Veterinary Medicine and Science

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Dogs

Retrospective Analysis of Clinical Presentation, Prognostic Factors and Outcome in 32 Dogs with Subcutaneous Mast Cell Tumours Treated with a Curative-Intent Approach

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Received: 18 February 2024 | **Revised:** 11 December 2024 | **Accepted:** 17 February 2025

Funding: The authors received no specific funding for this work.

Keywords: curative-intent approach | Darier's sign | dogs | nodal metastases | subcutaneous mast cell tumours

ABSTRACT

Background: Subcutaneous mast cell tumours (ScMCTs) have been traditionally associated with a good prognosis, with low rates of recurrence and metastasis.

Objectives: This study aims to describe the clinical presentation, outcome, and prognostic factors in dogs diagnosed with ScMCTs and treated with a curative-intent approach.

Methods: Clinical and histopathological data were retrospectively collected from dogs diagnosed with ScMCTs after undergoing curative-intent surgery and complete staging between 2018 and 2023 in a single institution. Adjuvant and neoadjuvant therapies were allowed. The study's endpoints were the disease-free interval (DFI) and disease-specific survival time (DSST). Variables, including location, histopathological description, clinical stage, infiltrative behaviour, atypia, Darier's sign, surgical margins, mitotic count (MC) >4 in 10 high power fields (HPF), nodal status, and chemotherapy after surgery, were evaluated as potential influences on DFI and DSST.

Results: Thirty-two cases were included. Lymphadenectomies were performed in 18/32 (56.3%), and nodal metastases (early or overt) were documented in 12/32 (37.5%). The median follow-up was 405 days (range 79–1312). In 9/32 (28.1%), the disease progressed, and 7/32 (21.9%) died of ScMCT-related causes. The median DFI and DSST were not reached at 1312 days. The overall 1-year and 2-year survival rates were 80% and 70%, respectively. Patients presenting with Darier's sign, MC >4 in 10 HPF, and those who received chemotherapy had a higher risk of dying from the disease (hazard ratios of 14.9, 5.8 and 8.4, respectively).

Conclusions: Our results suggest that despite the overall good long-term prognosis of ScMCTs, they may exhibit a higher metastatic rate at presentation than previously reported. Additionally, patients with Darier's sign or a high mitotic count may be associated with a poorer prognosis.

1 | Introduction

Mast cell tumours (MCTs) are dogs' most frequent cutaneous malignancies, accounting for approximately 20% of all canine skin tumours (Meuten 2017). Most occur as solitary nodules in the skin or, less commonly, in the subcutis. MCTs frequently involve the regional lymph nodes (LNs) as specific metastatic sites. Distant metastases are less common and generally associated with a worse prognosis. The most affected sites are the spleen, liver, bone marrow and non-regional lymph nodes (Meuten 2017;

Ribeiro et al. 2022). Other primary locations, such as visceral organs, the gastrointestinal tract, and the oral cavity, can occur but are rare (Meuten 2017).

Subcutaneous MCTs (ScMCTs) originate from the subcutis and are typically situated beneath the dermis and epidermis, occasionally extending into the deeper dermis. In contrast, if the tumour is located in the epidermis or outermost dermis, it should be classified as a cutaneous mast cell tumour (cMCT) (Meuten 2017; Thompson et al. 2011). ScMCTs are generally non-encapsulated and infiltrative but may also be well-circumscribed or exhibit both patterns (Meuten 2017; Thompson et al. 2011). They usually present as soft masses, grossly resembling lipomas. These tumours can be located all over the body, but the legs, back, and thorax are the most common sites, accounting for 60% of all ScMCTs (Thompson et al. 2011; Newman et al. 2007). Multiple ScMCTs are uncommon but are described in 5% of cases in one study (Thompson et al. 2011).

ScMCTs should be considered a specific clinical entity regarding prognosis, recurrence rate, and metastasis. Approximately 90% of ScMCTs exhibit benign behaviour and can be controlled with surgery alone, even with affected margins (Thompson et al. 2011; Newman et al. 2007). Nevertheless, around 10% of affected dogs will die from ScMCT-related disease, 11% will develop *de novo* ScMCTs, 8–9% will experience recurrence, and 4–6% will metastasize (Thompson et al. 2011; Newman et al. 2007). According to the literature, recurrence seems independent of margin status, with only 8%–21% having recurrence with incomplete margins (Thompson et al. 2011; Newman et al. 2007; Gill et al. 2020). In a recent retrospective study involving 43 dogs with ScMCTs treated with surgery alone, the median disease-free interval and survival time were not reached for more than five years, indicating favourable long-term outcomes (Gill et al. 2020).

