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

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15 **Abstract**

16 Background

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

21 Materials and Methods

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

29 Results

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

35 Conclusion

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

40 **Introduction**

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

46 Various tumour subtypes such as fibrosarcoma, myxosarcoma, liposarcoma, perivascular
47 wall tumour, and peripheral nerve sheath tumour are included in the STS group.¹ A few
48 studies have described a trend that suggest fibrosarcoma and liposarcoma may carry a
49 worse prognosis than other STS subtypes.³⁻⁵ However, the histological subtype currently has
50 little bearing on the clinical management of these tumours as more studies are needed to
51 confirm and measure differences in prognosis among sufficient numbers of tumours of each
52 subtype.¹ In addition, an accurate identification of the STS subtype frequently relies on a
53 large panel of immunohistochemical markers, including those applied to frozen sections¹,
54 making this practice difficult to be routinely applied in the clinical setting.

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

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

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

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

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

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

84 **Materials and methods**

85 Samples

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

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

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

93 Grading and scoring of the tissue sections

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

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

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

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

119 **Results**

120 *Intra-observer study*

121 Of 77 cases assessed by the pathologist 7 were considered to not be a STS. Of the cases
122 that were considered STS, tumour grading between the two readings differed in 6/70 cases
123 (8.6%). Of these 6 cases 2 had a different mitotic score and 4 cases had different necrosis
124 scores, resulting in different final tumour grading. One case had a mitotic score difference of
125 '1' and the other case had a difference of '2' between assessment, based on previously
126 established assessment score.¹ The other four cases had a difference in necrosis score of
127 '1'.¹ All these six cases had the same differentiation score for both assessments.

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

131 *Inter-observer study*

132 Of the 77 samples previously diagnosed as STS 3 were unanimously assessed as non-STS
133 by all the pathologists. The presumptive diagnosis for these cases included histiocytic
134 sarcoma, haemangiosarcoma, round cell tumour, osteosarcoma, and amelanotic melanoma.
135 Of the 77 cases, disagreement of the diagnosis of STS (is it a STS?) was present in 8 cases
136 (10.4%) (Figure 1) (ie, the agreement between pathologists for the diagnosis of STS was
137 89.6%). The presumptive diagnosis of these cases included chondrosarcoma,
138 osteosarcoma, histiocytic sarcoma, amelanotic melanoma, haemangiosarcoma, suture

