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Splenic stromal sarcomas in dogs: Outcome and clinicopathological prognostic factors in 32 cases

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Abstract

Due to the low frequency and the changes in diagnostic techniques and terminology during the last few years, only little clinical information is available on splenic stromal sarcoma (SSS). This multi-institutional study aimed at gathering clinical cases of SSS in dogs and investigates their clinical behaviour, as well as analyse possible clinicopathological prognostic factors, including the use of adjuvant therapy. Dogs with a histologically confirmed SSS that underwent splenectomy were retrospectively included. To be included in the study, either FFPE tissue blocks or multiple tissue sections had to be available for histopathologic and immunohistochemical revision. Clinical and pathological variables, along with adjuvant therapy data, were collected. Cumulative incidence of metastatic disease was analysed through univariate and bivariate analyses. The impact of adjuvant chemotherapy on metastasis incidence and survival was assessed, considering an estimated propensity score. A total of 32 dogs were included. Among them, 22 developed metastases with an incidence of 37.5%, 59.38%, and 65.94% at 6, 12, and 24 months, respectively. Univariate analysis identified mitotic count, total scoring, and necrosis as prognostic factors. In bivariate analysis, mitotic count remained prognostic. The administration of adjuvant chemotherapy did not have an impact on metastasis incidence or survival time. The study found that dogs with SSSs are at high risk of metastasis, although a small subgroup may experience longer survival after splenectomy. Mitotic count was the only variable having a reliable prognostic impact. Adjuvant chemotherapy did not appear to decrease the incidence of metastasis or prolong survival in these dogs.

KEYWORDS
canine, chemotherapy, sarcoma, spleen, visceral

1 | INTRODUCTION

Primary splenic neoplasms can arise from intrinsic splenic components, including smooth muscle, fibrous, nervous, vascular, histiocytic, and lymphoid tissues.[1] In dogs, the most frequent primary splenic neoplasms include hemangiosarcoma and lymphoma.[1–4]

Canine splenic nonangiomatous-nonlymphomatous sarcomas (SNANLSTSs) account for 23% to 34% of primary splenic neoplasms.[1,5–8]

The most common canine SNANLSTSs include fibrosarcoma, leiomyosarcoma, undifferentiated sarcoma, and liposarcoma.[1,5–8]

The limited availability of clinical information and the wide range of median survival times, spanning from 60 to 599 days, can be attributed to the relatively low frequency of splenic sarcomas in dogs, the lack of comprehensive medical records, and the inclusion of heterogeneous entities in most studies.[6,8–14] Noteworthy, histiocytic sarcomas were originally included along with SNANLSTSs, but are currently considered as a different entity due to their myeloid (non-stromal) origin and their specific behaviour and treatment.[7,13–15]

With regard to their embryologic origin, tumours arising from the splenic stroma are now classified under the term 'Splenic Stromal Sarcomas' (SSS), replacing the previous term of SNANLSTSs.[11,15] This classification includes various histotypes such as fibrosarcoma, myxosarcoma, leiomyosarcoma, liposarcoma, and undifferentiated stromal sarcoma (whether arising from complex nodular hyperplasia (CNH) or not).[15] Additionally, the reclassification of splenic fibrohistiocytic nodules into complex nodular hyperplasia, lymphoid nodular hyperplasia, marginal zone lymphoma, high-grade B-cell lymphoma, histiocytic sarcoma, and stromal sarcoma may contribute to a better differentiation of these entities based on their origin and behaviour. This approach is expected to enhance our understanding of their biology, improve clinical management, and address the challenges associated with comparing different study results.[7,13,14]

Considering the low frequency observed in daily clinical practice, the limited number of cases described in the literature, and the evolving diagnostic techniques and terminology in recent years, only little information is available on prognostic factors. Mitotic count has been identified as the variable most strongly associated with the biological behaviour of SSS. Specifically, tumours with a mitotic count below 9 have longer survival times.[7,12,13] However, in many studies, the mitotic count was not standardised based on a specific tissue area as recommended by current guidelines.[16] Additionally, the inclusion of histiocytic sarcomas or other types of sarcomas in previous studies may have further complicated the interpretation of results and comparisons.[7,12]

It is indeed intriguing that the grading system commonly used for cutaneous and subcutaneous soft tissue sarcomas (STS) in dogs has shown prognostic relevance in splenic liposarcoma.[17–19] In a case series of 13 dogs, lower-graded tumours were associated with longer survival times, with grade I tumours having the longest survival (1009 days), followed by grade II (206 days) and grade III (74 days) tumours.[17] Another recent study has provided evidence that the STS grading system may have prognostic relevance also for a group of visceral sarcomas, which also included SSS.[12]

However, it is important to note that the management of patients with SSS remains challenging due to the limited and conflicting clinical and prognostic information available. Additionally, there is a lack of clear therapeutic guidelines for post-splenectomy management of SSS cases.

This multi-institutional retrospective study aimed at gathering clinical cases of SSS in dogs and investigates their clinical behaviour, as well as analyse possible clinicopathological prognostic factors, including the use of adjuvant therapy.

2 | MATERIALS AND METHODS

2.1 | Criteria for case selection

The members of the Italian Society of Veterinary Oncology (SIONCOV) retrospectively searched their medical records to identify dogs that had undergone splenectomy and were histologically confirmed to have SSS. To be included in the study, either FFPE tissue blocks or multiple tissue sections had to be available for histopathologic revision.

Collected information included signalment, presence and duration of clinical signs, presence or absence of distant metastasis at admission and during follow-up, results of hematologic and biochemical analyses, and whether adjuvant chemotherapy was administered.

As part of the study protocol, the initial staging had to be conducted either before or no later than 3 weeks after splenectomy using a total body CT scan (TBCT) or 3-view thoracic radiographs and abdominal ultrasound.

The choice of adjuvant chemotherapy regimen and the scheduling of follow-up visits varied according to individual cases and the decisions made by the attending clinicians and dog owners. In cases where metastatic disease was suspected either at the time of admission or during the follow-up period, additional diagnostic procedures such as cytology and/or histopathology were performed to confirm the presence of metastasis. In cases where multiple sites were present, the site that was easiest to sample was selected for analysis. If this site tested positive for metastasis, it was presumed that the other sites were also metastatic.