Although the behaviour of cutaneous MCTs (cMCTs) is predictable with the Patnaik and Kiupel grading systems (Kiupel et al. 2011; Patnaik et al. 1984), it is essential to highlight that these grading systems lack complete applicability and are not employed within the subcutaneous (Sc) subset. Nevertheless, several histopathological features may suggest more aggressive biological behaviour in this set of patients, such as multinucleation, infiltrative growth, mitotic count (MC) greater than 4 in 10 high-power fields (HPF), KIT cellular location pattern, high Ki67, argyrophilic nucleolar organiser region (AgNor) and proliferating cell nuclear antigen (PCNA) indices (Meuten 2017; Thompson et al. 2011). However, in the absence of these features, some ScMCTs behave more aggressively than expected, leading to poor outcomes. Identifying these patients can provide an even more accurate prognosis and guide the therapeutic approach.

Only a few studies have examined ScMCTs as a specific entity during the last two decades (Thompson et al. 2011; Newman et al. 2007; Gill et al. 2020). These studies could have some bias due to their retrospective nature, case selection (mainly from pathology laboratory files), and non-systematic staging and follow-up conducted on the selected population.

Recently, three studies by Marconato et al. (2023), Cherzan et al. (2023), and Treggiari et al. (2023) have provided new insights into the behaviour of ScMCTs. Marconato et al.'s (2023) research suggests that these tumours may be more aggressive than previously reported, with nodal metastasis found in 58% of cases, including both overt (34.9%) and early (23.1%) metastatic lymph nodes (LNs). Disease recurrence occurred in 18.6% of cases, but overall, a multimodal approach yielded a good long-term prognosis, with ScMCT-related deaths in 11.6% of cases.

Similarly, Cherzan et al.'s (2023) study indicates a notable level of aggressiveness in ScMCTs, with nodal metastasis identified in 26.7% of cases and disease recurrence in 28.8% of dogs. However, a multimodal approach also led to a favourable overall long-term prognosis, with ScMCT-related deaths occurring in 13.3% of cases.

According to Treggiari et al.'s (2023) findings, the recurrence rate was 15%, and 63% of the evaluated LNs were metastasic. Overall, the long-term prognosis was also good without negative prognostic factors.

Despite ScMCTs historically being associated with benign behaviour and low metastatic rates, our hypothesis suggests that nodal metastasis on presentation could be substantially higher than previously reported, as outlined in recent publications. This study aimed to determine the nodal or distant metastatic status in dogs with confirmed ScMCTs who had undergone a curative-intent approach following thorough staging, including assessment of sentinel or regional LNs and tumour extension. Another purpose was to identify potential histopathological prognostic variables and investigate potential associations between clinical presentations and their outcomes.

2 | Material and Methods

Clinical and histopathological data from dogs diagnosed with ScMCTs were collected retrospectively from a single institution (Anicura Glories Hospital Veterinari, Barcelona), between September 2018 and July 2023. The study included dogs with a first-occurring histopathologically confirmed ScMCT that underwent curative intent surgery, with or without regional or sentinel lymphadenectomy. According to Meuten et al. (2017), if the tumour arises from Sc tissue but extends dorsally into the deeper dermis, it should be considered an ScMCT, and this criterion was used in the case selection. Dogs also underwent complete staging, which involved the evaluation of sentinel or regional LNs through cytology and/or regional ultrasound and/or computed tomography (CT) scan prior to surgery. The clinical staging was established according to the World Health Organization (WHO) system, which was adapted for the Sc subset and slightly modified for this study (patients with 2 or more unrelated ScMCTs without LN involvement were considered stage I) (Table 1). Curative-Intent surgery was defined as the surgical excision of all gross disease, including regional LNs when indicated by the clinician based on gross tumour characteristics, cytology findings, LN enlargement, or confirmed metastasis on LN cytology. Cytological grading of the primary tumour was not evaluated in most cases because the current grading system is not applicable to ScMCTs (Camus et al. 2016) (Figure 1). Surgical margins were deemed incomplete when the histopathology report indicated the presence of neoplastic cells infiltrating the biopsy margin; otherwise, they were considered complete. The biopsy samples were sent to the same external laboratory but were reviewed by

TABLE 1 | World Health Organization (WHO) mast cell tumour staging system, slightly modified for this study.

Stage	Criteria
I	One or more unrelated tumours confined to subcutis without regional lymph node involvement[a]
II	One or more tumours confined to subcutis with regional LN involvement
III	Large infiltrating tumours with or without regional LN involvement
IV	Any tumour with distant metastasis including blood or bone marrow

[a]Patients with 2 or more unrelated SCMCTs without LN involvement were considered stage I and not stage III.

