Tumor Necrosis
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Contents

Introduction:

The purpose of guidelines is to provide standardized methods used to evaluate tumors in animals and accrue data so that, over time, large data sets with comparable information can be evaluated and studies validated uniformly. Ultimately this will enable meaningful conclusions and accurate prognostic information that will improve patient care. New methods and modifications of present methods are encouraged and should be described in such detail that others can replicate and validate results. Guidelines and protocols are “living” documents which will be modified as new information becomes available to authors. This Guideline is intended for all animal tumors.

Although it seems obvious that the means to assess various histologic parameters need to be defined prior to implementation, this has not always happened (e.g. the area in which MF were counted was never standardized). The percent of tumor necrosis in soft tissue tumors/soft tissue sarcomas (STT/STS) is included in grading schemes, yet the means to assess necrosis has not been clearly defined or standardized. Was the percent necrosis determined by examination of the tumor during gross sectioning and areas appearing necrotic confirmed microscopically? Was the percent necrosis used in the grading system based upon visual estimate of necrosis in random histologic tumor sections? Was a consistent portion of the tumor submitted for microscopic examination? A recent publication suggested preparation of 1 tissue block for each 2 cm diameter of soft tissue tumors.[1] Since no formulae for number of blocks/slides per tumor have been described in published grading systems for dogs this seems like a good starting point. Percent of tumor necrosis has been associated with increased risk of death due to tumor related causes in dogs with soft tissue sarcomas.[2] However, the means to assess and assign a numerical score or quantitate the percentage of tumor that is necrotic is defined poorly or not at all. Percent necrosis for human tumors has been determined by estimating the amount seen grossly and histologically.[3-6] Studies on dogs and cats did not indicate if gross observations were used in combination with histological assessment, as in humans, or if only histologic assessments were evaluated.[7,2,8] We presume only histology was used.

For necrosis to be assessed as a parameter for future grading schemes, new studies must determine if gross and histological assessment of necrosis can be documented in a standardized fashion and if this parameter correlates with outcome assessments. The presence of necrosis can only be *estimated* on gross examination, as tumor matrix, hemorrhage, edema, inflammation, cysts and other lesions could be misinterpreted as necrotic neoplastic tissue. Gross assessments must be confirmed with histology. Establishing a standard method of sample submission, such as 1 block per 2 cm diameter of tumor as suggested in a recent publication[1] should be evaluated for utility.

Although the presence of necrosis can be confirmed by histological examination, sectioning areas of tumor which appear grossly necrotic is not typically performed in most veterinary laboratories, biasing the extent of necrosis within histological sections. In human oncological studies, extent of tumor necrosis has been determined by both gross and histological assessment as well as with various imaging technologies.[9,3,4,10,5,6] Whole slide imaging with use of computer assisted technology has been used to evaluate necrosis in human tumors.[6] Imaging techniques and computer assisted whole slide imaging should be considered potential methods for future studies of tumor necrosis in animal tumors. This Guideline provides criteria to determine the percent necrosis of any tumor and recommendations for future studies (Note A).

Reporting format:

Tumor Type:
Location on body:

Tumor Size:
(Indicate if gross or histologic assessment)

Greatest dimension: _____

Additional dimensions: _____

Number of histological sections examined: _____

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 – list:

Necrosis Gross

(Estimated percent of tumor which is necrotic.) (Note A, B)

_____Record if assessment performed by technician, pathologist, others

_____ None observed

_____ <50%

_____ > 50%

_____Not assessed

_____Number of cuts (surfaces) examined

_____Number of sections submitted for slide preparation

_____ Necrosis estimated by imaging, state mode

Necrosis Histologic

(Estimated percent of tumor which is necrotic in sections examined.) (Note B, C)