2.2 | Histopathology and immunohistochemistry

The histopathology service of the Department of Veterinary Medicine and Animal Sciences of the University of Milan reviewed all slides. Paraffin-embedded tissue blocks were cut into multiple 5 microns sections.

Slides were routinely stained with Haematoxylin and Eosin (H&E) for morphologic reassessment and histologic grading based on tissue differentiation, mitotic count, and percentage of necrosis (assessed by evaluating all available sections).[19,20] The mitotic count was estimated on 10 contiguous high-power fields corresponding to a standard area of 2.37 mm^2 (field number of the ocular of 22 mm and a 40× objective).[16]

Additionally, 5-micron sections were put onto glued slides for immunohistochemistry (IHC). Immunohistochemistry was performed following established protocols as previously reported and utilising a panel of primary antibodies against vimentin, Factor VIII-RA, α-SMA, desmin, GFAP, and CD18 (detailed information in Supplemental Table 1 and Supplemental Figures 1–6).[21,22] Immunohistochemistry

markers to exclude lymphoma were not included in the panel because lymphomas were excluded from the study during the selection of cases. Among the included cases, none had a confirmed or suspected diagnosis of primary splenic lymphoma, as there were no tumours consisting of nodular to coalescing aggregates or diffuse sheets of round monomorphic cells.

To reach a definitive diagnosis, H&E and IHC stained slides were examined independently by a board-certified pathologist (PR) and a third-year resident in training (FG) and then reviewed conjunctively to reach an agreement.

Immunohistochemistry for vimentin and Factor VIII-RA were performed to confirm the diagnosis of sarcoma (vimentin-positive) and exclude hemangiosarcoma (Factor VIII-RA-negative), respectively. Histologic diagnoses were obtained as previously described by combining the criteria of.[11,13,15,23] Tumours were diagnosed as SSS if they were composed of a predominant (>50%) population of mesenchymal neoplastic cells, with residual lymphocytes present as small loosely aggregated fading follicular structures or diffuse infiltrates. SSS were further classified based on the presence of specific histologic growth patterns and immunohistochemical reactivity as: (1) leiomyosarcoma, if characterised by bundles of mesenchymal cells intersecting at 45 to 90 ° angles, with no stroma, in association with alpha-SMA positivity and GFAP negativity (regardless of desmin reactivity) (Supplemental Figures 4A,B); (2) nerve sheath tumour (NST), if characterised by the presence of palisading, whorls, Antoni A and Antoni B patterns and Verocay bodies and GFAP positivity and simultaneous muscular markers negativity (Supplemental Figures 5A,B); (3) fibrosarcoma or myxosarcoma, if characterised by streams and fascicles of spindle cells separated by variable amounts of collagenous or myxoid stroma in association with negativity to muscular markers and GFAP (Supplemental Figure 6); (4) liposarcoma, if composed of spindle to polygonal cells organised in bundles or dense areas and containing clear well distinct intracytoplasmic vacuoles. Splenic stromal sarcomas lacking specific and diagnostic histologic growth patterns (displaying generic fasciculated patterns) and characterised by vimentin only or vimentin and variable alpha-SMA and-or desmin positivity (Factor VIII-RA, GFAP, and CD18 negative) were diagnosed as undifferentiated SSS (uSSS) (Supplemental Figure 1).[13,15,24] For vimentin only positive cases, CD18 IHC was performed to exclude histiocytic sarcoma and to further support a diagnosis of SSS (Supplemental Figure 2). As suggested by Moore et al.[11] and according to previously reported criteria tumours retaining nodular architecture with various combinations of macrophages, small mature lymphocytes and plasma cells and the spindle cell component having clear morphological features of neoplasia were classified as stromal sarcomas arising from CNH.[11,13]

2.3 | Statistical analysis

To summarise clinical and pathologic characteristics, the median and range of the distribution were reported for continuous variables. In contrast, the number of subjects and corresponding percentages were reported for categorical variables. Time to death was calculated from the date of surgery to the date of death. The cumulative incidence of death was estimated by the Kaplan-Meier method. Median follow-up was estimated by the reverse Kaplan-Meier method[25] where the probability to remain in follow-up was calculated considering only time to death as censored. The primary study end-point was the incidence of metastatic disease. Time to metastases (TTM) was calculated from the date of surgery to the first evidence of metastases.

Death unrelated to the tumour prevented the observation of the primary end-point (TTM), therefore a method for competing risk was applied to estimate the cumulative incidence of metastases and Fine and Grey regression model was used to estimate the effect of covariates on cumulative incidence of metastases.[26]

Continuous numerical variables in their original measurement scale were included in the Fine and Grey regression model, considering a possible non-linear effect by regression cubic splines. Categorical variables were included in the regression model by dummy coding.

According to the suggestion on the number of primary events per variable to be used in Fine and Grey model, only models with two variables can be considered in multivariate analysis for TTM.[27]

The impact of chemotherapy on the incidence of metastasis and death was also analysed by adjusting the treatment effect for propensity score.[28] For the incidence of metastases, the Fine and Grey regression model was used by including chemotherapy and propensity score as independent variables. For the incidence of death, the Cox regression model was used by including the two above-mentioned variables. The propensity score is the estimated probability of being treated with chemotherapy, given each dog's baseline clinical/pathologic characteristics. The propensity score was estimated by a logistic model with response variables chemotherapy (yes or no) and the variables as predictors.

The statistical analysis was performed with *R*-Software (www.r-project.org). Significance was set at $p < .05$.

2.4 | Cell line validation statement

No cell lines were used.

