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Evaluation of leukocyte counts and neutrophil-to-lymphocyte ratio as predictors of local recurrence of feline injection site sarcoma after curative intent surgery

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(Article begins on next page)

1 **EVALUATION OF LEUKOCYTE COUNTS AND NEUTROPHIL-TO-LYMPHOCYTE**
2 **RATIO AS PREDICTORS OF LOCAL RECURRENCE OF FELINE INJECTION SITE**
3 **SARCOMA AFTER CURATIVE INTENT SURGERY**

4
5
6 **Abstract**

7 Local recurrence (LR) is the major concern in the treatment of feline injection-site sarcoma (FISS).
8 Pretreatment leukocyte counts and ratios have been reported as diagnostic and/or prognostic
9 markers in human and canine oncology. The aim of this retrospective study was to explore the
10 prognostic impact on LR and overall survival time (OST) of pretreatment neutrophil-to-lymphocyte
11 ratio (NLR), white blood cell count (WBCC), neutrophil count (NC) and lymphocyte count (LC) in
12 cats with surgically excised FISS.

13 Eighty-two cats with histologically confirmed FISS at first presentation, without distant metastases,
14 and with available pretreatment hematological analyses were retrospectively enrolled. The
15 correlation of NLR, WBCC, NC, LC with tumor variables and patient variables was explored. NLR
16 was correlated with tumor size (P 0.004), histological pattern of tumor growth (P 0.024) and
17 histotype (P 0.029), while WBCC and NC were associated with ulceration (P 0.007, P 0.011) and
18 pattern of growth (P 0.028, P 0.004). No significant relationships emerged between LC and any of
19 the considered variables. The impact of NLR, WBCC, NC, LC on LR and OST was then estimated
20 in univariate and multivariate analysis. In univariate analysis, NLR, WBCC, NC were significant
21 prognostic factors for both LR and OST. NLR, WBCC, NC remained prognostic in multivariate
22 analysis for LR but not for OST. When NLR, WBCC and NC were jointly analyzed, WBCC was
23 the marker with the greater impact on LR. Preoperative NLR, WBCC, and NC may aid in
24 identifying cats at higher risk of LR.

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37 **Introduction**

38 Feline injection-site sarcoma (FISS) is among the four most common feline skin cancers.¹ Although
39 it was initially hypothesized that the etiology of FISS is strictly related to vaccination², it is
40 nowadays widely accepted that this tumor can develop following any stimulus that causes chronic
41 local inflammation of the subcutis or muscles.³⁻⁷ Despite the relatively low incidence of distant
42 metastasis (0–28%)⁸⁻¹¹, FISS tends to be locally aggressive and local recurrence (LR) represents the
43 major concern, with reported rates as high as 14%–42%,^{8,12-15} therefore, achieving adequate control
44 of local disease through wide-margin/radical surgical excision is the cornerstone in oncological
45 management of the tumor^{14,16}.

46 The prognostic impact of different variables on LR and overall survival time (OST) after wide-
47 margin/radical excision has been widely explored in the last two decades. Histological grading is
48 considered one of the most important prognostic factors for canine soft tissue sarcomas¹⁷ (STS);
49 nonetheless, the traditional three-tier grading system has shown limited value in predicting LR and
50 outcome for FISS.^{11,13,17-18}

51 Completeness of excision is indeed crucial in obtaining long-term control of the local disease;
52 however, LR has been reported even in the presence of histologically non-infiltrated margins,
53 suggesting that factors other than completeness of excision may be implicated in tumor
54 relapse.^{11,13,19}

55 Although other variables, such as size and location of the tumor, expertise of the surgeon, recurrent
56 tumors, p53 expression, mitotic count, and differentiation, have been proposed as predictors of LR,
57 there is still no consensus on which factors other than completeness of excision should be taken into
58 account to predict prognosis.^{8,13,15,20,21} Hence, readily available and accurate variables that can aid
59 in identifying cats at higher risk of LR are desirable to improve oncological management of these
60 patients.

61 Pretreatment neutrophil-to-lymphocyte ratio (NLR) is a marker of systemic inflammatory response
62 that has been reported to be a useful prognostic tool for several solid tumors in human medicine,
63 including STS.²² Recently, the veterinary literature has reflected growing interest in peripheral
64 blood cell abnormalities as diagnostic and prognostic markers for both neoplastic and inflammatory
65 conditions. A few papers have explored the prevalence of different leukocyte populations and T-
66 lymphocyte subsets in tumor-bearing versus healthy dogs.²³⁻²⁶ Furthermore, leukocyte counts and
67 ratios have been proposed as diagnostic and prognostic tools for dogs with lymphoma,
68 osteosarcoma, mast cell tumors, and STS.²⁷⁻³¹ Yet, the prognostic impact of peripheral leukocyte

69 counts and ratios, including NLR, has not been assessed in feline medicine. Given the promising
70 results described in canine oncology, this retrospective study aimed to explore the prognostic
71 impact on LR of pretreatment white blood cell count (WBCC), neutrophil count (NC), lymphocyte
72 count (LC), and NLR in cats with newly diagnosed, surgically excised FISS. As a secondary aim,
73 the impact of leukocyte counts and NLR on OST was assessed.

74 **Materials and Methods**

75 Records (January 2002 to December 2017) from the XX and YY were searched for client-owned
76 cats with histologically confirmed FISS. The main inclusion criteria were: FISS at first presentation,
77 absence of distant metastasis (assessed by total body contrast-enhanced CT or thoracic radiography
78 and abdominal ultrasound), and treatment by wide-margin/radical surgical excision with three to
79 five cm lateral margins and two deep fascial planes or limb or tail amputation. To be eligible for
80 inclusion, presurgical complete hematological data with leukocyte differential (within 45 days
81 before surgery) had to be available. Exclusion criteria were: (1) neoadjuvant chemotherapy and/or
82 radiotherapy, (2) adjuvant radiotherapy, (3) FIV and/or FeLV positivity, (4) administration of
83 antibiotics and/or corticosteroids within two months before surgery, and (5) lack of availability of
84 postoperative follow-up information.

