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Intra- and inter-observer agreement in histological assessment of canine soft tissue sarcoma

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Abstract

Background

The diagnosis of canine soft tissue sarcoma (STS) is based on histological assessment. Assessment of criteria such as, degree of differentiation, necrosis score and mitotic score, gives rise to a final tumour grade, which is important in the recommendation of treatment and prognosis of patients.

Materials and Methods

Previously diagnosed cases of STS were independently assessed by three board-certified veterinary pathologists. Participating pathologists were blinded to the original results. For the intra-observer study, the cases were assessed by a single pathologist six months apart and slides were randomized between readings. For the inter-observer study, the whole case series was assessed by a single pathologist before being passed onto the next pathologist. Intraclass correlation coefficient (ICC) and Fleiss's Kappa (ƙ) for the intra- (single observer) and inter-observer agreement.

Results

Strong agreement was observed for the intra-observer assessment in necrosis score, mitotic score, total score and tumour grading (ICC between 0.78 to 0.91). The intra-observer agreement for differentiation score was rated perfect (ICC 1.00). The agreement between pathologists for the diagnosis and grading of canine STS was moderate (ƙ = 0.60 and 0.43 respectively).

Conclusion

Histological assessment of canine STS had high reproducibility by an individual pathologist. The agreement of diagnosis and grading of canine STS was moderate between pathologists. Future studies are required to investigate further assessment criteria to improve the specificity of STS diagnosis and the accuracy of the STS grading in dogs.

Introduction

Soft tissue sarcoma (STS) includes a heterogeneous group of mesenchymal neoplasms derived from soft tissues.[1] It is reported to represent 15% of all skin and subcutaneous tumours in dogs and 7% in cats.[2] STSs are typically considered locally invasive and potentially metastatic tumours. However, rates of local recurrence and metastasis are variable, making accurate prognostication in individual cases difficult.[1]

Various tumour subtypes such as fibrosarcoma, myxosarcoma, liposarcoma, perivascular wall tumour, and peripheral nerve sheath tumour are included in the STS group.[1] A few studies have described a trend that suggest fibrosarcoma and liposarcoma may carry a worse prognosis than other STS subtypes.[3-5] However, the histological subtype currently has little bearing on the clinical management of these tumours as more studies are needed to confirm and measure differences in prognosis among sufficient numbers of tumours of each subtype.[1] In addition, an accurate identification of the STS subtype frequently relies on a large panel of immunohistochemical markers, including those applied to frozen sections[1], making this practice difficult to be routinely applied in the clinical setting.

Genetic alterations have been showed in STS in human and dogs.[6,7] The advances in this field have improved diagnostic accuracy in human STS.[6] These alterations included chromosomal translocation, chromosomal numerical changes, oncogenic mutations, gene amplifications or deletions.[6-7] Veterinary research in the genetic alterations in STS is vastly limited; however, future research in this field may provide objective diagnosis, improve prognostication as well as the development of possible targeted gene therapy.

Presently the most used histological parameter to prognosticate STSs in dogs is the tumour grade, which is calculated based on cellular differentiation, mitotic rate and percentage of tumour necrosis. The application of these histologic criteria allows individual STSs to be categorized into three grades (I-low grade, II-intermediate grade or III-high grade).[3,8-11]

Previous studies have indicated that the grade is strongly predictive for local recurrence in marginally excised canine STSs.[11]

Multivariable analysis indicated that mitotic rate and percentage of tumour necrosis are the only statistically significant prognostic elements of the grading scheme.[3] The mitotic rate is predictive for distant metastasis,[3] survival time,[1,3,4] and local recurrence.[4] Tumour necrosis, is prognostic for survival time after surgery.[3] To date, no single histological criterion has been shown to be a consistent prognostic factor in local recurrence and other patient outcome assessments (such as distant metastasis, survival time and disease free interval) amongst studies. Histologically incomplete margin was significantly associated with decreased disease-free intervals and survival times.[12] There was also a trend of shorter survival time and higher local recurrence with larger tumour size; however, a statistical significance was not found.[4-12]

Comparatively, histological grading is the most important prognostic factor for STS in human patients.[9] Despite this, grade discrepancies are reported in 25 – 34.6% human adult STS cases.[8,10] In addition, the agreement for the diagnosis of non-visceral STS in humans was reported to be 78% between pathologists.