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

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

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

154 **Discussion**

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

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

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

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

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

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

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

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

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

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

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

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

250 **Conclusion**

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

255 **Acknowledgement**

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

257 **Table 1: Histological criteria assessed and scores assigned¹**

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

260 **Table 2: Grade assigned1**

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

261

262 **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

263

264

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

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

266

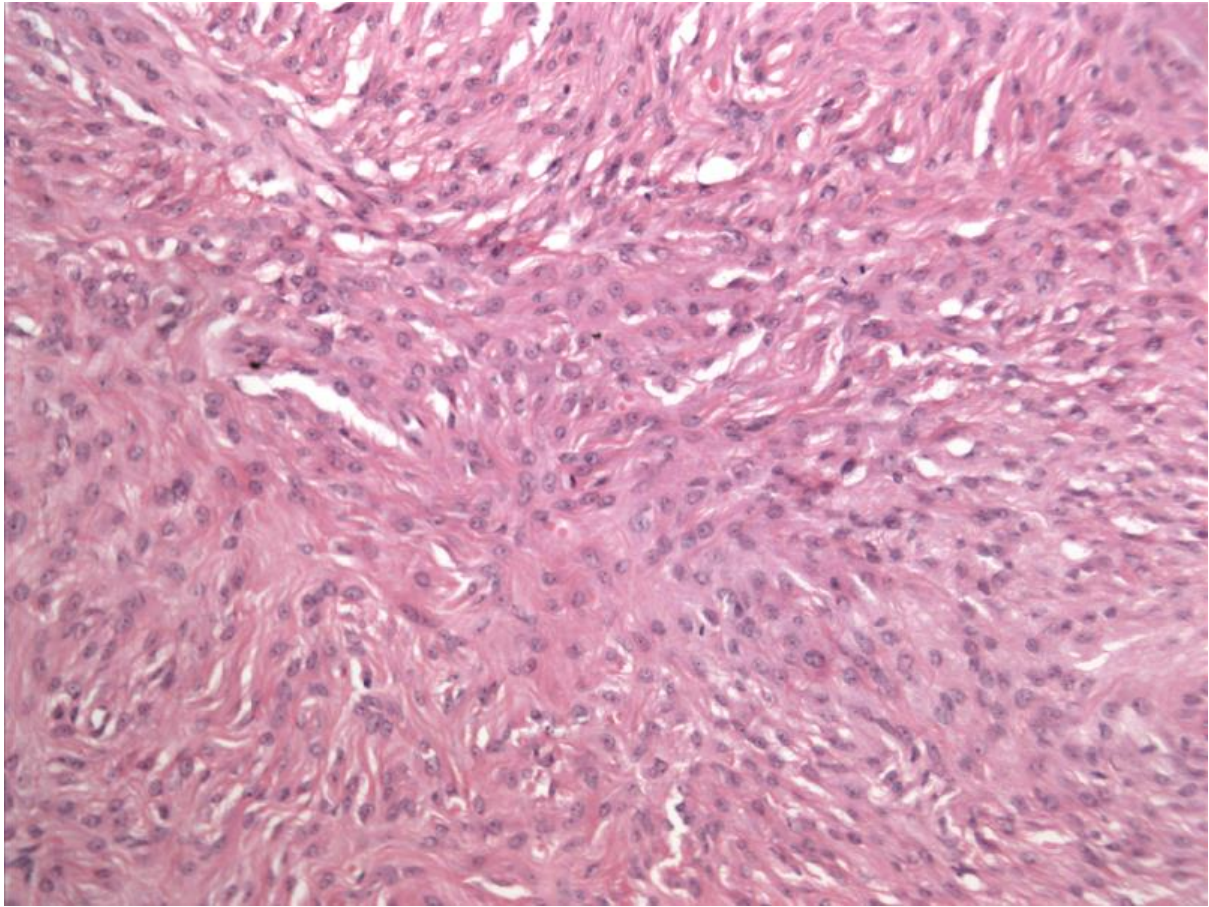
267 **Table 5: Kappa values (inter-observer agreement)**