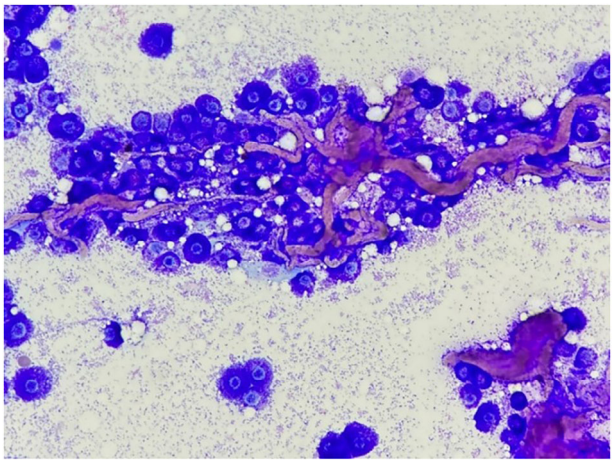

FIGURE 1 | Fine needle aspirate of aScMCT without metastatic tendency. Modified Wright—Giemsa stain; 20x objective. The sample shows a highly granulated mast cells with mild anisocytosis and anisokaryosis. Moderate amounts of pale pink, fibrillar material consistent with degraded collagen are present. Occasional mesenchymal cells are also noted. Findings are compatible with a well-differentiated mast cell tumour, collagenolysis, and fibroplasia.

different board-certified pathologists. Adjuvant and neoadjuvant therapies were allowed, and dogs were eligible for inclusion regardless of the treatment protocols received. These could involve surgical revision, adjuvant or neoadjuvant chemotherapy (tyrosine kinase inhibitors or traditional cytotoxic drugs), electrochemotherapy (ECT), radiation therapy (RT), or various combinations of these treatments. Patients with cutaneous and subcutaneous involvement in histopathology, concurrent neoplasia, severe comorbidity, and distant metastasis on presentation (surgery was not recommended for stage IV patients), as well as those with a lack of staging and follow-up data, were excluded. However, previously operated low-grade cutaneous mast cell tumours and concurrent multiple ScMCTs were allowed for inclusion in the study.

One of the authors (X.E.) collected data retrospectively through the review of medical records, follow-up calls to owners and referring veterinarians, and clinical check-ups. The information collected included age at diagnosis, breed, sex, previous chemotherapy or steroid treatments, date of diagnosis and first surgery, location of the ScMCT (head and neck, trunk or limbs), clinical stage, blood work, staging diagnostics (including regional and abdominal ultrasound, aspirates of spleen and liver, and CT scan with or without lymphangiography), cytological lymph node evaluation according to Krick et al. (2009) (if performed), type of surgical procedure (wide vs. narrow; wide surgery was defined as having gross surgical margins of 3 cm and extending one fascial plane deep; narrow or marginal surgery was performed in selected cases due to tumour location or the owner's refusal to undergo a more aggressive procedure), methylene blue mapping for sentinel lymph nodes (if performed), systemic signs on presentation, Darier's sign (defined as severe mast cell degranulation, producing swelling, oedema, and bleeding following scratching of the affected area due to itching caused by the release of bioactive constituents, such as histamine, heparin, and proteases, which may occur before, during, or after surgery). (Blackwood et al. 2012), histopathological description (well-differentiated or poorly differentiated ScMCT), histopathological features including infiltrative behaviour (encapsulated, expansive, and circumscribed tumours vs. infiltrative tumours; using criteria from Thompson et al. (2011); tumours having both patterns were classified as infiltrative), atypia (defined as the presence of one or more of the following features: anisocytosis, anisokaryosis, multinucleated cells, variation in nuclear size, and prominent and multiple nucleoli), MC (with a cutoff of >4 or ≤4 in 10 HPF based on previous data) (Thompson et al. 2011; Marconato et al. 2023), and surgical margins. Histopathological nodal status was recorded when lymphadenectomy was performed, according to Weishaar et al. (2014). Only HN2 LNs (early metastatic) and HN3 LNs (overtly metastatic) were considered as metastatic in the statistical analysis. The data also included the date of surgical revision, chemotherapy regimen, RT and ECT protocols, date of first relapse (local recurrence, nodal recurrence, or distant metastasis) according to the response evaluation criteria for solid tumours in dogs (RECIST) (Nguyen et al. 2015), and date and cause of death. Proliferation indices (AgNORs, PCNA and Ki67) and cKIT staining were not performed in the study population.

Regarding the outcome, the study's endpoints were the disease-free interval (DFI) and disease-specific survival time (DSST). DFI was calculated from the surgery date to the date of the first local recurrence, nodal relapse, or distant metastasis. Patients who showed no evidence of recurrence at the time of data collection, during the last follow-up, or at the time of death or euthanasia were censored. DSST was defined as the survival time between surgical excision and death due to ScMCT. In the survival analysis, dogs that died due to causes other than ScMCT, were alive at the last follow-up, or were lost to follow-up were censored. DFI and DSST were assessed using Kaplan–Meier (KM) curves, and the log-rank test was used to compare outcomes between different groups. Variables evaluated as potential influences on DFI and DSST were location, histopathological description, clinical stage, infiltrative behaviour, atypia, Darier's sign, surgical margins, MC (>4 or ≤4), nodal status, and chemotherapy after surgery. Univariate and multivariate Cox proportional hazards regression model analyses were performed to investigate further potential interactions between the effects of risk factors on DFI and DSST. Data were analysed using the commercial IBM SPSS

Statistics 25 software (IBM Corp., Armonk, NY, USA). p-values of ≤0.05 were considered statistically significant.