The objective of this study was to assess intra- and inter-observer agreement of histological assessment of canine STS; included criteria were tumour differentiation, mitotic rate, percentage of tumour necrosis, final diagnosis of STS and overall tumour grade.

Materials and methods

Samples

Haematoxylin and eosin stained histology slides of tissue samples previously diagnosed as canine STS were collected from two veterinary referral hospitals. Soft tissue sarcomas excised for curative intent, for cytoreductive intent or for excisional biopsies were included.

Oral and visceral STS as well as mesenchymal tumours such as synovial cell sarcoma, osteosarcoma, haemangiosarcoma and round cell tumour were excluded.

The laboratory identification codes on the histology slide were covered for anonymization and all the cases were numbered sequentially.

Grading and scoring of the tissue sections

Previously diagnosed cases of STS were independently assessed by three board-certified veterinary pathologists. Participating pathologists were blinded to the original results, e.g. tumour subtype and grade, and each other's conclusions. In order to minimise inconsistency among pathologists, previous recommendations on the assessment of STS were adopted.[1] These recommendations included assessment of areas that were well-fixed and not overly complicated by inflammation and/or haemorrhage; mitotic index was assessed within the most cellular part of the tumour and the area with the highest mitotic activity; differentiation represented the histologic type and true differentiation of the tumour but that uncertainty regarding histogenesis had no bearing on degree of differentiation.[1]

For the intra-observer study, the cases were assessed by a single pathologist six months apart and slides were randomized between readings. For the inter-observer study, the whole case series was assessed by a single pathologist before being passed onto the next pathologist.

Initial evaluation targeted confirmation of the original diagnosis of STS. Cases where there was disagreement on the diagnosis of STS were excluded for the subsequent assessment of each canine STS histological parameter.[1] Criteria encompassed tumour differentiation, mitotic rate and percentage of tumour necrosis.[1] In concordance with the grading system, a correlating score was assigned for each criterion (Table 1).[1] A final tumour grade was assigned based on the cumulative score from these three criteria (Table 2).[1] The histologic subtype of the tumour was not assessed.

Statistical analyses were performed using a web-based program (StatsToDo at www.statstodo.com). Intraclass correlation coefficient (ICC) and Fleiss's Kappa (κ) for the numerical and categorical values were calculated for the intra- (single observer) and inter-observer agreement (three observers). Interpretation of the ICC and the κ values is indicated in Table 3.

Results

Intra-observer study

Of 77 cases assessed by the pathologist 7 were considered to not be a STS. Of the cases that were considered STS, tumour grading between the two readings differed in 6/70 cases (8.6%). Of these 6 cases 2 had a different mitotic score and 4 cases had different necrosis scores, resulting in different final tumour grading. One case had a mitotic score difference of '1' and the other case had a difference of '2' between assessment, based on previously established assessment score.[1] The other four cases had a difference in necrosis score of '1'.[1] All these six cases had the same differentiation score for both assessments.

Strong agreement was observed for the intra-observer assessment in necrosis score, mitotic score, total score and tumour grading (ICC between 0.78 to 0.91) (Table 4). The intra-observer agreement for differentiation score was rated perfect (ICC = 1.00).

Inter-observer study

Of the 77 samples previously diagnosed as STS 3 were unanimously assessed as non-STS by all the pathologists. The presumptive diagnosis for these cases included histiocytic sarcoma, haemangiosarcoma, round cell tumour, osteosarcoma, and amelanotic melanoma. Of the 77 cases, disagreement of the diagnosis of STS (is it a STS?) was present in 8 cases (10.4%) (Figure 1) (ie, the agreement between pathologists for the diagnosis of STS was 89.6%). The presumptive diagnosis of these cases included chondrosarcoma, osteosarcoma, histiocytic sarcoma, amelanotic melanoma, haemangiosarcoma, suture

material reaction and granulation tissue. Five of these cases had disagreement between STS and another malignant tumour; 3 of these cases had disagreement between STS and a benign process (suture material reaction or granulation tissue). The 3 cases unanimously assessed to be a non-STS and the 8 disagreed cases were excluded for the subsequent part of the study (assessment of the histological criteria).

