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BRIEF COMMUNICATION

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Correlation between cytologic features and histologic grades in cutaneous and subcutaneous soft tissue sarcomas in dogs—A pilot study

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Abstract

Currently, canine soft tissue sarcoma (STS) grading is based on histopathology. In humans, several studies have demonstrated concordance between cytologic grading systems for STS and histologic grade. The aim of this study was to correlate several cytologic parameters (smear cellularity, anisokaryosis, nucleolar malignancy score, multinucleation, and the number of mitotic figures per 200 cells) that form part of a human STS cytologic grading system, with histologic grades of canine cutaneous and subcutaneous STS. Three observers (blinded) reviewed the cytologic preparations independently from cases with confirmed histologic diagnoses of STS. A cytologic grading score was assigned for each parameter. Correlations between cytologic grading scores (averaged between observers) and histologic grades were assessed using Spearman's correlation coefficient, with statistical significance defined as $P < .05$. Twenty-one cases were included in the study (10 Grade I STS, nine Grade II STS, and two Grade III STS). The number of mitotic figures (≥3) per 200 cells was the only parameter that showed a significant but weak, positive correlation with histologic grade ($r_s = .469$; $P = .032$). No Grade I tumors had ≥3 mitotic figures per 200 cells; however, ≥3 mitotic figures per 200 cells were only observed in 33% of Grade II tumors and 50% (one out of two) of the Grade III tumors. This pilot study suggests that an increased number of mitotic figures seen on cytology might correlate with higher grade STS; however, the sensitivity of this parameter for grading STS appears to be low.

KEYWORDS

cellularity, cytology, grading, mitotic figures, soft tissue sarcoma

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Soft tissue sarcomas (STS) are a heterogeneous group of tumors derived from tissues of mesenchymal origin with similar clinical behaviors. These neoplasms arise from tissues of mesenchymal origin on any part of the body, although the skin and subcutaneous tissues are the most commonly affected sites. These neoplasms can recur locally after surgical removal, and the likelihood of recurrence has been associated with the histologic grade and the completeness of surgical excision; low grades and clean margins are independent predictors of non-recurrence.[1]

Currently, STS tumor grading is based on histopathology. Tumor grading is considered more useful than histologic subtyping for predicting prognosis and planning treatment strategies. The histologic grading system described for canine STS classifies STS Grades from I to III, with Grade III being the most malignant.[2] The likelihood of a distant metastasis ranges from low to moderate, with an increased likelihood of metastasis being associated with higher grade STSs.[2] In people, several studies have evaluated cytologic grading systems for STS, which incorporate cytologic parameters including nuclear atypia, nuclear overlap, mitotic figures, necrosis, and cellularity. These studies have demonstrated a concordance between cytologic and histologic grading systems of 55%-92%,[3-6] which suggests that cytologic findings can be useful for grading STS. However, to the authors' knowledge, no studies have attempted to develop similar cytologic grading systems for STS in dogs.

The aim of the present pilot study was to evaluate the correlation between the cytologic features that are included in a human STS cytologic grading system and the histologic grade of canine cutaneous and subcutaneous STS. Cytologic parameters that correlate with the histologic grade might then form part of a cytologic grading system for canine STS.

Cases with a histologic diagnosis of canine cutaneous and subcutaneous STS that were presented to the Queen's Veterinary School Hospital between January 2014 and March 2018 were identified. Cases without corresponding cytologic samples from fine-needle aspirates of a mass were excluded. In addition, cases with cytologic specimens considered unsuitable for cytologic evaluation were also excluded. Suitability of the aspirate smear preparations for cytologic evaluation was characterized by the presence of sufficient numbers of well-preserved cells (>200) to permit cytologic evaluation. Cases included had fine-needle aspirate cytologic preparations that had been previously stained with a Wright-Giemsa stain using an automated slide stainer (Aerospray; Wescor). The cytologic smears were randomly numbered (by a person who did not perform cytologic scoring) and independently assessed by three clinical pathologists with different levels of clinical pathology experience: one intern with an interest in clinical pathology, one clinical pathology resident, and one board-certified clinical pathologist. The observers did not have any information about patient identifications, previous cytologic diagnoses, or the assigned histologic grades.

The cytologic features of mesenchymal cells were scored by each observer using a cytologic grading system adapted from Jones et al[4] (Table 1). When the scores for a specific parameter differed between observers by 1 point, then the scores were averaged to obtain the overall score for that parameter. If the cytologic score given for an individual parameter differed by 2 points between observers, then the smears were reviewed by the three observers together, and a consensus score was obtained. If the slides were deemed not cellular enough to evaluate by one observer, then the scores for the other two observers were averaged.