____ Manual (visual) light microscopy with glass slide evaluation

____ Computer assisted whole slide imaging

_____ 0 - none seen in any histological section

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

_____ 10 – <50%

_____ > 50%

_____Not assessed

_____ Was morphometry or other objective means used to quantitate? Describe

Scoring system for necrosis

recommendation;if no gross data provided use histology

_____ 0 no necrosis seen grossly or histologically

_____ 1 minimal or no necrosis seen on gross exam and histologic necrosis estimated at < 50%

_____ 2 multiple large areas of necrosis seen grossly and histologically > 50% necrosis

Discussion:

The first mention of necrosis as a diagnostic criterion in the grading of soft tissue sarcomas is in a 1984 report by Trojani et al.[5] In this study of 155 human cases of soft tissue sarcoma (STS), seven pathologic 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, only three (tumor differentiation, MC, and presence of necrosis) correlated with patient survival and tumor metastasis. In this particular study, a numerical score of 0-2 was given for the presence of necrosis which was assessed grossly and histologically - zero points were assigned when necrosis in a given sample was absent, no necrosis within the given tissue, 1 point for tumor necrosis comprising less than 50% of the slides, and 2 points for

necrosis of over half the sample. A grade of two could also be assessed for any neoplasm whose gross appearance was described as “mainly necrotic” by a surgeon or pathologist even if no necrosis was seen on the submitted sections.[3] We do not recommend this last criterion be adopted for animal tumors and later authors and grading systems as well as current College of American Pathologist protocol for assessment of soft tissue tumors in humans require microscopic confirmation/validation of macroscopic evidence suggesting necrosis.[3,4]

Even for an experienced pathologist, the macroscopic diagnosis of necrosis may be problematic. Areas of edema or exudate may be quantitated as areas of necrosis grossly, and areas of hemorrhage, which are often associated with necrosis, may far exceed the boundaries of actual necrotic tissue. These problems are further compounded by the interpretation of tumor tissue, especially heterogeneous tumors such as sarcomas, which demonstrate areas of myxomatous change and formation of cystic spaces in addition to the edema, hemorrhage, and exudate. Was the percent necrosis determined by examination of the tumor during gross sectioning and areas appearing necrotic confirmed microscopically? Was a consistent portion of the tumor submitted for microscopic examination? Gross/macroscopic assessment of necrosis requires histologic confirmation which, in large tumors, may not be practical. The number of sections examined at trimming and or submitted for histopathology is likely far fewer in veterinary than human pathology. If gross assessment is to be used as a parameter, numerous confounders must be clarified in future studies. This requires documentation of systematic sampling of both necrotic and viable tissue during the gross examination and confirmation of necrosis by histological evaluation. Alternatively, we can abandon use of gross assessment and only use light microscopy. The percent necrosis in histologic sections may be easy to estimate If targets of greater than or less than 50% are thresholds. This can be performed visually with glass slides or by using image annotation software in histologic images. The manual or visual method can be readily compared to the results from software annotation of WSI, but inconsistent sampling of the tumor, purposely avoiding areas of necrosis in tissue selection can skew any determination of percent necrosis in histologic sections. This would be straightforward but if gross assessment of tumor necrosis improves the discrimination of

grading systems, then it would be lost as a parameter. The usual practice of only sampling viable tissue for histological examination might bias the utility of tumor necrosis as an independent parameter or a component in grading systems. This may be the only histologic parameter that we purposefully bias by avoiding the parameter we are trying to measure. 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 histologically must be documented. Based on size of tumor a recommendation is needed for how many sections should be examined grossly and microscopically. For example, it has been suggested to prepare one tissue block for each 2 cm diameter of soft tissue tumors.[1]

With microscopic evaluation, necrosis may result from other causes that exempt it from consideration as part of the grading process. Previous fine needle aspiration, incisional biopsy, the effects of previous chemotherapeutic or radiation treatment, ulceration, and even patient trauma may also result in areas of necrosis wholly unrelated to a neoplastic grade. Areas of hemorrhage or hyalinization may be misinterpreted histologically as areas of necrosis (Notes C and E).