85 Data retrieved from the medical records of included cats were: signalment (breed, sex, age, weight),
86 characteristics of the primary tumor (location, size at clinical examination, ulceration), presurgical
87 laboratory data (leukocyte counts, hyperglycemia as indicator of stress), concomitant diseases if
88 present, histological findings (histotype, necrosis, grading, pattern of growth, margin status), and
89 outcome (time to LR, time to metastases, cause of death, OST).

90 For pretreatment hematological analysis, blood was collected in EDTA, and CBC parameters,
91 including leukocyte differential, were measured with the same laser-based analyzer at the laboratory
92 of one of the two institutions (ADVIA®120 Hematology System, Siemens Diagnostics).
93 Instrumental differential was also checked microscopically on May-Grunwal-Giemsa stained
94 smears. NLR was calculated as the ratio of the absolute count of neutrophils to lymphocytes. When
95 the biochemistry profile was available, hyperglycemia was retrieved and considered as a possible
96 marker of stress that could have altered the absolute values of both neutrophils and lymphocytes
97 and, as a consequence, the NLR. Variations from normal values of WBCC, NC, and LC were
98 defined based on the reference intervals reported in Table 1.

99 Histopathological specimens were processed and examined by the same pathologist at each
100 institution (XX, XY). Variables retrieved from the histological reports were: histotype, pattern of
101 growth³², necrosis (semiquantitatively scored as 0 when absent, 1 when <25%, 2 when 25%-50%,
102 and 3 when >50%), histological grading¹⁷ if available, and status of surgical margins¹³ (infiltrated

103 or non-infiltrated). Surgical margins were evaluated combining, at trimming, two techniques: radial
104 sectioning along the longest axis of the sample (perpendicular margins)³³, and tangential (*en face*)
105 sectioning/3D technique^{13,33}.

106 Follow-up information was obtained from the clinical records or by telephone conversations with
107 cat owners or referring veterinarians. Time to LR and OST were the primary and secondary
108 endpoints, respectively. Time to LR was calculated from the date of surgery to the date of LR,
109 defined as a cytologically or histologically confirmed FISS growing within two cm from the scar of
110 previous excision. OST was calculated from the date of surgery to the date of death or euthanasia;
111 cause of death was classified as either tumor related or tumor unrelated.

112

113 *Statistical analysis*

114 Statistical analysis evaluated the relationship between NLR, WBCC, NC, and LC and the following
115 variables: age, glycemia, tumor size, ulceration, concomitant diseases, tumor necrosis, tumor
116 histotype, and histological pattern of growth. The impact of NLR, WBCC, NC, and LC on LR and
117 OST was then assessed.

118 Pearson coefficient (r) was used to assess the linear correlation between NLR, WBCC, NC, and LC
119 and continuous variables (tumor size, glycemia, age). Results were reported as estimated correlation
120 coefficient and 95% confidence intervals (CI). The strength of the correlation was interpreted
121 following the rule of thumb.³⁴ The null hypothesis of the absence of correlation between two
122 variables was tested by t statistics.

123 For categorical variables (ulceration, concomitant diseases, necrosis, histotype, pattern of growth),
124 the distributions of NLR, WBCC, NC, and LC for each modality were reported as: minimum, Q1
125 (25%), mean, median, Q3 (75%), and maximum. As NLR, WBCC, NC, and LC were non-normally
126 distributed, non-parametric tests were used to compare the distributions of the above-mentioned
127 variables in the classes of categorical variables. Wilcoxon rank sum test with continuity correction
128 was used for the variables with two modalities and Kruskal-Wallis test for the variables with three
129 modalities.

130 The probability of being free of LR during follow-up was estimated by the Kaplan-Meier method.
131 For cats that died without developing LR, times to death were censored to the death date, assuming
132 independence between time to LR and time to death without LR. Patients lost to follow-up were
133 censored at the time of the last contact. However, since the above cited independence assumption
134 could not be determined on the basis of the available follow-up data, we also reported the bounds in
135 which the correct estimates of LR free survival it is expected to lie, avoiding the assumption of
136 independence.³⁵

137 The probability of surviving during follow-up was estimated by the Kaplan-Meier method. Cox
138 regression model was used to explore the prognostic impact of NLR, WBC, NC, LC and all other
139 examined clinical and pathological variables on LR and OST. Firstly, univariate analysis was
140 performed; then, a multivariate model was used to evaluate the prognostic role of each
141 hematological value, adjusted for the clinical and pathological variables which resulted statistically
142 significant in univariate analysis. For OST analysis, the maximum number of variables that could
143 be included in the model was determined following the EPV rule.³⁶ For LR analysis, a less
144 conservative rule³⁷ was applied, thus results of this analysis should be considered preliminary. A
145 backward selection procedure was used to obtain a final model that included only statistically
146 significant variables. To assess the robustness of multivariate analysis, bivariate models were also
147 performed adjusting NLR, WBCC, NC for each one of the clinical and pathological variables.
148 Categorical variables were included into the model as dummy variables, while continuous variables
149 were included in their original measurement scale. For these latter, the possible non-linear
150 relationship, was evaluated by regression cubic splines and the contribution of non-linear terms was
151 tested by the likelihood ratio test. Results were reported as cause-specific hazard ratios for LR and
152 hazard ratios for OST, and 95% CI. The Wald test was used to assess the significance of the
153 regression coefficients of the model. No competing risks were considered for LR, as this event was
154 recorded regardless of the occurrence of distant metastases. Predictive accuracy of the survival
155 model was determined by calculating the area under the curve (AUC) extended for survival analysis
156 (Harrell C statistic). Time-dependent ROC curves were used to find the best cut-off values for NLR,
157 WBCC, and NC on the basis of the Youden Index (i.e. sensitivity + specificity).³⁸
158 Median follow-up time was estimated with the reverse Kaplan-Meier method.³⁹
159 All statistical analyses were performed with a software package (R-Software; www.r-project.org)
160 and a *P* value ≤ 0.05 was considered significant.