3 | Results

Thirty-two dogs met the inclusion criteria. The most represented breeds were Beagle (n = 4; 12.5%), Labrador Retriever (n = 4; 12.5%), French Bulldog (n = 3; 9.4%), Bernese Mountain Dog (n = 2; 6.2%), American Staffordshire Terrier (n = 2; 6.2%), and Maltese (n = 2; 6.2%). Eight (25%) were mixed-breed, and seven breeds were represented just once. The median age at presentation was nine years (range, 4–14). There were five intact males (15.6%), seven neutered males (21.9%), six intact females (18.7%), and 14 spayed females (43.7%). ScMCTs were located in the trunk (n = 15; 46.9%), limbs (n = 15; 46.9%), and head and neck (n = 2; 6.2%). Four dogs (12.5%) had previously undergone surgery for low-grade cMCT; one dog (3.1%) developed *de novo* cMCT and underwent surgery; and two dogs (6.2%) developed *de novo* ScMCT, which were also surgically removed and considered a novel disease unrelated to their previous ScMCTs. One dog (3.1%) had two concurrent ScMCTs on presentation. One dog had systemic clinical signs (vomiting and diarrhoea) on presentation, which were assumed to be related to the ScMCT. Fourteen dogs (43.7%) were in stage I, eight dogs (25%) were in stage II, and ten dogs (31.3%) were in stage III. All dogs had complete blood work performed, and the results were unremarkable (abnormalities seen were equivalent to VCOG-CTCAE v2 grade 1 or grade 2). (LeBlanc et al. 2021) Twenty-seven dogs (84.4%) had abdominal ultrasounds and ultrasound-guided aspirates of the liver and spleen performed. No evidence of mast cell disease was identified in any of the aspirates. Ten dogs (31.3%) had regional LN ultrasounds, and 12 (37.5%) underwent advanced imaging with a CT scan. Six dogs (18.7%) had regional LN fine-needle aspirates, and five had either probable or certain metastasis, according to (Krick et al. 2009). All five cases were confirmed as metastatic in histopathology. Twenty-six dogs (81.2%) underwent wide surgery, while six (18.8%) had marginal surgery. Margins were assessed for all dogs, with 24 (75%) having complete surgical margins and 8 (25%) with incomplete margins. Two dogs (6.2%) had complete margins after scar revision surgery. Five of the eight dogs with affected margins underwent wide surgery, and three had marginal excisions. In one case, adjuvant RT was performed on the scar and regional LNs (45 Gy in 15 fractions), while in another case, ECT was applied (intravenous administration of 15,000 IU/m^2 of bleomycin followed by electroporation with 1000 V/cm). In the remaining cases, owners declined adjuvant therapies following surgery. Only two (25%) of the eight incompletely excised ScMCTs had local recurrences. None of the relapsed casesreceived any treatment after surgery; however, masitinib (10–12 mg/kg every 24 h) was started following tumour recurrence, and both dogs eventually died from tumour-related causes.

Twenty-Two dogs (68.8%) had infiltrative ScMCTs, while ten dogs (31.2%) had circumscribed tumours. In addition, 28 dogs (87.5%) had well-differentiated ScMCTs, whereas only four dogs (12.5%) were classified as poorly differentiated on histopathology. The median MC was 0.5 (range, 0–9), and three dogs (9.4%) had a MC >4; two of them died due to ScMCT progression after a median of 210 days (range, 147–435). Regional rather than sentinel LNs were removed in 18 dogs (56.3%). Regarding LN mapping, methylene blue was used in 6/32 dogs (18.8%), and computed tomographic lymphangiography was performed in three dogs (9.4%). The sentinel LN was identified in six dogs (33.3% of all removed LNs), with distribution as follows: two lumbar aortic, two popliteal, one cervical superficial, and one inguinal, considering both mapping techniques. Fourteen dogs (77.8%) had one regional LN removed, while four (22, 2%) had two LNs removed. A total of 22 LNs were removed, distributed as follows: six cervical superficial, five popliteal, five inguinal, four axillary, one accessory axillary, and one lumbar aortic. According to Weishaar et al. (2014), there were 6 (27.3%) non-metastatic (HN0) LNs, three (13.6%) pre-metastatic (HN1), 8 (31.8%) early metastatic (HN2), and five (22.7%) overtly metastatic (HN3). Overall, 12 dogs (37.5%) had at least one metastaticLN (HN2 or HN3), and in five of them (15.6%), the LN was overtly metastatic (HN3). Among these 12 dogs, 3 (25%) had tumour recurrence, and 2 (16.7%) died due to ScMCT. Among the five dogs with overtly metastatic LNs, two (40%) experienced tumour recurrence, and both died due to tumour-related causes.