3 | RESULTS

Between 2009 and 2022, 32 dogs from 8 institutions fulfilled the inclusion criteria. There were 14 (43.9%) mixed-breed dogs, 3 (9.5%) Labrador Retrievers, 2 (6.3%) Cocker Spaniel, and one (3.1%) each of the following: Bedlington Terrier, Spitz, Border Collie, Giant Schnauzer, Beagle, Rottweiler, Bolognese, Dobermann, German Shepherd, English Setter, Pekingese, Staffordshire Bull Terrier, and Jack Russel Terrier. Seventeen (53.1%) dogs were males, and 15 (46.9%) were females.

The median age was 11 years (range, 7–15 years), and the median weight was 21 kg (range, 7–40.6 kg).

The splenic mass was an incidental finding in 6 (18.8%) dogs. The other 26 dogs presented with one or more clinical signs for a median time of 14 days (range 6–75 days) before surgery (Supplemental Table 2). The most frequent clinical signs were lethargy and weight loss, both reported in more than 20% of symptomatic dogs. Twenty-one (65.6%) dogs showed one or more biochemistry abnormalities (Supplemental Table 2). Specifically, increasing of alkaline phosphatase, alanine transferase, and C-reactive protein were diagnosed in more than 20% of dogs with biochemistry alterations. Twenty-one (65.6%) dogs presented one or more CBC abnormalities and in particular anaemia was reported in 81% and neutrophilia in 57% of them (Supplemental Table 2).

Ten (31.3%) dogs were staged by TBCT, while 22 (68.7%) underwent abdominal ultrasound and thoracic radiographs. Only one showed hepatic involvement, as documented by an intraoperative biopsy. During surgery, a small to moderate amount of abdominal effusion was reported in 6 (18.7%) dogs.

At the ultrasonography examination, the splenic mass was complex in 20 (62.5%) cases, hypoechoic in 7 (21.9%), and hyperechoic in 1 (3.1%) case. The ultrasonographic feature was not available for 4 (12.5%) cases.

Regarding histopathology and IHC, all cases were vimentin-positive and factor VIII-RA-negative. Undifferentiated SSS was diagnosed in 10 (31.3%) cases, while SSS from CNH was diagnosed in 14 (43.8%) dogs. Six (18.7%) cases were diagnosed as leiomyosarcoma, while a NST and a fibrosarcoma were each diagnosed in 1 (3.1%) dog. Undifferentiated SSS and SSS arising from CNH showed variable α-SMA and desmin positivity (from 0% to 60% of positive neoplastic cells). The positivity of neoplastic cells to α-SMA and/or desmin excluded the diagnosis of histiocytic sarcoma in 29 out of 32 cases. Three cases (morphologically diagnosed as SSS from CNH, uSSS, and fibrosarcoma, respectively) were characterised by α-SMA, desmin, and GFAP negativity (vimentin-only cases). In these cases, IHC for CD18 was performed to exclude histiocytic sarcoma. All neoplastic cells in all 3 neoplasms were CD18-negative, thus excluding a diagnosis of histiocytic sarcoma. Tumours were characterised by scattered to disseminated infiltration of positive inflammatory and reactive cells (macrophages, lymphocytes, and neutrophils). No liposarcomas were diagnosed in this study.

The median mitotic count was 11 (range, 0–106). Using the mitotic score proposed by Dennis and colleagues (2011), 14 (43.8%) SSS had score 1 (0–9 mitosis), 9 (28.1%) had score 2 (10–19 mitosis), and 9 (28.1%) had score 3 (>19 mitosis). Tumour necrosis was absent in 13 (40.6%) cases (score 0), ≤50% in 16 (50%) cases (score 1), and >50% in 3 (9.4%) cases (score 2). Regarding the differentiation score, 26 (81.3%) SSS lacked specific differentiation (score 3), 3 (9.4%) SSS had a specific histotype (score 2), and 3 (9.4%) were similar to normal tissue (score 1). The median total scoring was 5. Histologically, 2 (6.3%) SSS were grade 1, 18 (56.3%) were grade 2, and 12 (37.5%) were grade 3.

Fourteen (43.8%) dogs did not receive any adjuvant treatment after surgery, including corticosteroids and NSAIDS. Eighteen (56.2%) dogs received chemotherapy (7 SSS from CNH, 5 leiomyosarcomas, 4 uSSS, 1 Nerve Sheath Tumour, and 1 fibrosarcoma): 11 were treated with metronomic therapy consisting of cyclophosphamide at the dosage of 10–15 mg/m^2 orally once daily and piroxicam at the dosage of 0,3 mg/kg or firocoxib at the dosage of 5 mg/kg orally once daily, 7 dogs received intravenous doxorubicin as a single agent at the dosage of 30 mg/m^2 every 3 weeks for 5 times. Among the latter, 3 dogs switched to metronomic therapy.

Median follow-up was 14.7 months, follow-up was less than 18 months for 10% of dogs (range, 30–1476 days).

By excluding the dog with metastasis at admission, 22 dogs developed metastases during follow-up (Figure 1). The most common site of metastasis was the liver ($n = 19$), followed by lungs ($n = 3$), peritoneum ($n = 3$), and bones ($n = 1$).

Five dogs, all with a diagnosis of SSS from CNH, were alive without metastasis at the end of the study, with a median follow-up of 840 days, whereas 27 died (Figure 2). Four dogs died from unrelated causes, and the other 23 died due to metastatic disease.

Summary of the significant specific data for each included dogs were reported in the Supplemental Table 3.

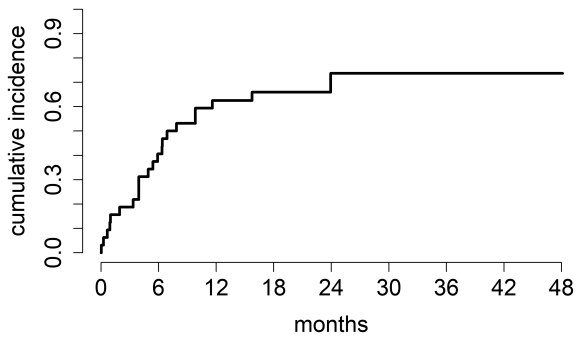

FIGURE 1 Cumulative incidence of metastasis in the sample population estimated by method for competing risks. The incidence of metastases at 6, 12, and 24 months was 37.5% (95% CI 20.97%–54.01%), 59.38% (95% CI 39.95%–74.36%), and 65.94% (95% CI 46.03%–79.97%), respectively.