161

162

163 **Results**

164 Eighty-two cats fulfilled the inclusion criteria of the study, including 41 neutered females, 38
165 castrated males, two intact females, and one intact male. Cat breeds included 71 domestic
166 shorthairs, three Persians, three Carthusians, two Siamese, two Norwegians, and one Maine Coon.
167 Median age at presentation was 11 years (range 6–18 years), and median weight was 4.5 kg (range
168 2.5–8.5 kg).

169 Tumor size at the longest diameter was available in 81 cats, and median tumor diameter was four
170 cm (range 0.7–15 cm). Tumor location was interscapular in 53 (64%), thoracic wall in 17 (21%),

171 abdominal wall in 8 (10%), and tail and limbs in 4 (5%). In 8 cats (10%) the tumor was ulcerated at
172 presentation.

173 Pretreatment hematology analyses were performed a median of 16.5 days before surgery (range 1 –
174 45 days) and revealed leukocytosis in 6 (7%) cats, leukopenia in 22 (27%), neutrophilia in 6 (7%),
175 neutropenia in 12 (15%), and lymphopenia in 48 (59%); 29 patients (35%) had leukocyte values
176 within the normal ranges. Glycemia was available for 78 patients (95%), of which 23 (30%) were
177 hyperglycemic and 46 (59%) normoglycemic.

178 Concomitant diseases were recorded in 25 cats (30%) at the time of surgical consultation and
179 included: chronic kidney disease (n=9), hyperthyroidism (n=4), chronic rhinitis (n=2), urinary tract
180 infection (n=2), cardiac hypertrophy (n=2), benign keratin cyst (n=1), gastroenteropathy (n=2),
181 diabetes (n=1), and allergic dermatitis (n=2).

182 Total body contrast-enhanced CT was performed in all but three patients that had thoracic
183 radiographs and abdominal ultrasound. Seventy cats (85%) were treated with surgery alone, and 12
184 (15%) received adjuvant chemotherapy (Doxorubicin 1 mg/kg IV every 3 weeks for 4 cycles in 6
185 cats; Carboplatin 200 mg/m² IV every 3 weeks for 4 cycles in 6 cats).

186 Histopathological report described 52 fibrosarcomas (64%), 11 pleomorphic sarcomas (13%), and
187 19 malignant fibrous histiocytomas (23%). Pattern of growth was available in 79 cases and was
188 considered expansile in 36 tumors (46%) and infiltrative in 43 (54%). Necrosis was scored 0 in 15
189 tumors (18%), 1 in 8 (10%), 2 in 29 (35%), and 3 in 28 (34%); in 2 cases the percentage of necrosis
190 was not reported. Histological grading was available in 62 reports: 9 tumors were classified as
191 grade I (15%), 26 as grade II (42%), and 27 as grade III (43%). Surgical margins were non-
192 infiltrated in 65 specimens (79%) and infiltrated in 17 (21%).

193 At the end of the study, ten cats were alive without signs of local and/or distant relapse, 14 were lost
194 to follow-up, and 58 were dead, including 24 that died of tumor-related causes and 34 that died of
195 causes other than FISS, including chronic kidney disease (n=16), hypertrophic cardiomyopathy
196 (n=3), intestinal lymphoma (n=4), car accident (n=3), acute pancreatitis (n=2), oral squamous cell
197 carcinoma (n=2), recurrent urethral obstruction (n=2), meningioma (n=1), transitional cell
198 carcinoma of the urinary bladder (n=1). Of the 24 cats that died because of FISS, 17 had a LR, three
199 had LR and pulmonary metastasis, and the remaining four cats had pulmonary metastasis alone.
200 Median time to LR was not reached (Figure 1). The estimates reported in Figure 1 are based on the
201 assumption of independence between time to relapse and time to death, which could not be
202 evaluated. However, it is possible to obtain intervals (bounding) into which the estimates are
203 expected to lie if the assumption were not tenable. For example, at 550 and 1100 days the Kaplan-

204 Maier estimated probability of being free from LR is 78% and 70% respectively, with a
205 corresponding bounding of 57-81% and 43-78%.

206 Median OST was 975 days (Figure 2). Median follow-up time was 1790 days.

207

208 *White blood cell count, neutrophil count, lymphocyte count and neutrophil-to-lymphocyte ratio*

209 WBCC and NC were significantly higher in ulcerated tumors (P 0.007; P 0.011). WBCC, NC and
210 NLR were significantly higher in histologically infiltrative FISS (P 0.028; P 0.004; P 0.024).
211 (Tables 2 and 3). NLR was also significantly higher in fibrosarcomas (P 0.029) and was correlated
212 with tumor size (r 0.3215; P 0.004). None of the tested variables showed a relationship with LC
213 (Tables 2 and 3).