The histologic preparations were re-examined by a single boarded anatomic pathologist (FCC) blinded to cytologic scores. The histologic grade and classification for each STS were given using the system previously mentioned.[2] All tumor sections were collectively evaluated microscopically, considering the following features: the degree of neoplastic cell differentiation, number of mitoses, and amount of tumor necrosis. Differentiation was considered by the architecture or histologic type and how closely neoplastic cells resembled normal mesenchymal cells. Mitotic counts were measured in 10 HPFs (2.37 mm^2) in the most cellular areas of the tumor sections, where the highest mitotic rate could be found. Tissue necrosis was assessed by the percent of sections examined from each tumor.

Correlations between the individual scores for each parameter and the histologic grades were assessed using Spearman's rank correlation coefficient. Statistical significance was defined as a $P < .05$.

Twenty-one histologically diagnosed STS cases with prior adequate fine-needle aspirate preparations were eligible for inclusion. Histologic diagnoses included perivascular wall or peripheral nerve sheath tumors (14), perivascular wall tumors (two), peripheral nerve sheath tumors (two), fibrosarcomas (two), and fibrosarcoma or peripheral nerve sheath tumor (one). Ten STS tumors were low grade (I), nine were intermediate grade (II), and two were high grade (III). Table 2 records the average scores obtained using the cytologic grading system compared with the histologic grade of the 21 cases. Cytologic scores were recorded for each parameter by the individual observers before and after consensus scores were reached, as shown in Tables S1 and S2, respectively. To reach a consensus score, five preparations required re-evaluation due to major discrepancies amongst the observers (Tables S1 and S2).

The number of mitotic figures per 200 cells was associated with the histologic tumor grade ($r_s = .469$; $P = .032$; Table 3). No Grade I tumors (n = 10) had ≥3 mitotic figures per 200 cells; however, ≥3 mitotic figures per 200 cells were only observed in 33% of Grade II tumors (n = 9) and 50% (one out of two) of the Grade III tumors

TABLE 1 Parameters assessed in the cytologic grading of soft tissue sarcomas, adapted from Jones et al[4]

Criteria for cytologic grading of soft tissue sarcomas	
Features scored	**Points assigned**
Cellularity	1 (low), 2 (moderate), 3 (high)
Number of mitotic figures/200cells	1 (0-2), 2 (3-5), 3 (>5)
Anisokaryosis	1 (mild <25% variability), 2 (moderate 25%-50% variability), 3 (marked >50% variability)
Multinucleation	1 (absent), 2 (occasional), 3 (frequent)
Nucleolar malignancy (based on number, size and shape of nucleoli)	1 (mild), 2 (moderate), 3 (severe)

TABLE 2 The average scores for each parameter in all cases with the corresponding histologic grade and histologic diagnosis

		Nuclear atypia					
Case no	Cellularity	Anisokaryosis	Multinucleation	Nucleolar malignancy	No mitotic figures/200 cells	Histology grade	Histologic diagnosis
1	2	2.7	2.7	2.7	1	I	PVWT/PNST
2	1.7	1	2	1.4	1	III	Fibrosarcoma/PVWT
3	1	1.4	2.7	1.4	1	I	PVWT/PNST
4	3	1.4	2.4	1.4	1	I	PVWT/PNST
5	2.4	3	1.4	1	1	II	Fibrosarcoma
6	1.7	2	2.7	2	1	I	PVWT
7	3	1.7	2.7	1	1	II	Fibrosarcoma
8	3	1.7	1.4	1	1	II	PVWT
9	1.4	1.4	2.7	1.4	1	II	PVWT/PNST
10	2.7	1.7	2.7	2	1.4	II	PVWT/PNST
11	1	1.5	1	1	1	II	PVWT/PNST
12	3	2.4	1.4	1.4	1.4	III	PVWT/PNST
13	3	2.4	3	2.4	1	I	PNST
14	2	3	2	3	1.4	II	PVWT/PNST
15	1.4	1.4	2.7	1.4	1	I	PNST
16	2.4	1.7	1.4	1.7	1	I	PVWT/PNST
17	2	1.7	2	2.4	1	I	PVWT/PNST
18	1	1	1.5	1	1	I	PVWT/PNST
19	1.4	1.4	1.4	1.7	1	II	PVWT/PNST
20	1	1.4	1.7	1.7	1	I	PVWT/PNST
21	3	2	1.4	1.7	1.7	II	PVWT/PNST

Abbreviations: PNST, peripheral nerve sheath tumor; PVWT, perivascular wall tumor.