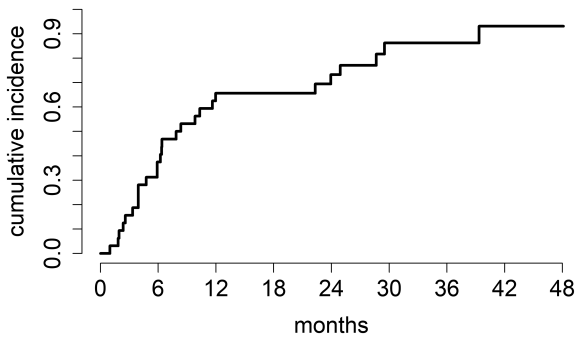

FIGURE 2 Cumulative incidence of death in the sample population estimated by Kaplan–Meier method. The death incidence at 6, 12, 24, and 48 months was 31.25% (95% CI 16.13%–47.64%), 62.50% (95% CI 42.92%-77.01%), 69.44% (95% CI 49.22%–82.90%) and 93.12% (95% CI 48.55%–99.30%), respectively.

TABLE 1 Univariate analysis for the occurrence of metastatic disease. Estimates by Fine and Grey regression model for competing risks. Results of Fine and Grey model are reported in terms of sub-distribution hazard ratio (SDHR) with 95% confidence interval and Wald test. SDHR are not simple to be interpreted in terms of a measure of clinical impact, nevertheless they are directly related to the cumulative incidence of metastases. For categorical variables a value of SDHR significantly greater than 1.00 implies a significant greater incidence of metastases for the category of the variable in comparison to the reference one. For variables on numerical scale a value of SDHR significantly greater than 1 for each unit increase of the variable implies a significant progressive increase of the incidence of metastases with the increase of the variable values.

Variable	SDHR	95% C.I.	*p*-value
Age			
1 year increase	1.092	0.891–1.34	.400
Sex			
F versus M	1.204	0.542–2.671	.650
Weight			
1 kg increase	1.001	0.966–1.038	.950
Symptoms			
Yes versus No	2.171	0.648–7.279	.210
CBC Abnormalities			
Yes versus No	2.53	0.982–6.52	.055
Anaemia			
Yes versus No	1.329	0.574–3.074	.510
Neutrophilia			
Yes versus No	1.649	0.674–4.034	.270
Biochemistry abnormalities			
Yes versus No	1.205	0.56–2.587	.630
US Appearance			
Complex mass + hyperecoic versus hypoechoic mass	2.088	0.804–5.424	.130
Abdominal effusion			
Yes versus No	1.2	0.364–3.957	.760
Mitotic count			
1 mitosis increase	1.022	1.006–1.038	**.005***
Mitotic count			
>9 versus < =9	2.319	1.018–5.28	**.045***
Mitosis scoring			
2 versus 1	1.512	0.542–4.214	.43
3 versus 1	2.637	1.508–8.772	**.004***
Necrosis scoring			
1 versus 0	0.275	0.114–0.663	**.004***
2 versus 0	0.416	0.157–1.102	.078
Total score			
1 score increase	1.563	1.156–2.04	**.003***
Histological grading			
3 versus 1 + 2	2.047	0.99–4.234	.053
Histotype[a]			
SSS from CNH versus uSSS	0.71	0.301–1.678	.44
Leiomyosarcoma versus uSSS	0.641	0.213–1.933	.43
Chemotherapy			
Yes versus no	1.078	0.468–2.482	.86

Abbreviations: *, significant *p*-value; 95% C.I., 95% confidence interval; CNH, complex nodular hyperplasia; *p*-value, *p*-value of the Wald statistic; SDHR, sub-distribution hazard ratio; SSS, splenic stromal sarcoma; uSSS, undifferentiated splenic stromal sarcoma.

[a]The categorisation 'others' was not included in the statistical model due to the presence of only two cases. The variable 'histologic differentiation scoring' has not been included due to the high frequency in score 3 than score 1 and 2 (respectively, 26 vs. 3 and 3 cases).

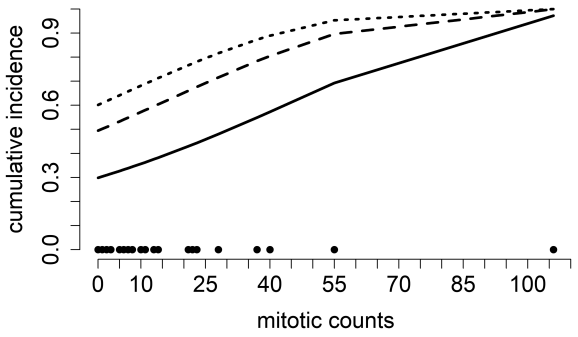

FIGURE 3 Fine and Grey model estimated cumulative incidence of metastasis at 6, 12, 24 months based on the mitotic count. Dotted line = 6 months; Dashed line: 12 months; Continuous line: 24 months. The points above the x axes are the observed values of mitotic counts in the sample. The non-linear effect, modelled by regression splines, was not significant ($p = .12$); thus, only the results of the model with linear effect were reported. At 6, 12, and 24 months, for 1 mitotic count, the estimated cumulative incidence was 30%, 50%, and 61%, respectively, and increased to 57%, 80%, and 89%, respectively, for 40 mitotic counts.

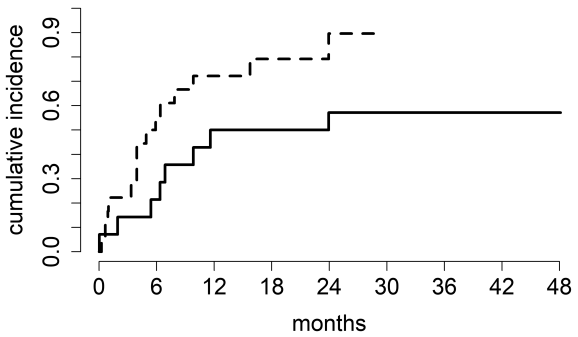

FIGURE 4 Cumulative incidence of metastasis for mitosis considering the cut-off of 9 estimated by method for competing risks. Dashed line: >9 mitotic count; Continuous line: <=9 Mitotic counts.