214 In univariate analysis, a statistically significant prognostic impact on LR was found for WBCC (P
215 0.003), NC (P 0.003), NLR (P 0.015) but not for LC. The hazard of LR increased with increasing
216 values of WBCC, NC and NLR (Table 4). With regards to diagnostic accuracy, for WBCC AUC
217 was 0.695 at one year, 0.614 at two years and 0.599 at three years, with best estimated cut-off of
218 $10.270 (\times 10^3/\mu\text{L})$ at one year (sensitivity=0.543; specificity=0.777), $11.240 (\times 10^3/\mu\text{L})$ at two years
219 (sensitivity=0.380; specificity=0.827), and $11.460 (\times 10^3/\mu\text{L})$ at three years (sensitivity=0.287;
220 specificity=0.840). For NC, AUC was 0.731 at one year, 0.653 at two years and 0.664 at three
221 years, with best estimated cut-off of $4.960 (\times 10^3/\mu\text{L})$ at one year (sensitivity=0.831;
222 specificity=0.542) and two years (sensitivity=0.695; specificity=0.523), and $6.940 (\times 10^3/\mu\text{L})$ at
223 three years (sensitivity=0.482; specificity=0.791). For NLR, AUC was 0.630 at one year, 0.568 at
224 two years, and 0.585 at three years. The optimal cut-off value for NC to predict LR at one year was
225 1.823, which yielded a sensitivity of 0.947 and a specificity of 0.296; the optimal estimated cut-off
226 at two and three years was 3.654 (sensitivity=0.525; specificity=0.660) and 3.654
227 (sensitivity=0.523; specificity=0.669).

228 WBCC (P 0.011), NC (P 0.014) and NLR (P 0.028) were prognostic for OST as well, with
229 increasing hazard of death for increasing values of WBCC, NC and NLR (Table 4). LC was not
230 prognostic for OST.

231 Of the examined clinical and pathological variables, concomitant diseases, ulceration, histological
232 pattern of growth and margins status were prognostic in univariate analysis for LR, while age,
233 ulceration, tumor size, and margins status were prognostic for OST (Tab 4).

234 In multivariate analysis, WBCC (P 0.003), NC (P 0.004) and NLR (P 0.016) remained
235 independently prognostic for LR (Table 5). However, when WBCC, NC and NLR were considered
236 together in Cox regression model, only WBCC remained prognostic for LR (P 0.012). Concomitant
237 diseases, ulceration, and margin status remained prognostic as well, while pattern of growth

238 (expansile VS infiltrative) was removed from the model by backward selection procedure because it
239 was no longer statistically significant (Table 5). Bivariate models confirmed the results of the
240 multivariate analysis.

241 For OST, none of the clinical/pathological variables that were significant in univariate analysis
242 (age, ulceration, tumor size and margins) was removed from the multivariate model by the
243 backward procedure. WBCC, NC and NLR were not confirmed to be prognostic for OST in the
244 multivariate model. Age, ulceration, margins and tumor size remained prognostic (Table 6).

245

246

247

248 **Discussion**

249 In the study population, pretreatment NLR, WBCC, and NC had a prognostic impact in univariate
250 analysis on both LR and OST in cats with FISS at first presentation that underwent curative-intent
251 surgery. LC, conversely, was not useful in predicting LR or OST. However, NLR, WBCC and NC
252 were not confirmed to be prognostic for OST in the multivariate model, considering other clinical-
253 pathological variables.

254 Surprisingly, when WBCC, NC, NLR were considered together in the Cox regression model, only
255 WBCC remained prognostic for LR, while NLR and NC lost their significance. This finding is in
256 contrast with previous reports in human and canine oncology.^{22,27,31} Indeed, NLR and other
257 leukocyte ratios reportedly have higher prognostic impact than leukocyte counts due to their greater
258 stability and lower susceptibility to fluctuations of single cell populations caused by
259 pathophysiological changes.^{27,31} Since NLR takes into account both the impact of variations of NC
260 and LC, the fact that NLR was inferior in predicting LR may be due to the lack of significance of
261 LC; inclusion of this variable in the ratio may have reduced the prognostic significance of NLR.
262 The fact that, despite having a higher proportion of lymphopenic rather than neutrophilic cats in the
263 study population, LC was not correlated with LR nor OST, further corroborates this consideration.
264 Similarly, as neutrophils constitute the majority of WBC in cats, the higher impact of WBCC
265 compared with NLR may simply reflect the higher contribution of NC to this value. A recent paper
266 evaluated NC and morphology in 517 cats with various diseases and reported a higher mortality for
267 neutrophilic patients.⁴⁰ Although it is difficult to extrapolate whether oncological cats were included
268 in the study, this finding corroborates the role of neutrophilic immune response in the feline
269 species.⁴⁰ Nonetheless, in light of these considerations, it is surprising that NC lost its significance
270 as well; however, this result should be considered cautiously, and further studies on a wider sample
271 size are warranted to confirm the superiority of WBCC in predicting LR in FISS.

272 Among the examined tumor variables, pattern of tumor growth was the only one that showed a
273 significant correlation with all the hematological variables in univariate analysis; indeed, patients
274 with infiltrative tumors tended to have higher values of pretreatment WBCC, NC, and NLR. This
275 result may suggest a relationship between pattern of tumor growth and the clinical behavior of
276 FISS, with invasive tumors showing a more aggressive behavior that triggers an immune response
277 with higher values of WBCC, NC, and NLR. Although histological pattern of growth has not been
278 previously studied in FISS, this finding is consistent with a previous report on canine STS, where
279 histologically invasive variants were associated with higher recurrence rates.³² However, this
280 consideration should be considered with caution, as in multivariate analysis tumor pattern of growth
281 was not prognostic for LR, and the prognostic impact of this variables should be further
282 investigated in future studies.

283 While NLR did not show any correlation with tumor ulceration, both NC and WBCC were
284 associated with such variable. It might be argued that the better predictive accuracy of the leukocyte
285 count is due to its correlation with tumor ulceration, which is a well-known prognostic factor for
286 other solid tumors⁴¹; however, even though in our study ulceration was prognostic for both LC and
287 OST, its significance in FISS should be confirmed in further studies in order to accept or refuse this
288 hypothesis.