For 35/66 (53%) of the cases, at least 1 pathologist disagreed with the tumour grading. The disagreement was only between adjacent grades (i.e. between grade I and II, and between grade II and III); there was no disagreement between tumour grade I and III. . Most cases of disagreement (27/35) were between grade I and grade II.

The agreement for the diagnosis of STS, mitotic scores, necrosis scores and tumour grades were moderate among observers (κ between 0.43 to 0.60) (Table 5). The differentiation score had poor agreement (κ = 0.11) and the total score for all histological criteria had poor agreement (κ = 0.20) among observers (Table 5). If differentiation score was removed from the calculation of the total score, the inter-observer agreement improved to κ value of 0.44; this was an improvement of total score from fair to moderate agreement.

Discussion

Histological assessment remains the main tool for the diagnosis canine STS. As a result, discrepancies of the diagnosis as well as grading of STS can have a profound effect on case management and prognosis. Agreement in the diagnosis of STS in our study was 89.6% between pathologists, compared favourably to that reported in human non-visceral soft tissue sarcomas (78%).[13] In general, misdiagnosis of tumours may lead to increased patient morbidity or mortality through inappropriately tailored treatment, either the absence or administration of unnecessary treatment, such as aggressive surgical excision with or without follow-up radiotherapy in a benign lesion. Misdiagnosis of other tumours as STS may also affect prognostication of patients (eg, haemangiosarcoma vs STS).

Tumour grading has been regarded as the most important prognostic factor and best indicator of metastatic risk in human adult STS.[9] In veterinary medicine, STS tumour grading has been shown to be a predictive factor for local recurrence.[11, 14] The agreement on STS histological grading in this study was moderate (κ = 0.44), which was similar to that in human medicine (κ = 0.49).[8] Histological grading of STS can influence the recommendation of surgical margins, with margins of <1 – 3 cm being described as acceptable for low grade STS.[15,16] In comparison, other authors advised at least 3 cm peripheral margins and a deep margin of fascial plane for STS excision.[17] Tumour grading can also affect recommendations on further treatment for marginally excised STS; some authors supported a more conservative approach for marginally excised grade I and II STS.[11] If conservative approach is adopted for a marginally excised high grade STS misdiagnosed as a lower grade tumour, local recurrence is likely. Local recurrence has been shown to be associated with tumour-related death.[14] On the other hand, an animal with low grade STS misdiagnosed as high grade STS may receive unnecessary adjunctive treatment, such as aggressive scar excision or adjunctive radiotherapy. At the author's institution, tumour excision is recommended for grade III tumours, followed by adjunctive therapy. In contrast, grade I tumours (and often grade II) are excised with margins and are monitored without further adjunctive treatment. Tumour grade of STS may also play a role in metastasis. In some studies, grade III STS has been shown to be significantly associated with metastasis (regional and distant).[3,18] In these studies, metastasis was reported to be as high as 44% of the grade III cases. [3,18] This further emphasised the importance of consistent and accurate tumour grading, especially for prognostication of an individual patient.

Inconsistency in histological diagnosis and grading of canine STS also has a bearing on interpretation of published and future literature. Currently, majority of the literature on canine STS investigated patient outcome based on retrospective information histological diagnosis of STS and the grading. Clearly, inconsistency in these histological assessments may raise question over the results produced by these studies. In addition, the inconsistency might

also play a role in the variable results between studies, explaining why histological criteria, such as mitotic rate and degree of tumour necrosis, have been shown to be prognostic factors for various patient outcome assessment in some studies, but not in others.[1,3,11] Other factors that may result in variation in results between studies include different sample sizes, different methodology in case selection and patient outcome assessment.

In the intra-observer study, final tumour grading differed in 6 cases. In these cases, differences in mitotic and necrosis scores between the assessments resulted in final tumour grade disagreement. Interestingly, the differentiation score was consistent throughout these six cases, despite it being the most subjective criterion of the 3 in the inter-observer study (poor agreement). The marked difference in agreement of the differentiation score for intra-observer (perfect) and inter-observer (poor) could reflect the subjectivity in individual's interpretation of 'degree of resemblance of sarcoma tissue to normal adult mesenchymal tissue'.