3.1 | Prognostic variables

In univariate analysis (Table 1), the cumulative incidence of metastases significantly increased with increasing mitotic count (Figure 3). Mitotic

TABLE 2 Bivariate analysis for the occurrence of metastatic disease. Estimates by Fine and Grey regression model for competing risks. Results of Fine and Grey model are reported in terms of sub-distribution hazard ratio (SDHR) with 95% confidence interval and Wald test. SDHR are not simple to be interpreted in terms of a measure of clinical impact, nevertheless they are directly related to the cumulative incidence of metastases. For categorical variables a value of SDHR significantly greater than 1.00 implies a significant greater incidence of metastases for the category of the variable in comparison to the reference one. For variables on numerical scale a value of SDHR significantly greater than 1 for each unit increase of the variable implies a significant progressive increase of the incidence of metastases with the increase of the variable values.

Variables	SDHR	95%C.I.	*p*-value
Mitotic count and necrosis			
1 mitosis increase	1.023	1.01–1.036	**<.01***
Necrosis score 1 versus 0	1.851	0.767–4.446	.17
Necrosis score 2 versus 0	2.447	0.329–18.196	.38
Mitotic count and total score			
1 mitosis increase	1.013	0.989–1.037	.3
1 score increase	1.388	0.972–1.981	.071
Mitotic count and grading			
1 mitosis increase	1.015	0.991–1.039	.23
Histological grade 3 versus 1–2	1.617	0.585–4.471	.35
Necrosis and total score			
Necrosis score 1 versus 0	1.205	0.461–3.153	.7
Necrosis score 2 versus 0	0.831	0.106–6.543	.86
1 total score increase	1.538	1.158–2.043	**.003***
Necrosis and grading			
Necrosis score 1 versus 0	1.449	0.573–3.664	.43
Necrosis score 2 versus 0	1.217	0.158–9.356	.85
Histological grade 3 versus 1–2	2.182	1.016–4.686	**.045***
Total score and grading			
1 total score increase	1.639	0.893–3.01	.11
Histological grade 3 versus 1–2	0.836	0.177–3.941	.82

Abbreviations: *, significant *p*-value; 95% C.I., 95% confidence interval; HR, sub-distribution hazard ratio; *p*-value, *p*-value of the Wald statistic.

count was a significant prognostic factor for metastases also by applying a cut-off of 9 ($p = .045$; Figure 4). When the mitotic count was classified into three categories by scoring, the cumulative incidence of metastases increased with the increasing score, but only the incidence for score 3 was significantly higher than the incidence for score 1 ($p = .004$). The cumulative incidence of metastatic disease also increased significantly with the increase of the total score ($p = .003$).

Conversely, the increasing of the necrosis score seemed to decrease the metastatic probability, but only the cumulative incidence

TABLE 3a Propensity score analysis: association of each variable to the chemotherapy proposal by clinicians. Results of univariate logistic regression model.

Variable	OR	95% C.I.	p-value
Age			
1 year increase	0.605	0.391–0.937	**0.024***
Sex			
F versus M	1.333	0.327–5.434	.688
Weight			
1 kg increase	1.037	0.97–1.109	.288
CBC Abnormalities			
Yes versus No	0.167	0.029–0.968	**.046***
Anaemia			
Yes versus No	0.255	0.057–1.138	**.073***
Neutrophilia			
Yes versus No	0.214	0.046–0.994	**.049***
Biochemistry abnormalities			
Yes versus No	1.5	0.354–6.347	.582
Abdominal effusion			
Yes versus No	0.106	0.011–1.05	**.055***
Mitotic count			
1 mitosis increase	1.016	0.975–1.058	.457
Mitosis scoring			
2 versus 1	0.278	0.048–1.623	.155
3 versus 1	1.111	0.19–6.492	.907
Necrosis scoring			
1 versus 0	0.804	0.181–3.57	.774
2 versus 0	0.313	0.022–4.413	.389
Total score			
1 score increase	0.745	0.414–1.340	.325
Histological grading			
3 versus 1 + 2	2.5	0.548–11.41	.237

Abbreviations: *, significant *p*-value; 95% C.I., 95% confidence interval; OR, odds ratio; *p*-value, *p*-value of the Wald statistic.

TABLE 3b Propensity score analysis – reduce model. Results of the backward selection procedure.

Variable	OR	95% C.I.	*p*-value
Age			
1 year increase	0.562	0.338–0.935	**.027***
Neutrophilia			
Yes versus No	0.132	0.018–0.971	**.047***
Abdominal effusion			
Yes versus No	0.122	0.01–1.552	**.105***

Abbreviations: *, significant *p*-value; 95% C.I., 95% confidence interval; OR, odds ratio; *p*-value, *p*-value of the Wald statistic.

TABLE 4a Prognostic impact of chemotherapy on metastasis adjusted for the propensity score. Results of the Fine and Grey regression model for competing risks. When the variable 'chemotherapy' had been adjusted for the propensity score the SDHR increase (2.11 vs. 1.078) and although not yet statistically significant, the *p*-value tended to decrease (.19 vs. .86) therefore the impact of chemotherapy on the incidence of metastases tended to be reduced.

Variable (on metastasis)	SDHR	95% C.I.	*p*-value
Chemotherapy			
Yes versus no	2.11	0.699–6.375	.19
Propensity score			
1 score increase	0.78	0.622–0.997	.031

TABLE 4b Prognostic impact of chemotherapy on survival adjusted for the propensity score. Results of Cox regression model. When the variable 'chemotherapy' had been adjusted for the propensity score the HR increase (1.909 vs. 0.96) and although not yet statistically significant, the *p*-value tend to decrease (.22 vs. .91) therefore the impact of chemotherapy on the incidence of death tended to be reduced.