Nine dogs (28.1%) received chemotherapy after surgery due to affected margins, nodal metastasis, or disease progression. Of these, 5 out of 9 patients received just one drug post-surgery: two received vinblastine (2 mg/m^2 every week for 4 treatments, followed by every other week for 4 more treatments, with the dose progressively escalated up to a maximum of 2.4 mg/m^2), and three received masitinib (10–12.5 mg/kg every 24 h). The remaining four dogs received different first-line treatments: toceranib phosphate (n = 1; 2.75 mg/kg 3 days per week), lomustine (n = 1; 55 mg/m^2 every 3 weeks), and masitinib (n = 2; 10–12.5 mg/kg every 24 h). Various rescue treatments were used, including masitinib, toceranib phosphate, vinblastine, imatinib (10 mg/kg every 24 h), and chlorambucil (20 mg/m^2 every two weeks). Two of these dogs were on masitinib before surgery, which was stopped 48 hours prior and restarted one week post-surgery. Additionally, as previously mentioned, one dog received adjuvant RT, and another underwent ECT due to affected margins. ECT was also applied to the scars of two more dogs with narrow margins. Darier's sign, as described previously, was observed in 7 dogs (21.9%); among them, five dogs died due to ScMCT after a median of 180 days (range, 79–581).

The mean follow-up was 442 days, with a median follow-up of 405 days. Disease recurrence occurred in 9 dogs (28.1%) after a median of 127 days (range, 50–506). Of these, seven dogs (77.8%) had local recurrence, while two dogs (22.2%) had nodal recurrence. KM's median DFI and DSST were not reached at 1312 days (Figures 2 and 3). The overall 1-year and 2-year survival rates were 80% and 70%, respectively. At the final data analysis, 19 dogs (59.4%) were still alive, with a median follow-up of 464 days (range, 147–1044). Seven dogs (21.9%) died of ScMCT-related disease after a median of 180 days (range, 79–581), three dogs (9.4%) died from other non-related causes, and three dogs were lost to follow-up.

In the survival analysis, factors such as location, histopathological description, infiltrative behaviour, atypia, wide surgery, surgical margins, and nodal status did not demonstrate a statistically significant impact on DFI and DSST (Table 2).

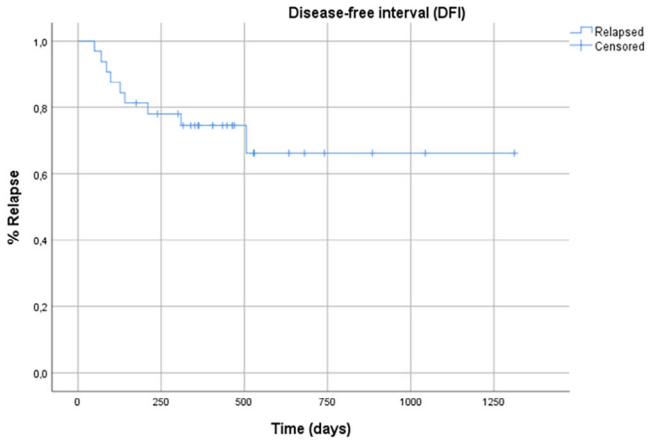

FIGURE 2 | Disease-free interval (DFI) Kaplan–Meier (KM) curve analysis in 32 dogs with ScMCT treated with curative-intent approach. DFI was calculated from the surgery to the date of the first local recurrence, nodal relapse, or distant relapse. Censored patients were those who showed no evidence of recurrence at the time of data collection, during the last follow-up, or at the time of death or euthanasia. The median DFI was not reached at 1312 days.

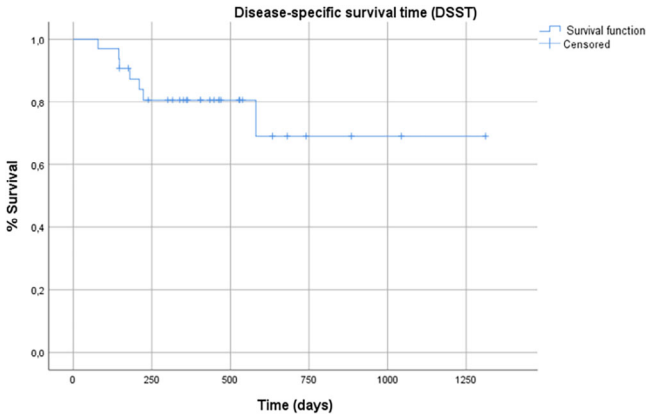

FIGURE 3 | Disease-specific survival time (DSST) Kaplan–-Meier (KM) curve analysis in 32 dogs with ScMCT treated with curative-intent approach. DSST was defined as the survival time between surgical excision and death due to ScMCT. In the survival analysis, dogs that died due to causes other than ScMCT, were alive at the last follow-up, or were lost to follow-up, were censored. The median DSST was not reached at 1312 days.