289 Other histological and clinical tumor variables were inconsistently correlated with the above-
290 mentioned parameters, with presence of ulceration influencing WBCC and NC but not NLR, and
291 tumor histotype and size influencing NLR. Tumor necrosis was not correlated with any of the
292 above-mentioned hematological parameters, perhaps because necrosis remains circumscribed
293 within the tumor pseudo capsule, that may hide it to the immunity system, thus precluding an
294 immune response against it.

295 In the present study, we proposed different cut-offs for NLR, WBCC, and NC for prediction of LR
296 at one, two, and three years, as the different follow-up times of the included patients precluded the
297 determination of a single cut-off value. However, since the majority of events happened during the
298 first year of follow-up, the cut-off at one year should be considered the most reliable, as suggested
299 by the higher sensitivity and specificity of this value compared with the cut-offs at two and three
300 years. These values should be considered barely preliminary, and more reliable cut-offs need to be
301 determined in prospective studies on a larger population in order to allow validation on an
302 independent sample.

303 The estimated cut-offs for leukocyte counts in our study population fall in their physiological
304 ranges. However, it should be noticed that in the study design it was decided to consider leukocyte
305 counts as continuous rather than categorical variables, and this decision was mainly due to the

306 explorative nature of the study. In fact, since to the authors' knowledge no previous papers have
307 examined the significance of leukocyte fluctuations in feline oncology, it would have been
308 misleading to establish empiric cut-offs. As a consequence, our results and the estimated cut-offs on
309 the present study population suggest that for each increase in the WBCC and NC determines an
310 increase in the hazard of LR, regardless of absolute alterations in the physiological ranges of
311 leukocyte counts.

312 Cut-off values for NLR, WBCC, and NC were estimated for the prediction of LR but not OST. LR
313 was a measurable outcome, even in the retrospective setting of this report, as cytological or
314 histological biopsies were always performed to confirm it; on the other hand, OST may have been
315 influenced by the decision of the owners to euthanize their cat and was thus considered a less
316 reliable endpoint.

317 Which variables should be considered when planning treatment and predicting prognosis in FISS is
318 an open debate; however, several papers have shown that the recurrence rate is significantly lower
319 for cats with histologically non-infiltrated margins versus infiltrated margins, and completeness of
320 excision has been reported to be a prognostic factor for survival.^{11,13,15} Consistently with previous
321 studies, margin status resulted prognostic for both LR and OST in our report. In the bivariate model
322 that was performed as a more robust analysis to confirm the results of the multivariate model, the
323 prognostic impact of margin status on both LR and OST was confirmed. Furthermore, even though
324 our primary aim was to evaluate the prognostic impact of leukocyte counts and NLR on LR and
325 OST, other potentially prognostic clinical and pathological variables were included in the survival
326 analysis. Ulceration resulted significantly prognostic for both LR and OST. Although ulceration has
327 not been previously reported to be prognostic for other canine or feline soft tissue sarcomas, it is
328 considered as negative prognostic factor for other solid tumors, such as canine mast cell tumors⁴¹. It
329 may thus be hypothesized that this characteristic is correlated with a more aggressive clinical
330 behavior that causes a rapid tumor growth and subsequent disruption of the cutis.

331 Other clinical-pathological variables that had a prognostic impact were concomitant diseases for
332 LR, and age and tumor size for OST; however, given the low numerosity of events in this study
333 population, it would be speculative to draw conclusions from these results, especially considering
334 that such variables resulted prognostic for only one of the considered end-points. Further studies are
335 thus needed to assess the real impact of such variables on LR and OST.

336 To the authors' knowledge, this is the first report to evaluate the prognostic value of leukocyte
337 counts and ratios in tumor-bearing cats. The prognostic impact of different leukocyte populations,
338 however, has previously been assessed in both human and canine oncology. In human medicine,
339 higher values of pretreatment NLR have been correlated with poorer prognosis for several solid

340 malignancies, including STS.²² The prognostic/diagnostic value of different leukocyte populations,
341 such as neutrophils, lymphocytes, eosinophils, and monocytes, and their ratios has been explored in
342 canine solid and lymphoproliferative neoplasms.^{26-31,42} In a recent paper, NLR was significantly
343 higher in dogs with STS than in those with benign soft tissue lesions, although the parameter was
344 not predictive of tumor grade.³⁰ In two further studies focusing on canine mast cell tumor, higher
345 NLR was predictive of higher tumor grade and was correlated with poorer outcome in univariate
346 analysis, although it was not confirmed as an independent prognostic factor for survival in
347 multivariate analysis.^{29,31} Neutrophilia has also been linked with tumor-associated systemic
348 inflammatory response for different canine malignancies, such as oropharyngeal cancer and acute
349 leukemia.^{24,42} In a paper evaluating the prevalence of leukocyte populations in healthy, older, and
350 tumor-bearing dogs, the authors reported that tumor-bearing dogs had a higher number of WBC and
351 a higher percentage of neutrophils.⁴³ Such findings are consistent with the results in the present
352 study.