Variable (on survival)	HR	95% C.I.	*p*-value
Chemotherapy			
Yes versus no	1.909	0.676–5.396	.22
Propensity score			
1 score increase	0.759	0.597–0.965	.025

of metastases for score 1 (≤50% of necrosis) was significantly lower than the cumulative incidence of metastases for score 0 (absent of necrosis) ($p = .004$).

Given the main clinical/pathologic interest, the bivariate models included the following variables: mitotic count, necrosis, total score, and grading. In bivariate analysis, mitotic count, histologic grading, and total score resulted a significant prognostic factor for the incidence of metastases when adjusted for necrosis (Table 2).

The administration of chemotherapy did not impact survival ($p = .91$, HR 0.96, 95% CI 0.451–2.044). Chemotherapy was offered more often to young dogs (the propensity decreased with higher age) and those without CBC abnormalities, anaemia, neutrophilia, or abdominal effusion (Table 3a). When all these variables were jointly included in the model, and a reduced model was found by backward procedure, only age, neutrophilia, and abdominal effusion were maintained (Table 3b). Considering the same propensity score, dogs undergoing adjuvant chemotherapy had greater incidence of death compared with those undergoing surgery alone. Nevertheless, metastases incidence tended to decrease with the increased propensity score (Tables 4a and 4b).

4 | DISCUSSION

The findings of this study align with the existing literature, which indicates that SSS are relatively rare in dogs.[12–14] Over a period of 13 years, this study collected only 32 cases from multiple institutions, highlighting the uncommon nature of this tumour in the canine population.

The study's results confirm the aggressive biological behaviour of SSS, with the majority of dogs (19 out of 32; 59.4%) succumbing to metastatic disease within 12 months of diagnosis. However, there was a small subset of dogs that survived or died of tumour-unrelated causes for over 450 days from diagnosis. This variability in survival times may contribute to the heterogeneous survival data reported in previous studies, which often lacking comprehensive clinical staging and therapeutic information, as well as different histotype inclusion.[8–14] In the earlier reports, predating the reclassification of splenic fibrohistiocytic nodules, and documented survival times ranging from 2 to 9 months.[6,8,9] A study by Moore and colleagues[11] reported a median survival time of 488 days in a small cohort of 8 dogs with confirmed SSS.[11] Another recent study involving 33 dogs with uSSS and SSS from CNH reported a median post-splenectomy survival time of 439 days.[13] A larger study on 59 cases reported a median survival time of 166 days, although no specific staging or therapy data were analysed.[14] Conversely, a separate study on 42 visceral sarcomas, including 20 SSSs (of which 8 uSSSs, 5 STS, 3 leiomyosarcomas, 2 fibrosarcomas, 1 myxosarcoma, and 1 PNST), reported a median survival time of 599 days.[12] This discrepancy in survival outcomes further emphasises the challenge in assessing the biological behaviour of SSS and the complex nature of its histotype diagnosis and clinical management.

The study findings reinforce the importance of including mitotic count as an independent prognostic factor in the pathology report of SSS. Mitotic count has been consistently identified as a reliable indicator for the development of metastasis and should be routinely assessed to provide valuable prognostic information for guiding treatment decisions. The cut-off of 9 for the mitotic index was first proposed by Spangler et al.[7] in a case series of 76 SNANLSTS and subsequently confirmed by Linden and colleagues[12] in dogs with visceral sarcomas.[7,12] The significance of the mitotic count in predicting survival outcomes and metastatic development in SSS is further supported by the study conducted by Wittenberns and colleagues,[13] who also utilised a mitotic count cut-off of 9. Their findings demonstrated a significant association between the mitotic count and survival in SSS. In line with these findings, the present study also identified the cut-off of 9 mitoses per 10 high power fields (equivalent to 2.37 mm^2) as a significant predictor of metastatic development. The use of a prognostic cut-off implies that all cases below that value will exhibit a uniformly better prognosis, while all cases above it will have a uniformly worse prognosis (or vice versa). However, in the present study, an increased incidence of metastasis was observed with each incremental increase in mitotic count, indicating that the use of a cut-off value of 9 may not accurately distinguish between two prognostic groups. Therefore, the effectiveness of the cut-off value for stratifying prognosis may be limited in this context. The incidence of metastasis constantly increased at 6, 12, and 24 months for each increase of 1 mitotic count up to the count of 40. The acknowledgement of the limited number of cases with high mitotic counts and the potential

complexity of the prognostic relationship for values higher than 40 is an important consideration. The presence of only two cases with mitotic counts above 40 indicates a rarity of such occurrences in the study population. It is indeed ideal to have a uniform distribution of values across the entire range of mitotic counts to accurately assess the relationship between mitotic count and metastasis risk. However, due to the limited number of cases and the rarity of high mitotic counts, obtaining a balanced distribution becomes challenging. The removal of the exceptionally high mitotic count of 106 from the overall statistical analysis to test its influence and finding that the slope of the relationship was negligibly affected suggests that this outlier did not significantly impact the overall findings (data not shown). However, cautious interpretation is still warranted, considering the limited number of cases with high mitotic counts.

The histologic grading used here was taken from that used for STS.[18–20] In a recent paper including visceral splenic and non-splenic STS, dogs with grade III were more likely to develop metastatic disease, and the histologic grading system was associated with survival.[12] The finding that histologic grade showed an impact on the risk of metastasis in the univariate analysis, but not in the bivariate analysis when adjusted for necrosis, suggests that the relationship between histologic grade and metastasis in SSS is influenced by other factors such as necrosis and mitotic count. Based on the assumption that histologic grading is based on the concurrent evaluation of necrosis, mitotic count, and differentiation, mitotic count had a major impact on metastatic risk. This consideration also held for the variable 'total score', which analysed the sum of necrosis, mitotic count, and differentiation score in a continuous scale.