This brings us to the dilemma of how to currently approach reporting tumor necrosis. Given no current guidance, the pathologist can estimate necrosis either visually with glass slides or with annotation software in WSI. If WSI has drawing software simply outline the entire tumor circumference (X) as well as the areas of necrosis (Y), followed by calculation of (Y/X) x 100 = % necrosis in one section (Fig 1). In the absence of software or if using a microscope then visually estimate with varying magnifications (to confirm areas are indeed necrotic) if the percent necrosis is <50%>. The range of <50%> seems like a wide target and perhaps that is sufficient for estimates. We “assume” prior studies that estimated necrosis in canine tumors only used histology. But how representative the slide(s) is(are) of overall tumor necrosis is unknown and inconsistent sampling of the tumor, purposely avoiding areas of necrosis in tissue selection can skew any determination of percent necrosis in histologic sections. Incredibly, the parameter we are trying to “measure” is purposefully avoided

during trimming. Given the wide target of greater than or less than 50% necrosis, it may be possible to assess this level of necrosis histologically, even with inconsistent sampling. However, determining the 10% threshold of necrosis, as reported in one study[2] may prove problematic.

Future studies could compare pathologist estimates of % necrosis with computer assisted measured percent areas aiming to assess reproducibility, accuracy and usefulness of the method. Data could be tabulated as actual % necrosis as well as grouped by <50%>, or various other cutoffs (10%) or present vs absent and statistically compare 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). It seems unnecessary for pathologists but definition of necrosis for studies should be included to aid reproducibility and validation among study pathologists (e.g. *H&E-stained sections: area of increased eosinophilia with homogeneous clusters and sheets of degenerating and dead cells – some of these features present: nuclear shrinkage, fragmentation and disappearance, shadows/ghosts of tumor cells. Areas of hemorrhage, edema, tumor matrix and hyalinization should be skipped. The areas of necrosis do not need to be contiguous).* If simple details are not included then methods may not be reproducible, which may bias results such that the method appears it is not useful to predict outcome, independently or in a grading system.

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 independently or as part of a grading system. 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 veterinary 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 grossing personnel are instructed to search and report the percent of entire tumor that appears necrotic. How

pathologists who used this parameter in prior reports determined when greater or lesser than 50% of the tumor was necrotic, especially at the 10% threshold 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 and or computational pathology (see Guideline11 CPATH) of histological sections and compare this to subjective assessment of the percent necrosis (Figure 1). There are reports that visual estimates of necrosis were comparable to computer assisted evaluations, this was at a cutoff of 10% necrosis.[6] Future study considerations are listed at the end of this Guideline. Veterinary studies have also never determined how many sections of a tumor can be considered sufficient to determine necrosis, margins, or MC. As with other parameters, the utility of necrosis as a feature of tumor behavior must be compared to patient outcomes.

Notes:

A. The percent of necrosis within a tumor is a parameter used in grading schemes in humans and animals.[3,7,2,8,5] The principle is that the greater the amount of necrosis presumably the more aggressive the tumor but data on that for tumors in animals is not definitive or the techniques to assess are not described. None of the animal studies reviewed stated if gross assessment was included or how necrosis was evaluated. The grading scheme in one canine study of STS assigned scores for necrosis using a 50% threshold similar to the French system but changed the assigned scores used in the human scoring system (no necrosis changed from 0 to 1, <50% from 1 to 2 and $\geq$50% from 2 to 3) to grade the tumors but in the results described a 10% threshold for necrosis, indicating that dogs with tumors with > 10% necrosis were 2.7 times more likely to die of tumor related causes.[2] The data relating to the 10% necrosis threshold was not reported.[2] 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.[2] Future studies should try to validate that a 10% threshold is or is

not prognostic for STT/STS, or other tumors. Any parameter used must be described in such detail that others can reproduce the method and validate results.