353 The mechanism responsible for the relationship between leukocyte counts and ratio alterations and
354 prognosis in human and veterinary oncology remains unclear. Indeed, tumor microenvironment and
355 type of host immune response against cancer play a role in tumor development and progression.
356 Neutrophils have both pro- and antitumor activities, but in cancer patients, neutrophilia has been
357 linked with angiogenesis and tumor progression. Moreover, granulocytes and granulocyte-
358 macrophage colony-stimulating factors are produced by some solid tumors and are known to
359 modulate their progression in people⁴⁴⁻⁴⁵. On the other hand, T-lymphocyte subsets are crucial in
360 specific antitumor immunity, and lymphopenia has been correlated with a worse prognosis in
361 human patients with solid tumors.⁴⁶ As a consequence, a high NLR may reflect a less effective
362 immune response against cancer, with a predominant neutrophilic response and relative
363 lymphopenia, which promotes tumor growth and dissemination.^{23,43,47}

364 In the light of such considerations, the absence of a correlation between LC and both LR and OST
365 in the sampled population is surprising; however, it may be hypothesized that the efficacy of the
366 host immune response against cancer is more influenced by the subpopulations of lymphocytes than
367 by the absolute lymphocyte count, as suggested by results of previous studies on dogs.^{23,25,43}

368 This report has several limitations, mainly related to its retrospective nature. The relatively low
369 number of included cats resulted in a low number of events (24/82), which compromised the
370 robustness of multivariate analysis. This consideration holds particularly true for multivariate
371 analysis on LR, where the number of variables to be included in the model was decided following a
372 less restrictive rule than the traditional EPV rule. However, a more robust bivariate model was
373 performed and confirmed the results of multivariate analysis. Furthermore, when the relationship

374 between NLR, WBCC, NC, and LC and factors that may have altered leukocyte populations was
375 explored, age, presence of concomitant diseases, and hyperglycemia were not associated with any
376 of the above-mentioned hematology values, suggesting that such variables are unlikely to have an
377 impact on them.

378 The decision to include cats that received adjuvant chemotherapy was mainly dictated by the fact
379 that, to date, there is no strong evidence that such treatment is effective against LR, and it would
380 thus have been unlikely to influence this endpoint.⁴⁸⁻⁵¹ In the sampled population, (4/6) 30% of cats
381 treated with chemotherapy developed LR, while 18/76 (24%) of the cats that received only surgery
382 reached this endpoint, suggesting a lack of impact of this treatment against LR. It might be argued
383 that adjuvant chemotherapy may prevent distant metastasis, thus influencing OST, which was the
384 secondary endpoint of the study; however, of the 24 cats that died due to tumor-related causes, only
385 two had distant relapse, while the remaining 22 all experienced LR, suggesting that the impact of
386 distant metastases on OST is negligible. Finally, in univariate analysis adjuvant chemotherapy was
387 not significant for neither of the endpoints, suggesting the low impact of such treatment on
388 outcome.

389 Strengths of this report are the homogeneity of patient management and long-term follow-up.
390 Indeed, all cats were routinely staged, with most patients receiving a preoperative total body CT,
391 definitive treatment consisted of curative-intent surgery for all patients, while cats that underwent
392 neoadjuvant treatments were excluded; furthermore, we excluded patients that received
393 radiotherapy as such treatment is reportedly effective against tumor recurrence.^{12,50,52} Finally, the
394 median follow-up of 1790 days allowed for a reliable observation of LR, including long-term
395 recurrences.

396 In conclusion, pretreatment NLR, WBCC, and NC may be of value in identifying cats at higher risk
397 of LR after curative-intent surgery for FISS. NLR, WBCC, and NC are readily available, cost-
398 effective, and objective prognostic tools that can be easily retrieved from routine preoperative
399 hematological work-up without the need for invasive examinations or adjunctive cost for the
400 owners. However, the exploratory nature of this study impeded the identification of reliable
401 thresholds, and further prospective studies are warranted to confirm the prognostic impact of these
402 parameters on surgically excised FISS and to determine more accurate cut-offs.

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564 **Tables**

565

566 **Table 1.** References intervals for WBC, NC, LC in the cat.

Hematological parameter	Reference Interval
White Blood Cells (X 10 ³ /μL)	6.0 - 17
Neutrophil Count (X 10 ³ /μL)	3 – 13.4
Lymphocyte Count (X 10 ³ /μL)	2 – 7.2

567

568 **Table 2.** Comparison of Neutrophil/Lymphocyte ratio (NLR), Absolute Neutrophil Count (NC),
 569 White Blood Cell Count (WBCC), †Lymphocyte Count (LC) distribution in the categories of
 570 ulceration, necrosis, histology, concomitant disease and margins.

571

Variable	median,(Q1,Q3)	Mean(s.d.)	P
NLR vs Ulceration [§] Absent Present	3.069(1.756, 4.730) 3.199(2.555, 9.736)	4.952(6.547) 7.476(8.009)	0.226
NC vs Ulceration [§] Absent present	3.482 (4.765, 6.970) 6.052 (7.250, 10.820)	6.095(4.191) 12.730(14.007)	0.011*
WBC vs Ulceration [§] Absent Present	7.260(5.467, 10.400) 10.680(9.018, 14.900)	8.680(4.822) 16.010(13.880)	0.007*
LC vs Ulceration [§] Absent Present	1.565(1.002, 2.530) 2.120(1.720, 2.425)	1.877(1.140) 2.056(0.713)	0.407
NLR vs Expansile/Infiltrative [§] Infiltrative Expansile	3.405(2.331, 6.359) 2.444(1.594, 3.749)	6.695(8.531) 3.536(3.742)	0.024*