Darier's sign was associated with a shorter DFI and DSST. Patients with Darier's sign had a median DFI of 141 days (range, 50–405), whereas dogs without Darier's sign had not reached the median DFI at 1312 days ($p < 0.05$). Similarly, patients with Darier's sign had a median DSST of 210 days (range, 79–581), compared to dogs without Darier's sign who had not reached the median DSST by 1312 days ($p < 0.05$) (Figures 4, 5, and 6).

Patients who received chemotherapy after surgery were associated with a shorter DFI ($p = 0.08$) and DSST ($p = 0.03$). The median DFI was 310 days (range, 50–470), and the median DSST was 581 days (range, 79–581). The Clinical stage demonstrated an association with DFI ($p = 0.015$) but not with DSST, despite approaching significance ($p = 0.054$). Patients with an MC >4 were found to have a shorter DSST ($p = 0.022$), with a median DSST of 210 days (range, 147–435). However, the association with DFI was not statistically significant, although it approached significance ($p = 0.072$) (Table 2).

In univariate Cox proportional hazard analysis, variables associated with an increased risk of a shorter DFI (tumour progression) were clinical stages II and III (hazard ratio [HR] = 8.75, confidence interval [CI] = 1.1–70, $p = 0.04$) compared to stage I, Darier's sign (HR = 17.04, CI = 3.38-85, $p = 0.001$), and chemotherapy after surgery (HR = 5.6, CI: 1.3-23, $p = 0.018$). Variables associated with an increased risk of a shorter DSST (ScMCT-related death) were clinical stages II and III (HR = 8.97, CI = 0.9–82, $p = 0.05$), Darier's sign (HR = 14.94, CI = 2.8–79, $p = 0.001$), chemotherapy after surgery (HR = 8.38, CI: 2-44, $p = 0.012$), and MC >4 (HR = 5.8, CI: 1-32, $p = 0.043$) (Tables 3 and 4). In the multivariate analysis, only Darier's sign remained significant.

4 | Discussion and Conclusions

Most research has focused on cMCTs, resulting in few dedicated studies on the ScMCT subset. Historically, ScMCTs have been associated with a good prognosis, demonstrating low recurrence rates, nodal involvement, and distant metastases when managed with surgery alone, even when histological margins were affected.

Recent publications have introduced a paradigm shift by revealing a more aggressive behaviour in ScMCTs, demonstrating higher rates of nodal metastasis and recurrence than previously reported. These new findings challenge the traditional understanding of ScMCTs. The results of our study align with this emerging trend, as we observed a notable proportion of cases (37.5%) presenting with nodal metastasis at the time of diagnosis, with 15.6% of these cases being overtly metastatic. While only a subset of our study population (56.3%) underwent lymphadenectomies, assessment of LNs was conducted in all patients.

This observation suggests that if previous studies had included a systematic evaluation of LNs, the reported rates of nodal metastasis at presentation could have been more closely aligned with our study's findings and the recent figures reported by Marconato et al. (2023) (58%), Cherzan et al. (2023) (26.7%), and Treggiari et al. (2023) (63%). This stands in contrast with the historically mentioned rates of 4%–6% (Thompson et al. 2011; Newman et al. 2007) or the 2% reported by Gill et al. (2020).

As described by Weishaar et al. (2014), LN metastasis is a well-known negative prognostic indicator in canine cMCTs. Recently, LN metastasis was also described as a poor prognostic indicator for dogs with ScMCTS (Cherzan et al. 2023). In Marconato et al. (2023), the presence of at least one overtly metastatic LN (HN3) was associated with an increased risk of tumour progression but not with tumour-related death. Additionally, all five dogs who died of ScMCT-related causes in Marconato et al. (2023) had at least one HN3 LN at admission. Surprisingly, patients with nodal metastases (HN2 or HN3) did not show an association with shorter DFI and DSST in our study, nor did an individualised analysis of patients with overt metastasis. Moreover, it is essential

TABLE 2 | Results of log-rank tests comparing DFI and DSST based on clinical variables. Bolded *p*-values are statistically significant.