B. Until studies can determine the standard method of assessing necrosis and demonstrate the utility of this parameter, diagnostic labs should develop grossing procedures which include descriptions of the extent of apparent tumor necrosis. Gross description should indicate location of samples submitted for microscopic examination (e.g. if the apparent necrotic area was submitted). Unless the extent of what appears to be necrosis at trimming (consider photographs), is reported, no future study will be able to use this parameter since the data will be lost. We recommend that one or more sections identified with necrosis grossly be submitted for microscopic confirmation. Pathologists should report the extent of necrosis in histological sections examined and correlate gross and histological observations. Individuals or labs that have a protocol which addresses how to assess necrosis grossly are encouraged to send their protocol to the authors of this Guideline.

C. Currently there is no established method for reporting percent of necrosis in histologic slides. 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 in one section (Fig 1).

b. Microscope – visually estimate how much necrosis is seen like “a” above.

c. The range of <50%> seems like a wide target and therefore estimating as in “b” may be sufficient. The idea of narrowing the target to 10% range seems more difficult, however, there are reports on human tumors that used 10% thresholds.[6] Other reports have simply assessed necrosis as

present or absent. If we are to use necrosis as an independent variable or as part of a grade, the method needs to be established.

Future Considerations:

1. Determine if gross and/or histological assessment of necrosis can be documented in a standardized fashion and how this parameter correlates with outcome assessments.
    a. Develop a SOP that instructs technicians how to determine the extent of necrosis at trimming.
    b. Establish a standard method for number of samples submitted for histological evaluation. Assess utility of 1 sample for each 2 cm diameter of tumor.

2. Design a comparison of manual estimates vs computer measurements (Note E); evaluate the use of morphometry and or CPATH to quantitate necrosis and compare to subjective assessment by pathologist.

3. Develop a scoring system for necrosis.

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

5. Validate that a 10% threshold is prognostic for STT/STS.

6. Correlate size of tumor and type of tumor with necrosis and number of sections needed to accurately assess necrosis – seem like obvious observations that need clarification.

7. Assess utility of imaging studies to assess necrosis.

8. For routine diagnostic cases, reports should indicate the percent necrosis is based on assessment of a specific number of histologic sections. Should future studies establish a protocol permitting more accurate assessment of extent of tumor necrosis through standardization of systematic sampling of the tumor or other means, routine diagnostic reports should reflect this updated information.

Figures:

