson’s r] and as a categorical variable with specific cutoff point[s] of “X”% [k score]).

3. Determine in which types of tumors, if any, necrosis has prognostic value, independently or as part of a grading system.

4. Validate that a 10% threshold is prognostic for canine STT/STS and other tumors.

5. Correlate size of tumor and type of tumor with amount of necrosis, determine number of sections needed to accurately assess necrosis.

6. Assess utility of advanced clinical imaging studies to assess necrosis; this may have less broad application across the veterinary field as advanced clinical imaging is currently limited to selected veterinary hospitals. However, advanced imaging may allow for more accurate full representation of the tumor as opposed to relatively few histologic sections.

7. Routine diagnostic case reports should indicate the number of histologic sections assessed to determine the percent (or amount of) necrosis. If future studies establish a method to more accurately assess tumor necrosis, routine diagnostic reports should reflect this updated information.

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