NC vs Expansile/Infiltrative [§] Infiltrative Expansile	4.230(5.880, 8.410) 3.102(4.040, 5.628)	8.311(7.768) 5.092(2.957)	0.004*
WBC vs Expansile/Infiltrative [§] Infiltrative Expansile	8.620(6.66, 11.53) 7.025(5.245, 9.310)	10.98(8.171) 7.800(3.707)	0.028*
LC vs Expansile/Infiltrative [§] Infiltrative Expansile	1.690(1.080, 2.420) 1.775(1.182, 2.688)	1.875(1.194) 1.986(1.015)	0.472
NLR vs Necrosis [¶] 0 1 2 3	3.280(1.760, 4.770) 2.281(1.762, 3.488) 2.696(1.680, 4.374) 3.628(2.186, 7.356)	5.458(6.148) 2.671(1.232) 3.616(2.689) 7.243(9.827)	0.401
NC vs Necrosis [¶] 0 1 2 3	3.895(4.700, 7.450) 3.325 (4.165, 5.690) 3.810 (4.710, 6.200) 3.710 (5.550, 9.808)	6.579(4.170) 4.492(1.709) 5.654(3.316) 8.524(9.077)	0.498
WBC vs Necrosis [¶] 0 1 2 3	8.100(6.295, 10.200) 6.200(5.072, 7.650) 7.240(6.020, 9.900) 8.805(5.872, 13.260)	9.255(4.374) 6.726(2.278) 8.536(4.706) 11.130(9.301)	0.360
LC vs Necrosis [¶] 0 1 2 3	2.030(0.980, 2.840) 1.785(1.495, 2.320) 1.710(1.240, 2.540) 1.465(1.062, 2.455)	1.931(0.993) 1.895(0.827) 2.100(1.372) 1.719(0.9425)	0.799
NLR vs Histotype [§] Fibrosarcoma other	2.619(1.705, 4.110) 3.752(2.347, 7.674)	4.682(7.130) 6.092(5.860)	0.029*
NC vs Histotype [§] Fibrosarcoma other	3.385 (4.765, 6.920) 4.358 (5.535, 9.962)	5.674(3.376) 8.594(8.769)	0.104
WBC vs Histotype [§] Fibrosarcoma other	7.135(5.542, 10.170) 8.845(6.050, 11.730)	8.368(4.130) 11.170(9.123)	0.187
LC vs Histotype [§] Fibrosarcoma Other	0.980(1.855, 2.630) 1.105(1.515, 2.258)	1.985(1.193) 1.737(0.927)	0.528
NLR vs Concomitant			0.600

disease[§] Absent Present	3.279(2.050, 4.991) 2.539(1.823, 3.698)	4.801(4.844) 6.103(9.753)	
NC vs Concomitant disease[§] Absent Present	3.830(4.960, 6.940) 3.520 (5.540, 7.500)	6.861(6.791) 6.472(4.022)	0.698
WBC vs Concomitant disease[§] Absent Present	7.240(5.630, 10.950) 8.140(6.020, 10.560)	9.422(7.095) 9.332(5.058)	0.187
LC vs Concomitant disease[§] Absent Present	1.040(1.600, 2.470) 1.240(1.690, 2.620)	1.856(1.002) 1.982(1.327)	0.840
NLR vs Margins[§] Clean Dirty	2.700(1.710, 4.702) 3.405(2.539, 6.839)	4.676(5.367) 7.194(10.300)	0.1171
NC vs Margins[§] Clean Dirty	3.520 (4.960, 6.500) 4.230(6.900, 10.400)	6.435(6.360) 7.919(4.713)	0.108
WBC vs Margins[§] Clean Dirty	5.580(7.400, 9.500) 6.020(10.560, 11.820)	9.104(6.684) 10.51(5.852)	0.2146
LC vs Margins[§] Clean Dirty	1.080 (1.710, 2.540) 0.980 (1.240, 2.470)	1.898(0.971) 1.881(1.549)	0.3815

572 [§]Wilcoxon Rank sum test; [¶]Kruskall-Wallis rank sum test; * statistically significant at 5% level.

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575 **Table 3.** Association between Neutrophil-to-Lymphocyte Ratio (NLR), Neutrophil Count (NC),
576 White Blood Cells (WBC), Lymphocyte Count (LC) and size, glycemia and age.

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578

Variable	†r	95% CI	‡t (d.f.)	P
NLR vs Size	0.315	0.104 – 0.499	2.955(79)	0.004*
NC vs Size	0.090	0.131 - 0.302	0.805 (79)	0.423
WBC vs Size	0.054	-0.167 - 0.268	0.478 (79)	0.631
LC vs Size	- 0.176	-0.380 - 0.044	-1.592 (79)	0.115
NLR vs Glycemia	0.036	-0.818 - 0.256	0.315(76)	0.734
NC vs Glycemia	0.070	-0.154 - 0.288	0.619(76)	0.537
WBC vs Glycemia	0.074	-0.150 - 0.292	0.653(76)	0.515
LC vs Glycemia	0.042	-0.182 - 0.262	0.369 (76)	0.718
NLR vs Age	0.072	-0.1472 - 0.285	0.646 (80)	0.520
NC vs Age	0.047	0.1716 - 0.261	0.421 (80)	0.674
WBC vs Age	0.091	-0.128 - 0.301	0.818 (80)	0.418
LC vs Age	0.59	-0.159- 0.273	0.534 (80)	0.593

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599 †Pearson’s correlation coefficient; ‡t statistics (d.f.) degree of freedom; * statistically significant at
600 5% level

601

602 **Table 4.** Univariate analysis of Neutrophil-to-Lymphocyte Ratio (NLR), Neutrophil Count (NC),
603 White Blood Cells (WBC), Lymphocyte Count (LC) on Local Recurrence and Overall Survival.
604 Cox model results and area under ROC curve.