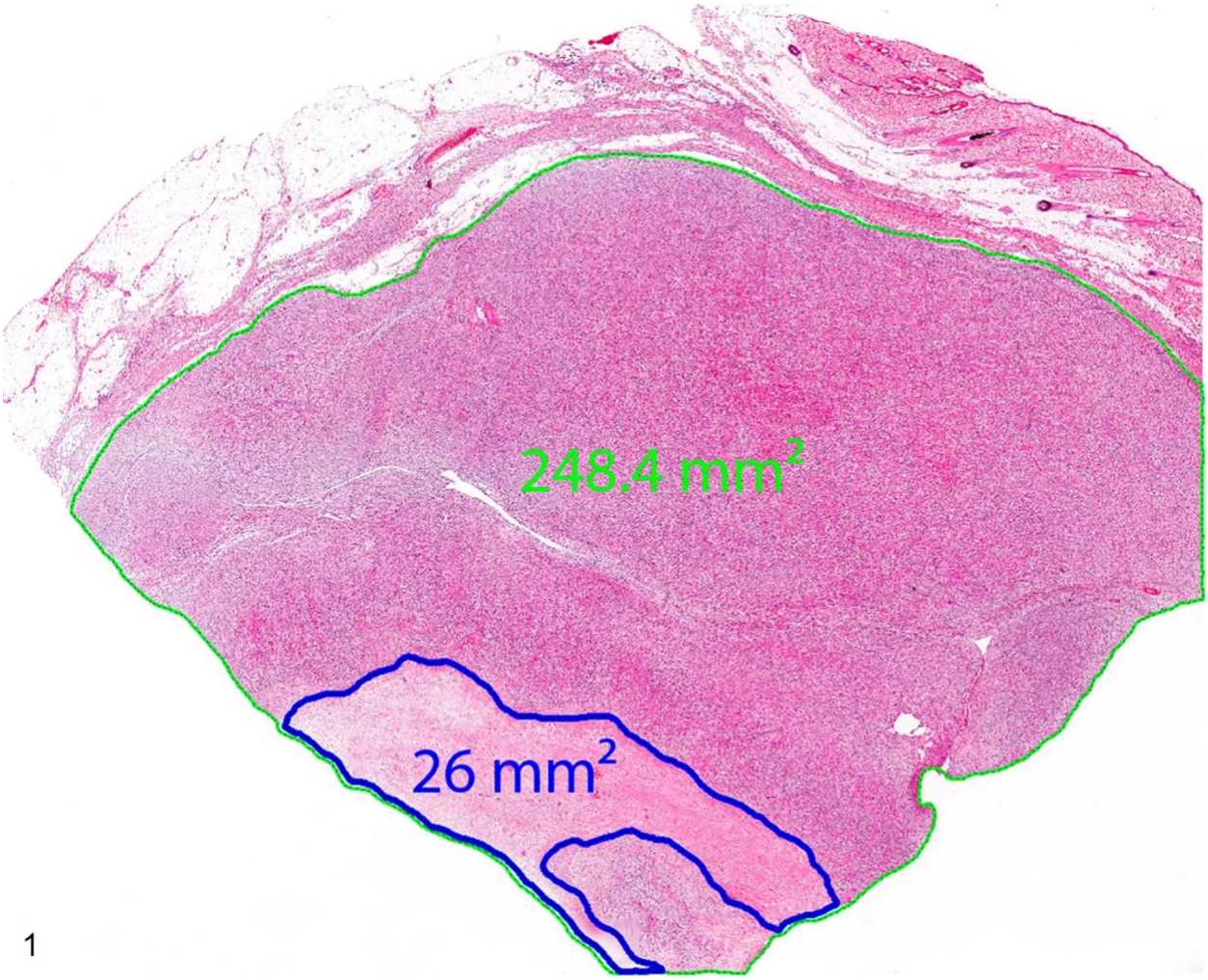

Figure1. Computerized measurement of necrosis area (blue line) and tumor area (green line) in a whole slide image (0.5 x magnification) of a canine, subcutaneous soft tissue sarcoma. The two regions were demarcated manually using a “pen tool” from the viewing software Aperio ImageScope (Leica Biosystems, Wetzlar, Germany) and the software accurately calculates the demarcated areas. In this case the necrosis area (26.0 mm²) is 10.5% of the tumor area (248.4 mm²) of the histological section. This technique accurately denotes the percent necrosis within an individual histologic section but inconsistent sampling of the tumor can skew the findings and result in incorrect estimate of overall tumor necrosis.