Mitotic score and degree of necrosis have been established to be prognostic indicators for survival time, distant metastasis and local recurrence.[3,4] These 2 criteria are more objective in comparison to the tumour differentiation. Despite the objectivity of these criteria, the agreement between observers was only moderate, in contrast to the strong intra-observer agreement. This could be due to variability in microscope field selection for the assessment as well as subjectivity in estimating the percentage of necrosis. In addition, the actual size of the field assessed for the mitotic scoring could vary based on different microscopes.[19] The variability in these criteria result in variability in the total cumulative score and resultant tumour grading. Unfortunately, this variability represents the 'real-life' situation in clinical setting as histological slides are assessed by different pathologists, at different laboratories and using different microscopes.

Agreement on tumour differentiation was poor among observers. This was likely to be secondary to the subjective nature of this assessment. However, microscope field selection,

again, could play a role in this. Interestingly, tumour differentiation score was perfect for the intra-observer study. This, along with strong to almost perfect agreement on other criteria, indicated consistency in single-pathologist evaluation in canine STS.

To minimise the undesired effect of inter-observer variability in the histological assessment of canine STS, recommendations have been made to minimise inconsistency among pathologists.[1] These recommendations included assessment of areas that are well-fixed and not overly complicated by inflammation and/or haemorrhage; mitotic index should be assessed within the most cellular part of the tumour and the area with the highest mitotic activity; differentiation should represent the histologic types and the true differentiation of the tumour; uncertainty regarding histogenesis has no bearing on degree of differentiation.[1] In addition to following these recommendations, the integration of more objective assessments may also be beneficial. The incorporation of immunohistochemical assessment, especially the non-morphological, proliferative markers such as Ki-67 counts and AgNOR assay, may improve the diagnostic specificity, and hence accuracy of prognostic advice on STS in dogs.[17,20,21] Further development in molecular genetics of canine STS and the use of automated mitosis detection may also provide consistent and accurate diagnosis. [6,22]

One of the limitations of this study is the lack of comparison of the results to the 'real' results (such as the 'real' tumour grading or the 'real' mitotic rate). The intra-observer study showed strong to prefect agreement in all parameters assessed, indicating a strong consistency or precision. The inter-observer study showed moderate agreement, hence precision, in most parameters assessed. However, precision differs from accuracy; accuracy refers to the proximity of a measured value to the actual/'real' value whereas the term precision refers to the repeatability of a measurement.[23] As a result, a single-pathologist's histological assessment of canine STS can be precise and accurate at the same time or it can be precise but inaccurate. Similarly, the moderate agreement and precision among pathologists did not necessary equate to moderate accuracy. The clinical application of this limitation is

unknown as there is currently no test that can provide perfect accuracy in the histological assessment of canine STS.

Another limitation of the study is the small numbers of pathologists recruited. A previous study assessing reproducibility of histological grading in human STS had 15 pathologists as well as an additional separate panel involved in the study.[8] Despite the smaller number of pathologists involved in our study, the inter-observer agreement on histological grading was comparable (κ values of 0.49 and 0.44) to that of the human study.8

Conclusion

Histological assessment of canine STS by an individual pathologist had high reproducibility. However, the agreement among pathologists for the diagnosis and grading of canine STS was moderate. Future studies are required to investigate further assessment criteria to improve the specificity of canine STS diagnosis and grading.

Acknowledgement

The study was approved by the Clinical Research and Ethical Approval, Animal Health Trust.

Table 1: Histological criteria assessed and scores assigned[1]

Differentiation score	
1	Sarcomas most closely resembling normal adult mesenchymal tissue, by type (eg, well differentiated perivascular wall or peripheral nerve sheath tumours, well-differentiated fibrosarcomas, or well-differentiated liposarcomas)
2	Sarcomas for which histologic type can be determined, although differentiation is poor (eg, poorly differentiated liposarcoma, fibrosarcoma, poorly differentiated perivascular wall tumour or peripheral nerve sheath tumour)
3	Undifferentiated sarcomas, sarcomas of unknown type
Mitosis score: mitoses per 10 high-power fields (400x)	
1	0 – 9
2	10 – 19
3	> 19
Tumour necrosis score	
0	No necrosis
1	≤ 50% necrosis
2	> 50% necrosis

Table 2: Grade assigned1

Grade	Cumulative scores of the three categories from Table 1
I	≤ 3
II	4 - 5
III	≥ 6