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

268

269

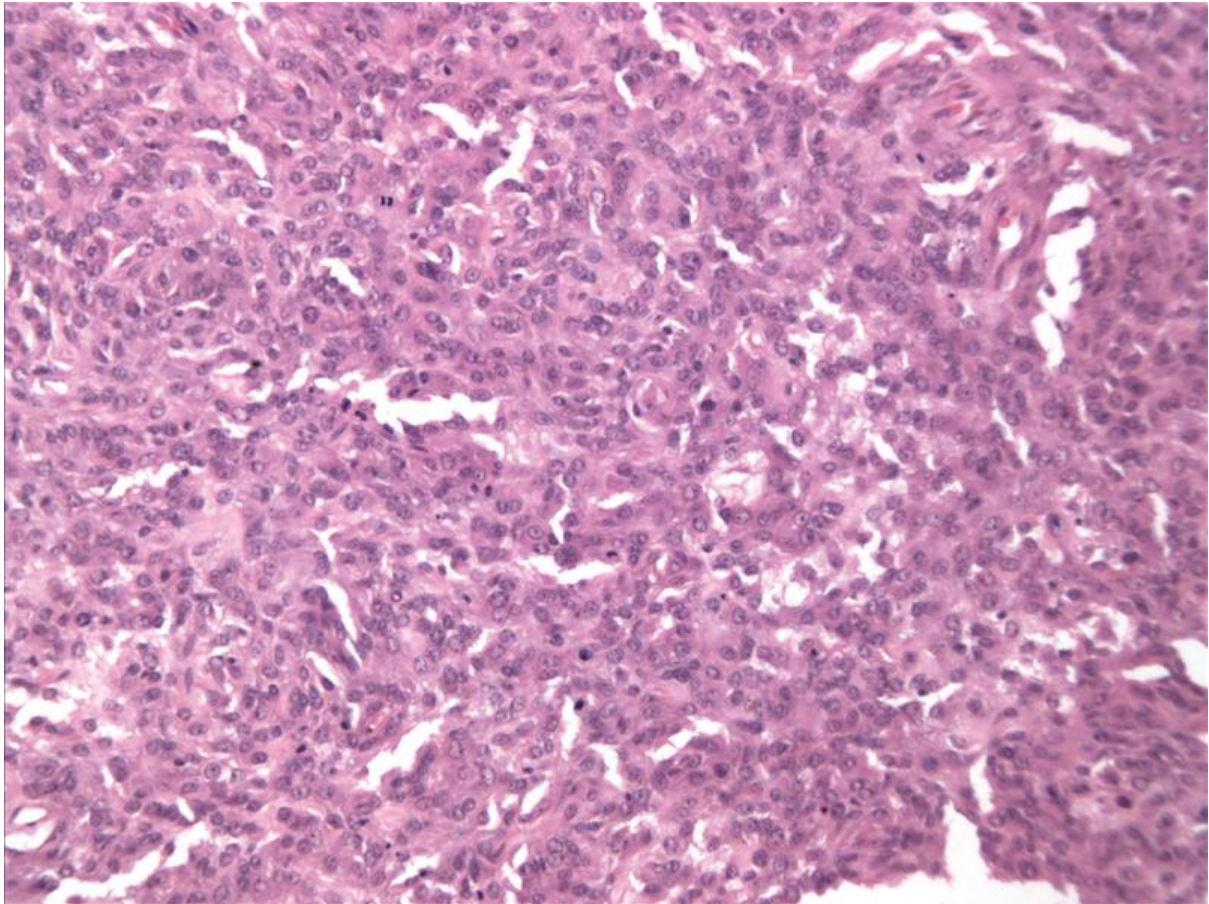
270 **Figure 1:** Two pathologists considered this as a soft tissue sarcoma (STS); for the third
271 pathologist (in no particular order), the differential diagnosis for this were histiocytic sarcoma
272 or haemangiosarcoma. Haematoxylin and eosin, objective lens x20.



273

274

275 **Figure 2:** Two pathologists graded this as a grade III soft tissue sarcoma (STS), 1
276 pathologist graded this as a grade II STS. Haematoxylin and eosin, objective lens x20.



277

278 **References**

- 279 1. Dennis MM, McSporran KD, Bacon NJ, Schulman FY, Foster RA, and Powers BE.
280 Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs.
281 *Veterinary Pathology* 2011; **48**: 73-84.
- 282 2. Theilen GH and Madewell BR. *Veterinary Cancer Medicine*. Philadelphia, Lea &
283 Febiger, 1979.
- 284 3. Kuntz CA, Dernell WS, Powers BE, Devitt C, Straw RC and Withrow SJ. Prognostic
285 factors for surgical treatment of soft-tissue sarcomas in dogs: 75 cases (1986–1996).
286 *Journal of the American Veterinary Medical Association* 1997; **211**: 1147–1151.
- 287 4. Bobstock DE and Dye MT. Prognosis after surgical excision of canine fibrous
288 connective tissue sarcomas. *Veterinary Pathology* 1980; **17**: 581-588.
- 289 5. McChesney SL, Withrow SJ, Gillette EL, Powers BE and Dewhirst MW. Radiotherapy
290 of soft tissue sarcomas in dogs. *Journal of the American Veterinary Medical*
291 *Association* 1989; **194**: 60-63.
- 292 6. Antonescu CR. The role of genetic testing in soft tissue sarcoma. *Histopathology*.
293 2006; **48**: 13-21.
- 294 7. Sargan DR, Milne BS, Aguirre Hernandez J, et al. Chromosome rearrangements in
295 canine fibrosarcomas. *J Hered*. 2005; **96**: 766-773.
- 296 8. Coindre JM, Trojani M, Contesso G, David M, Rouesse J, Bui NB, et al.
297 Reproducibility of a histopathologic grading system for adult soft tissue sarcoma.
298 *Cancer* 1986; **58**: 306-309.
- 299 9. Coindre JM. Grading of soft tissue sarcomas: review and update. *Archives of*
300 *Pathology & Laboratory Medicine* 2006; **130**: 1448-1453.
- 301 10. Guillou L, Coindre JM, Bonichon F, et al. Comparative study of the National Cancer
302 Institute and French Federation of Cancer Centers Sarcoma Group grading systems
303 in a population of 410 adult patients with soft tissue sarcoma. *Journal of Clinical*
304 *Oncology* 1997; **15**: 350-362.