Results of log-rank tests comparing DFI and DSST based on clinical variables				
	Disease-free interval (DFI)		Disease-specific survival time (DSST)	
Factor evaluated	**Cases (events[a])**	***p*-value**	**Cases (events[b])**	***p*-value**
Global	32 (9)		32 (7)	
Well-differentiated[c]	28 (8)	0.929	28 (6)	0.740
Poorly differentiated	4 (1)			
Location				
Head and neck	2 (0)		2 (0)	
Trunk	15 (6)	0.384	15 (5)	0.368
Limbs	15 (3)		15 (2)	
Clinical stage (WHO)				
1	14 (1)		14 (1)	
2	8 (2)	**0.015**	8 (2)	0.054
3	10 (6)		10 (4)	
Wide surgery				
Yes	26 (8)	0.612	26 (6)	0.792
No	6 (1)		6 (1)	
Infiltrative behaviour[d]				
No	10 (1)	0.127	10 (1)	0.221
Yes	22 (8)		22 (6)	
Mitotic count (MC)				
≤4	29 (7)	0.072	29 (5)	**0.022**
>4	3 (2)		3 (2)	
Atypia				
No	22 (6)	0.688	22 (4)	0.421
Yes	10 (3)		10 (3)	
Darier's sign				
No	25 (3)	**<0.05**	25 (2)	**<0.05**
Yes	7 (3)		7 (5)	
Lymphadenectomy				
Yes	18 (4)	0.511	18 (3)	0.509
No	14 (5)		14 (4)	
Surgical margins				
Yes	24 (7)	0.95	24 (5)	0.809
No	28 (2)		8 (2)	
Nodal metastasis				
HN0/HN1	20 (6)	0.944	20 (5)	0.779
HN2/HN3	12 (3)		12 (2)	
Chemotherapy after surgery				
Yes	9 (5)	**0.008**	9 (5)	**0.003**
No	23 (4)		23 (2)	

[a]Disease recurrence.
[b]ScMCT-related deaths.
[c,d]Histopathological description.

to highlight that removing overtly metastatic LNs could explain the improved outcome for these patients. Furthermore, the impact of removing LNs with early metastasis on the long-term outcome remains uncertain (Ferrari et al. 2018), which could explain the favourable prognosis observed in previous studies.

In this study, the sentinel LN was identified in only 6 out of 18 (33.3%) dogs that underwent lymphadenectomy. It is known that the regional LN may not correlate with the sentinel LN in up to 63% of cases. (Ferrari et al. 2020) Therefore, there is a possibility that some normal-sized metastatic LNs were not removed in our cohort of dogs, potentially contributing to an increased rate of disease progression. However, despite these findings, our study's overall long-term prognosis was favourable. In the survival analysis, the overall median DFI and DSST were not reached at 1312 days, and the overall 1-year and 2-year survival rates were 80% and 70%, respectively, consistent with previous research (Thompson et al. 2011; Newman et al. 2007; Gill et al. 2020; Marconato et al. 2023; Cherzan et al. 2023; Treggiari et al. 2023).

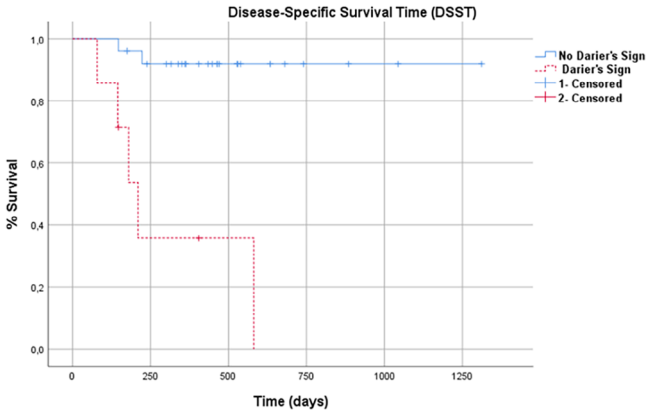

FIGURE 4 | Darier's sign effect on disease-specific survival time (DSST) Kaplan–Meier (KM) curve. DSST was defined as the survival time between surgical excision and death due to ScMCT. Darier's sign was defined as severe mast cell degranulation before, during, or after surgery.

ScMCT-related deaths were observed in 11.6% in Marconato et al. (2023) and 13.3% in Cherzan et al. (2023) of cases, consistent with rates reported previously by Gill et al. (2020) (7%) and Thompson et al. (2011) along with Newman et al. (2007) (approximately 10%). In our study, the mortality rate was slightly higher, with seven dogs (21.9%) dying from tumour-related causes. This discrepancy could be attributed to selection bias or more aggressive biological behavior, among other factors.