Table 3: Interpretation of intraclass correlation and Kappa values

ICC and Kappa values	Levels of agreement
≤ 0.2	Poor
0.21 to 0.40	Fair
0.41 to 0.60	Moderate
0.61 to 0.80	Strong
> 0.80	Almost perfect

Table 4: Intraclass correlation values (intra-observer agreement)

Parameters	**ICC**	**Agreement**
Necrosis score	0.79	Strong
Mitotic score	0.78	Stro ng
Differentiation score	1.00	Perfect
Total score	0.91	Almost perfect
Grade of STS	0.91	Almost perfect

Table 5: Kappa values (inter-observer agreement)

Parameters	**Kappa**	**Agreement**
Necrosis score	0.46	Moderate
Mitotic score	0.57	Moderate
Differentiation score	0.11	Poor
Total score	0.20	Fair
Grade of STS	0.43	Moderate
STS, yes or no?	0.60	Moderate

Figure 1: Two pathologists considered this as a soft tissue sarcoma (STS); for the third pathologist (in no particular order), the differential diagnosis for this were histiocytic sarcoma or haemangiosarcoma. Haematoxylin and eosin, objective lens ×20.

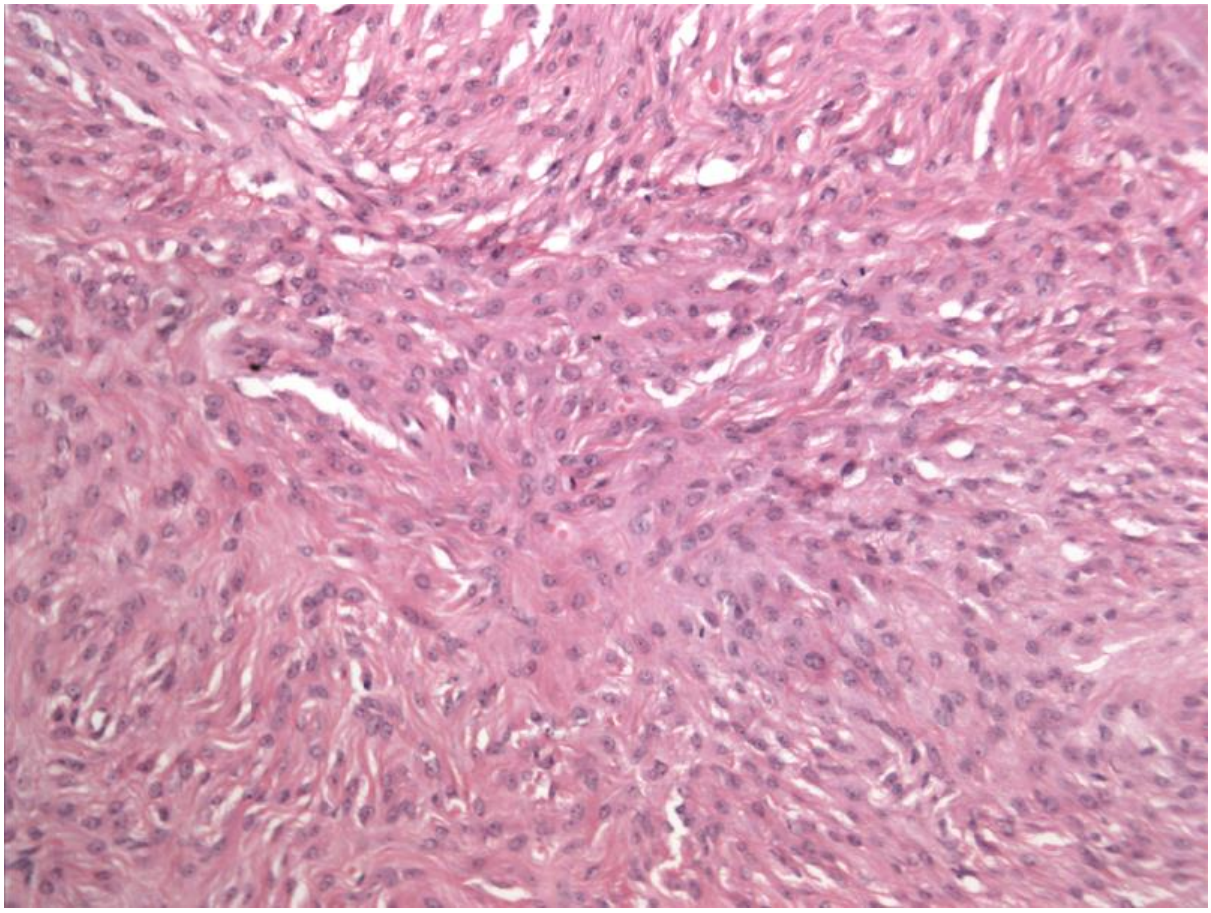
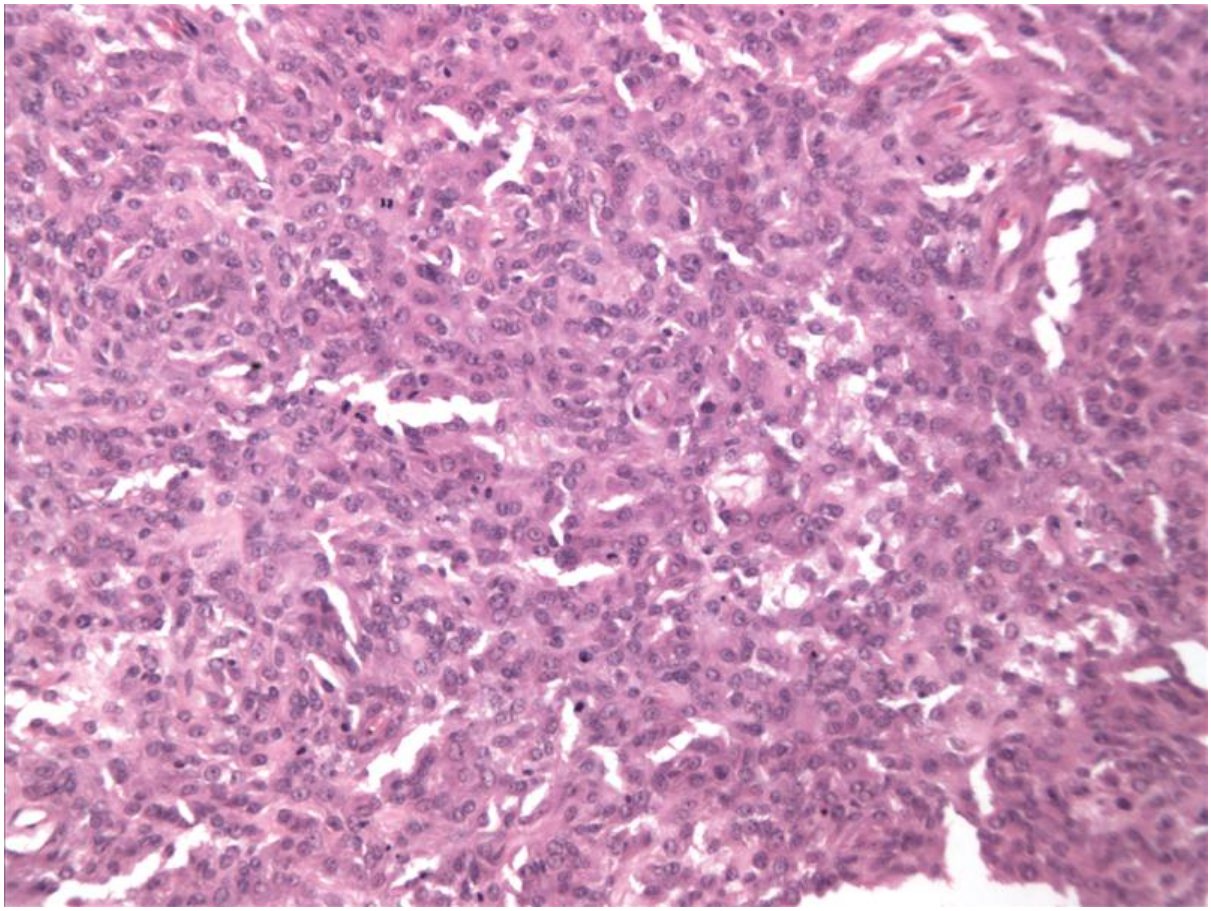


Figure 2: Two pathologists graded this as a grade III soft tissue sarcoma (STS), 1 pathologist graded this as a grade II STS. Haematoxylin and eosin, objective lens ×20.



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