Tumour necrosis leads to intra-tumour hypoxia, which is related to an increased metastatic potential.[29] Thus, it is reasonable to assume that an increased risk of metastases would accompany increasing intratumoral necrosis. Conversely, the results of the current study contradicted this finding. Increased necrosis score was associated with a decreased risk of metastases. There are some explanations for this finding. FFPE blocks were obtained from different institutions, potentially using different trimming techniques, which may have influenced the percentage of necrosis. It is plausible that certain necrotic areas were not sampled, while others were extensively included in the blocks, thereby resulting in under- or overestimation of the necrosis percentage. Alternatively, tumours with higher necrosis scores may be more susceptible to hypoxia, and neoplastic cells may be unable to develop more aggressive behaviour. Further studies standardising gross description and trimming methods are necessary to gain a better understanding of the influence of necrosis on metastatic risk.[30]

It is currently unknown whether chemotherapy improves the outcome. Twenty-three (71.9%) dogs developed metastases, suggesting that considering adjuvant chemotherapy may be prudent. Similarly, it is unknown which chemotherapeutic agent works best: doxorubicin was more commonly used, possibly because it has shown efficacy against splenic hemangiosarcoma.[31–34] Considering the absence of a significant influence of adjuvant chemotherapy on the occurrence of metastases, a separate analysis was conducted to evaluate its effect on survival time. However, it did not seem to reduce the metastatic risk or increase survival time in this case series. Given the retrospective nature of this study and the subjective nature of clinicians' and owners' decision-making regarding adjuvant treatment, a propensity score analysis was subsequently employed. The adjustment for the propensity score aimed at minimising the bias in comparing treatments caused by variations in the distribution of variables between patients who received treatment and those who did not.[27,35] Nevertheless, the adjustment for the propensity score did not alter the prognostic impact of adjuvant chemotherapy.

The diagnosis of SSS is often made in the case of mesenchymal tumours deriving from the stroma. However, the term SSS should be limited to tumours that do not have a specific morphology and phenotype (thus, better reported as uSSS). In this caseload, tumours had been assigned to a specific histotype, limiting the diagnosis of uSSS to those cases expressing only vimentin and devoid of specific morphologic features. No difference in metastatic rate was observed among the 3 more frequent histotypes. However, this data should be considered cautiously because of the low number of cases in each category. In addition, it is still unclear whether SSS arising from CNH and uSSS may represent a continuum of the same malignant transformation of the stromal component of complex nodular hyperplasia. The authors auspicate the continuous application of IHC to reach a specific diagnosis (whenever possible) before accepting the umbrella terminology of SSS. In addition, this approach would allow for the exclusions of tumours with a poorer prognosis or specific therapeutic indications, which should not be included into the 'umbrella' terminology of SSS, such as hemangiosarcoma, histiocytic sarcoma, and lymphoma. In cases where a definitive diagnosis cannot be established, a diagnosis of 'complex splenic nodule' should be assigned.[11,13,22]

Limitations of the study include the retrospective and multicentric nature. The optimal approach for prognostic analysis is to include the whole variable set in the model. However, this was impossible in this case series because of the small number of dogs developing metastases. A minimum number of events per variable are suggested to be 10 to obtain reliable model results in competing risks setting;[27] thus, only multivariate models with two variables were performed. Staging, restaging, and chemotherapy protocols were not standardised. Finally, the limited number of events implies a low statistical power, and possible prognostic factors may not have emerged.