(Table 2). No other cytologic parameters were significantly associated with tumor grade (Table 3).

The aim of the present study was to evaluate if any cytologic parameters would correlate with histologic tumor grades. The only cytologic grading parameter that was significantly correlated with the histologic tumor grade was the presence of ≥3 mitoses per 200 cells. The mitotic index is an indicator of the proliferative activity of the cells and was shown to be associated with more malignant tumors in a previous study.[2] In the present study, increased numbers of mitotic figures (≥3 per 200 cells) were only detected in four cases, which probably reflected the relatively low sensitivity of cytology for determining mitotic activity in neoplasia. Since increased numbers of mitotic figures were only detected on cytology in STSs with histologic Grades II or III, their presence might make Grade I STS less likely. However, further studies are needed to evaluate this premise in a larger number of dogs and to validate these findings. It is possible that the number of mitotic figures identified might be related to the presence of myxomatous or collagenous matrix within a tumor; however, this was not evaluated in the current study. Further studies are needed to investigate if a correlation between the number of mitoses and the presence of myxomatous or collagenous matrix on cytology exists.

It has been reported that more malignant tumors tend to exfoliate more readily, despite STS being a poorly exfoliative neoplasm[7]; however, in the present study, the cellularity of the smear was not significantly associated with the histologic tumor grade. The cellularity of the smears might also be influenced by inter-individual differences in the aspirate technique used to obtain the samples, and this might also have reduced the likelihood of detecting a statistically significant association between cellularity and histologic grade.

Anisokaryosis, multinucleation, and nucleolar malignancy score were also not associated with the histologic tumor grade. This lack of association with tumor grade might reflect the relative subjectivity of the parameters that contributed to the overall nuclear atypia score and the subsequently higher variability between observers. Also, in certain STS subtypes, such as perivascular wall tumors, multinucleation can be a characteristic morphologic feature regardless of the tumor grade, thus, limiting the use of the nuclear atypia for the cytologic grading of sarcomas.

The previous study by Jones et al[4] included the presence or absence of necrosis on cytology as a contributor to the overall cytologic score; however, we did not consider this parameter in the present study because cytologic evidence of necrosis was not observed in any of the slides evaluated. This was despite the fact that necrosis was observed on the histologic sections in 10 of the STS cases included, which suggests that cytology is an insensitive method for detecting necrosis in canine STS. Further studies including a larger

TABLE 3 Correlations between individual cytologic criteria and the histologic grade (n = 21). Correlations were assessed using Spearman's correlation coefficient

Cytologic criteria	r_s	Significance
Cellularity	.256	.262
Anisokaryosis	.122	.598
Multinucleation	−.399	.073
Nucleolar malignancy	−.306	.177
Mitotic figures per 200 cells	.469	.032

number of cases would be required to establish if the cytologic evidence of necrosis is associated with STS tumor grade; however, it seems likely that cytologic evidence of necrosis would be an insensitive indicator of malignancy based on our preliminary findings.

The main limitation of this study was the low number of cases that were included and particularly the low number of Grade III STS tumors. However, despite this limitation, we were able to determine that some cytologic parameters, such as the number of mitotic figures per 200 cells, might be worthy of a further investigation focusing on the cytologic markers of high-grade STS. An additional limitation was that the observers had different levels of expertise, which could have resulted in higher variability between observers and might have reduced the correlation between the cytologic scores and the histologic grades in the present study. Although, we could argue that this higher variablility strengthens our preliminary findings. Finally, it could be speculated that the opinion of the board-certified clinical pathologist might have biased the consensus scores agreed on when individual cytologic scores differed by two points among the observers (as was the case for seven parameters in five cases). However, the agreed-on consensus score only corresponded with the original score given by the board-certified clinical pathologist in three out of seven cases.

In conclusion, the results of this pilot study suggest that the number of mitoses per 200 cells correlates with the histologic grades of canine cutaneous and subcutaneous STS; however, the sensitivity of this parameter for the detection of higher grade (Grade II or III) STS is likely to be low. Further studies are required to confirm these findings in a larger cohort of dogs with cutaneous and subcutaneous STS.

DISCLOSURE

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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