- 305 11. McSporran KD. Histologic Grade Predicts Recurrence for Marginally Excised Canine
306 Subcutaneous Soft Tissue Sarcomas. *Veterinary Pathology* 2009; **46**: 928-933.
- 307 12. Chase D, Bray J, Ide A, Polton G. Outcome following removal of canine spindle cell
308 tumours in first opinion practice: 104 cases. *J Small Anim Pract.* **2009**; 50:568-574.
- 309 13. Harris M, Hartley AL, Blair V, Birch JM, Banerjee SS, Freemont AJ, *et al.* Sarcomas
310 in North West England: I. Histopathological peer review. *British Journal of Cancer*
311 1991; **64**: 315-320.
- 312 14. Yap FW, Abbondati E, Helm JR, Abbodanti E, Helm J, and Pratschke K. Glucose
313 transporter-1 (Glut-1) expression in canine soft tissue sarcomas. *Proceedings of the*
314 *24th Annual Scientific Meeting ECVS 2015*; 184.
- 315 15. Banks T, Straw R, Thomson M and Powers B. Soft tissue sarcomas in dogs: A study
316 assessing surgical margin, tumour grade and clinical outcome. *Australian Veterinary*
317 *Practitioner* 2004; **34**: 142-147.
- 318 16. Stefanello D, Morello E, Roccabianca P, Iussich S, Nassuato C, Martano M, *et al.*
319 Marginal excision of low-grade spindle cell sarcoma of canine extremities: 35 dogs
320 (1996-2006). *Veterinary Surgery* 2008; **37**: 461-465.
- 321 17. Ettinger SN. Principles of treatment for soft tissue sarcomas in the dog. *Clinical*
322 *Techniques in Small Animal Practice* 2003; **18**: 118-122.
- 323 18. Ettinger SN, Scase TJ, Oberthaler KT, Craft DM, McKnight JA, Leibman NF, *et al.*
324 Association of argyrophilic nucleolar organizing regions, Ki-67, and proliferating cell
325 nuclear antigen scores with histologic grade and survival in dogs with soft tissue
326 sarcomas: 60 cases (1996-2002). *Journal of the American Veterinary Medical*
327 *Association* 2006; **228**: 1053-1062.
- 328 19. Meuten DJ, Moore FM and George JW. Mitotic Count and the Field of View Area -
329 Time to Standardize. *Veterinary Pathology* 2016; **53**: 7-9.
- 330 20. Tomita Y, Aozasa K, Myoui A, Kuratsu S, Uchida A, Ono K, *et al.* Histologic grading
331 in soft-tissue sarcomas. An analysis of 194 cases including AgNOR count and mast-
332 cell count. *International Journal of Cancer* 1993; **54**: 194-199.

- 333 21. Choong PF, Akerman M, Willén H, Andersson C, Gustafson P, Baldetorp B, *et al.*
334 Prognostic value of Ki-67 expression in 182 soft tissue sarcomas. Proliferation--a
335 marker of metastasis? *Acta Pathologica, Microbiologica, et Immunologica*
336 *Scandinavica* 1994; **102**: 915-924.
- 337 22. Irshad H. Automated mitosis detection in histopathology using morphological and
338 multi-channel statistics features. *J Pathol Inform.* 2013; **4**:10-15.
- 339 23. Federer WT. *Statistics and Society – Data collection and interpretation.* New York,
340 Marcel Dekker, 1991.