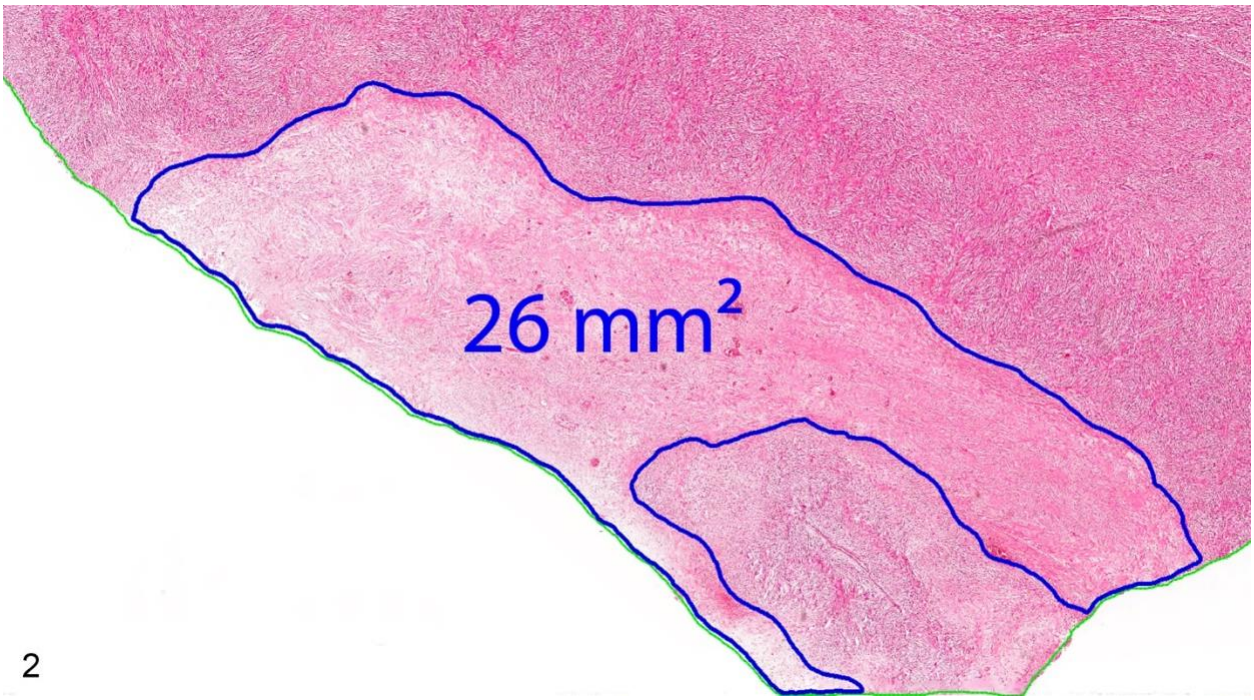

Figure2. Higher magnification of Figure 1.

References:

1. Roccabianca P, Schulman FY, G. A, et al. Tumors of Soft Tissue. 2020;Volume 3 ((Davis Thompson DVM Foundation))**:** 1-306.
2. Kuntz CA, Dernell WS, Powers BE, Devitt C, Straw RC, Withrow SJ. Prognostic factors for surgical treatment of soft-tissue sarcomas in dogs: 75 cases (1986-1996). *J Am Vet Med Assoc*. 1997;211**:** 1147-1151.
3. Coindre JM. Grading of soft tissue sarcomas: review and update. *Arch Pathol Lab Med*. 2006;130**:** 1448-1453.
4. Laurini JA, Blanke CD, Cooper K, et al. Protocol for the Examination of Specimens From Patients With Gastrointestinal Stromal Tumor (GIST). 2017.
5. Trojani M, Contesso G, Coindre JM, et al. Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. *Int J Cancer*. 1984;33**:** 37-42.
6. Vayrynen SA, Vayrynen JP, Klintrup K, et al. Clinical impact and network of determinants of tumour necrosis in colorectal cancer. *Br J Cancer*. 2016;114**:** 1334-1342.
7. Dobromylskyj MJ, Richards V, Smith KC. Prognostic factors and proposed grading system for cutaneous and subcutaneous soft tissue sarcomas in cats, based on a retrospective study. *J Feline Med Surg*. 2020**:** 1098612X20942393.
8. McSporran KD. Histologic grade predicts recurrence for marginally excised canine subcutaneous soft tissue sarcomas. *Vet Pathol*. 2009;46**:** 928-933.
9. Beddy P, Genega EM, Ngo L, et al. Tumor necrosis on magnetic resonance imaging correlates with aggressive histology and disease progression in clear cell renal cell carcinoma. *Clin Genitourin Cancer*. 2014;12**:** 55-62.
10. Monsky WL, Jin B, Molloy C, et al. Semi-automated volumetric quantification of tumor necrosis in soft tissue sarcoma using contrast-enhanced MRI. *Anticancer Res*. 2012;32**:** 4951-4961.