In conclusion, canine SSSs are generally associated with a high risk of metastasis, typically occurring within 12 months of surgery. However, a subset of dogs may have longer survival following splenectomy. Notably, mitotic count emerged as a constant prognostic factor maintaining its significance even when evaluated on a continuous scale. Adjuvant chemotherapy demonstrated no efficacy in averting metastatis or extending survival. Further prospective studies are warranted to validate the role of specific histotypes and ascertain effective therapeutics interventions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Spangler WL, Culbertston MR. Prevalence, type, and importance of splenic diseases in dogs: 1480 cases (1985-1989). *J Am Vet Med Assoc*. 1992;200:829-834.
2. Eberle N, Von Babo V, Nolte I, Baumgärtner W, Betz D. Splenic masses in dogs. Part 1: epidemiologic, clinical characteristics as well as histopathologic diagnosis in 249 cases (2000-2011). *Tierarztl Prax Ausg K Kleintiere Heimtiere*. 2012;40:250-260.
3. Wendelburg KM, Price LL, Burgess KE, Lyons JA, Lew FH, Berg J. Survival time of dogs with splenic hemangiosarcoma treated by splenectomy with or without adjuvant chemotherapy: 208 cases (2001-2012). *J Am Vet Med Assoc*. 2015;247:393-403.
4. Leyva FJ, Loughin CA, Dewey CW, Marino DJ, Akerman M, Lesser ML. Histopathologic characteristics of biopsies from dogs undergoing surgery with concurrent gross splenic and hepatic masses: 125 cases (2012-2016). *BMC Res Notes*. 2018;11:122.
5. Frey AJ, Betts CW. A retrospective survey of splenectomy in the dog. *J Am Anim Hosp Assoc*. 1977;13:730-734.
6. Westein MJ, Carpenter L, Mehlhaffschunk CJ. Nonangiogenic and nonlymphomatous sarcomas of the canine spleen:57 cases (1975-1987). *J Am Vet Med Assoc*. 1989;195:784-788.
7. Spangler WL, Culbertston MR, Kass PH. Primary mesenchymal (non-angiomatous /nonlymphomatous) neoplasms occurring in the canine spleen: anatomic classification, immunohistochemistry, and mitotic activity correlated with patient survival. *Vet Pathol*. 1994;31:37-47.
8. Day MJ, Lucke VM, Pearson H. A review of patological diagnoses made from 87 canine splenic biopsies. *J Small Anim Pract*. 1995;36:426-433.
9. Spangler WL, Kass PH. Pathologic factors affecting postsplenectomy survival in dogs. *J Vet Intern Med*. 1997;11:166-171.
10. Spangler WL, Kass PH. Pathologic and prognostic characteristics of splenomegaly in dogs due to fibrohistiocytic nodules: 98 cases. *Vet Pathol*. 1998;35:488-498.
11. Moore AS, Frimbergher AE, Sullivan N, Moore PF. Histologic and himmunoistochemical review of splenic fibrohistocytic nodules in dogs. *J Vet Intern Med*. 2012;26:1164-1168.
12. Linden D, Liptak JM, Vinayak A, Grimes JA, Sandey M, Smiley W. Outcomes and prognostic variables associated with primary abdominal visceral soft tissue sarcomas in dogs: a veterinary Society of Surgical Oncology retrospective study. *Vet Comp Oncol*. 2019;17:265-270.
13. Wittenberns BM, Thamm DH, Palmer EP, Regan DP. Canine non-Angiogenic, non-myogenic splenic stromal sarcoma: a retrospective Clinicopathological analysis and investigation of Podoplanin as a marker of tumour Histogenesis. *J Comp Pathol*. 2021;188:1-12.
14. Spröhnle-Barrera CH, McGhie J, Allavena RE, Owen HC, Palmieri C, Barnes TS. Epidemiology and survival of dogs diagnosed with splenic lymphoid hyperplasia, complex hyperplasia, Stromal Sarcoma and Histiocytic Sarcoma. *Animals*. 2022;12:960.
15. Valli VE, Bienzle D, Meuten DJ, Linder KR. Tumors of the hemolymphatic system. In: Meuten DJ, ed. *Tumors in Domestic Animals*. Vol 2016. John Wiley&Sons, Ames; 2016:203-321.
16. Meuten DJ, Moore FM, George JW. Mitotic count and the field of view area: time to standardize. *Vet Pathol*. 2016;53:7-9.
17. Gower KLA, Liptak J, Culp VTN, Bravo L, Powers B, Withrow SJ. Splenic liposarcomas in dogs:13 cases (2002-2012). *J Am Vet Med Assoc*. 2015;247:1404-1407.
18. Kuntz CA, Dernell WS, Powers BE, Devitt C, Straw RC, Withrow SJ. Prognostic factors for surgical treatment of soft-tissue sarcomas in dogs: 75 cases (1986-1996). *J Am Vet Med Assoc*. 1997;211:1147-1151.
19. Dennis MM, McSporran KD, Bacon NJ, Schulman FY, Foster RA, Powers BE. Prognostic factors for cutaneous and subcutaneous soft tissue sarcomas in dogs. *Vet Pathol*. 2011;48:73-84.
20. Avallone G, Rasotto R, Chambers JK, Miller AD, Behling-Kelly E, Monti P. Review of histological grading Systems in Veterinary Medicine. *Vet Pathol*. 2021;58:809-828.
21. Avallone G, Helmbold P, Caniatti M, Stefanello D, Nayak RC, Roccabianca P. The spectrum of canine cutaneous perivascular wall tumors: morphologic, phenotypic and clinical characterization. *Vet Pathol*. 2007;44:607-620.
22. Sabattini S, Rigillo A, Foiani G, Marconato L, Vascellari M, Greco A. Clinicopathologic features and biologic behavior of canine splenic nodules with stromal, histiocytic and lymphoid components. *Front Vet Sci*. 2022;9:962685.
23. Sabattini S, Lopparelli RM, Rigillo A, Giantin M, Renzi A, Matteo C. Canine splenic nodular lymphoid lesions: immunophenotyping, proliferative activity, and clonality assessment. *Vet Pathol*. 2018;55:645-653.
24. Roccabianca P, Schulman FY, Avallone G, et al. Growth patterns of soft tissue tumors. In: Kiupel M, ed. *Surgical Pathology of Tumors of Domestic Animals Volume3: Tumors of Soft Tissue*. Davis-Yhompson Foundation; 2020:32-49.
25. Shuster JJ. Median follow-up in clinical trials. *J Clin Oncol*. 1991;9:191-192.
26. Boracchi P, Roccabianca P, Avallone G, Marano G. Kaplan-Meier curves, cox model, and P-values are not enough for the prognostic evaluation of tumor markers: statistical suggestions for a more comprehensive approach. *Vet Pathol*. 2021;58:795-808.
27. Austin PC, Allignol A, Fine JP. The number of primary events per variable affects estimation of the subdistribution hazard competing risks model. *J Clin Epidemiol*. 2017;83:75-84.
28. Heinze G, Jüni P. An overview of the objectives of and the approaches to propensity score analyses. *Eur Heart J*. 2011;32(14):1704-1708.
29. Richards CH, Mohammed Z, Qayyum T, Horgan PG, McMillan DC. The prognostic value of histological tumor necrosis in solid organ malignant disease: a systematic review. *Future Oncol*. 2011;7:1223-1235.
30. Moore FM, Williams B, Bertram CA, et al. *Tumor Necrosis Guideline, version 1.2*. Veterinary Cancer Guidelines and Protocols; 2021 http://vetcancerprotocols.org
31. Sorenmo KU, Jeglum KA, Helfand SC. Chemotherapy of canine hemangiosarcoma with doxorubicin and cyclophosphamide. *J Vet Intern Med*. 1993;7:370-376.
32. Wendelburg KM, O'tolle TE, McCobb E, Price LL, Lyons JA, Berg J. Risk factors for perioperative death in dogs undergoing splenectomy

for splenic masses: 539 cases (2001-2012). *J Am Vet Med Assoc*. 2014;245:1382-1390.
33. Finotello R, Henriques J, Sabattini S, et al. A retrospective analysis of chemotherapy swich suggests improved outcome in surgically removed, biologically aggressive canine Haemangiosarcoma. *Vet Comp Oncologia*. 2016;10:493-503.
34. Matsuyama A, Poirier VJ, Mantovani F, Foster RA, Mutsaers AJ. Adjuvant doxorubicin with or without metronomic cyclophosphamide for canine splenic Hemangiosarcoma. *J Am Anim Hosp Assoc*. 2017; 53:304-312.
35. Ebrahim Valojerdi A, Janani L. A brief guide to propensity score analysis. *Med J Islam Repub Iran*. 2018;32:122.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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