Variable	Local recurrence		Overall survival	
	HR (95% C.I.)	P	HR (95% C.I.)	P
Sex M vs F	1.037 (0.449-2.395)	0.93	0.936 (0.558 -.570)	0.802
Age For each 1 year increase	1.046 (0.888-1.232)	0.59	1.15(1.034 -1.278)	0.01*
NLR For each 0.5 increase	1.066 (1.012- 1.122)	0.015*	1.045 (1.005-1.086)	0.028*
LC For each 100 cells increase	1.165 (0.776- 1.75)	0.462	1.103 (0.84-1.448)	0.482
NC For each 1000 cells increase	1.077 (1.025-1.132)	0.003*	1.048 (1.01-1.088)	0.014*
WBCC For each 1000 cells increase	1.078 (1.027 -1.132)	0.002*	1.048 (1.011-1.087)	0.011*
Glycemia For each unitary increase	1.002 (0.994-1.01)	0.631	0.999 (0.993-1.004)	0.68
Concomitant diseases Yes vs no	2.459 (1.02-5.929)	0.045*	1.637 (0.904-2.967)	0.104
Ulceration Yes vs no	5.062 (1.384-18.51)	0.014*	5.29 (2.233-12.54)	< 0.001*
Tumour Size For each cm increase	1.073 (0.954-1.208)	0.241	1.095 (1.025-1.171)	0.007*
Expansive/infiltrative Expansive vs infiltrative	0.267 (0.096-0.746)	0.012*	0.605 (0.351-1.043)	0.071
Necrosis 1 vs 0 2 vs 0 3 vs 0	1.003 (0.167-6.012) 1.051 (0.263-4.209) 1.844 (0.497-6.841)	0.643 0.998 0.944 0.360	0.853 (0.290-2.508) 1.173 (0.514-2.678) 1.522 0.675 3.433	0.541 0.772 0.705 0.311
Margins Dirty vs clean	2.584 (1.00- 6.674)	0.050*	2.531 (1.387-4.617)	0.002*
Histotype Other vs Fibrosarcoma	0.893(0.364-2.191)	0.805	1.174 (0.688-2.004)	0.557
Adjuvant. Therapies Yes vs no	1.629 (0.55-4.827)	0.378	1.393 (0.679-2.856)	0.366

605 *Statistically significant at 5% level.

606 **Table 5.** Multivariate analysis of Neutrophil-to-Lymphocyte Ratio (NLR), Neutrophil Count (NC),
 607 White Blood Cells (WBC), Lymphocyte Count (LC) on Local recurrence.
 608 Results of the Backward selection procedure applied to Cox model to adjust hematological
 609 parameters for the variables that were statistically significant in univariate analysis.

Model for NLR*		
Variable	HR (95% C.I.)	P
NLR For each 0.5 increase	1.064 (1.012 -1.118)	0.016*
Concomitant diseases Yes vs no	2.940 (1.168 -7.404)	0.022*
Ulceration Yes vs no	7.393 (1.835-29.786)	0.005*
Margins Dirty vs clean	2.659 (1.000 -7.072)	0.050*
Model for WBCC**		
Variable	HR (95% C.I.)	P
WBCC For each 1000 cells increase	1.085 (1.029 -1.144)	0.003*
Concomitant disease Yes vs no	3.647 (1.408 -9.444)	0.008*
Ulceration Yes vs no	5.046 (1.159-21.960)	0.031*
Margins Dirty vs clean	3.055 (1.152 -8.104)	0.025*
Model for NC**		
Variable	HR (95% C.I.)	P
Neu For each 1000 cells increase	1.084 (1.026 -1.144)	0.004*
Concomitant diseases Yes vs no	3.587 (1.387 -9.277)	0.008*
Ulceration Yes vs no	5.032 (1.143-22.145)	0.033*
Margins Dirty vs clean	3.019 (1.138 -8.007)	0.026*

610 *Statistically significant at 5% level. **Expansile/Infiltrative was removed by the Backward
 611 selection because not statistically significant.

612

613 **Table 6.** Multivariate analysis of Neutrophil-to-Lymphocyte Ratio (NLR), Neutrophil Count (NC),
 614 White Blood Cells (WBC), Lymphocyte Count (LC) on Overall Survival.

Model for NLR**		
Variable	HR (95% C.I.)	P
NLR For each 0.5 increase	1.028 (0.985-1.073)	0.206
Age For each 1 year increase	1.170 (1.051-1.303)	0.004*
Ulceration Yes vs no	7.352 (2.950-18.325)	<0.0001*
Margins Dirty vs clean	2.337 (1.256 -4.351)	0.007*
Tumour size For each 1 cm increase	1.101 (1.022 -1.186)	0.012
Model for WBCC**		
Variable	HR (95% C.I.)	P
WBCC For each 1000 cells increase	1.033 (0.993-1.075)	0.111
Age For each 1 year increase	1.171 (1.052 -1.303)	0.004*
Ulceration Yes vs no	6.521 (2.559-16.621)	<0.0001*
Margins Dirty vs clean	2.434 (1.305 -4.541)	0.005*
Tumour size For each 1 cm increase	1.115 (1.037-1.198)	0.003*
Model for NC**		
Variable	HR (95% C.I.)	P
Neu For each 1000 cells increase	1.032 (0.990-1.076)	0.141
Age For each 1 year increase	1.173 (1.054 -1.306)	0.003*
Ulceration Yes vs no	6.650 (2.614-16.917)	<0.0001*
Margins Dirty vs clean	2.395 (1.286-4.459)	0.006*
Tumor size For each 1 cm increase	1.114 (1.036-1.197)	0.003*

615 *Statistically significant at 5% level. **NLR, WBCC, NC were removed by Backward selection
 616 procedure because not statistically significant. The variable that made NLR, WBCC and NC not
 617 significant was ulceration.

618 **Figure legends**

619

620 **Figure 1**

621 Kaplan-Meier estimated local recurrence-free survival probability (continuous line) and 95%
622 confidence intervals (dotted lines).

623

624 **Figure 2**

625 Kaplan-Meier estimated survival probability (continuous line) and 95% confidence intervals (dotted
626 lines).