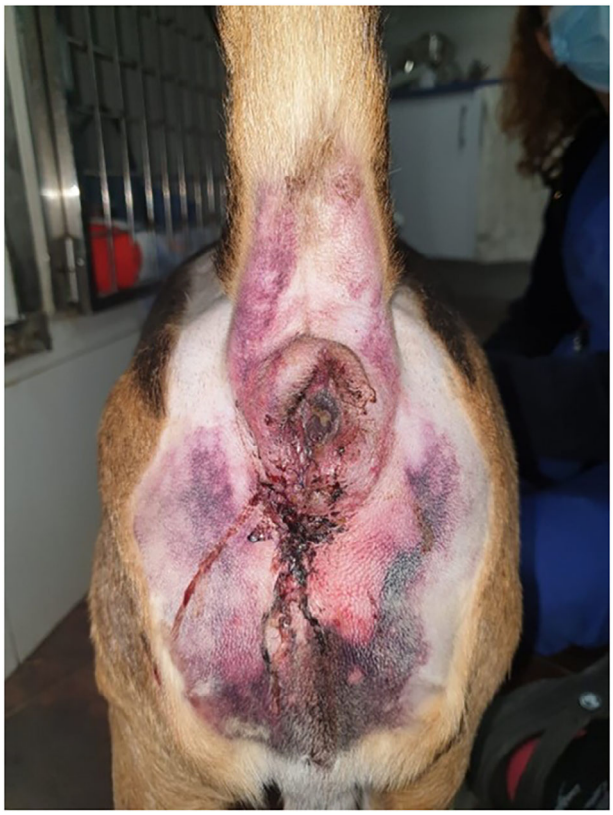

FIGURE 6 | Same patient Figure 5. Severe Darier's sign was observed after the surgical removal of a ScMCT in the perineal region. The disease-free interval (DFI) and disease-specific survival time (DSST) for this dog were 70 days and 145 days, respectively.

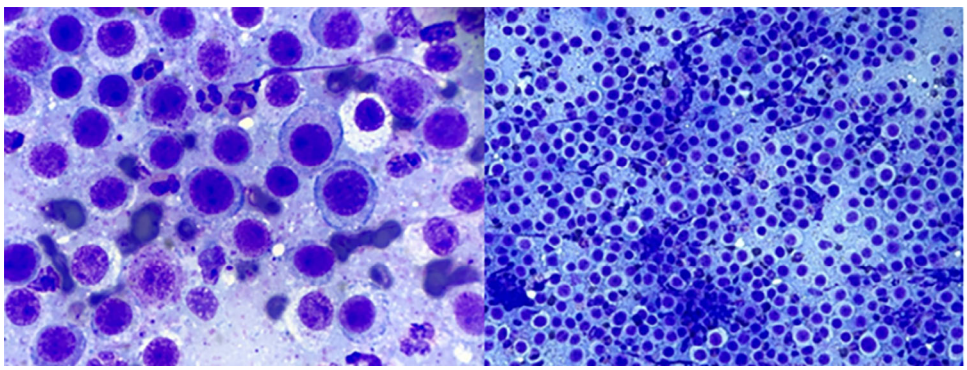

FIGURE 5 | Fine needle aspirate of a ScMCT tumour with aggressive behaviour and metastatic tendency. Modified Wright—Giemsa stain; (left) 100× objective, (right) 20× objective. The sample shows a slightly granulated mast cells with moderate anisocytosis and anisokaryosis, along with occasional binucleated forms. Mitotic figures are also present. Additionally, there is a high number of eosinophils. Small amounts of pale pink, fibrillar material consistent with degraded collagen are noted as well. Findings are compatible with a poorly differentiated MCT, eosinophilic inflammation, and collagenolysis.

TABLE 3 | Bolded *p*-values are statistically significant.

Univariate Cox proportional hazard analysis for disease free interval (DFI)			
Risk factor	**Hazard ratio**	**95% confidence interval**	***p*-value**
Poorly differentiated ScMCTs	1.1	0.1–8.9	0.92
Marginal surgery	1.7	0.212–13.6	0.6
Clinical stages 2 and 3	**8.75**	1.1-70	**0.04**
Darier's sign	**17.038**	3.38-85	**0.001**
Chemotherapy after surgery	**5.6**	1.3-23	**0.018**
Infiltrative behaviour	4.4	0.5-35	0.16
Mitotic count > 4	4.7	0.9-24	0.066

TABLE 4 | Bolded *p*-values are statistically significant.

Univariate Cox proportional hazard analysis for disease-specific survival time (DSST)			
Risk factor	**Hazard ratio**	**95% confidence interval**	***p*-value**
Poorly differentiated ScMCTs	1.136	0.68-12.29	0.74
Marginal surgery	1.328	0.16-11	0.793
Clinical stages 2 and 3	**8.97**	0.9-82	**0.05**
Darier's sign	**14.943**	2.8-79	**0.001**
Chemotherapy after surgery	**8.38**	2-44	**0.012**
Infiltrative behaviour	3.466	0.4-28	0.12
Mitotic count > 4	**5.8**	1-32	**0.043**

In terms of disease recurrence within this study, it was observed in 28.1% of the cases (6.3% metastatic recurrence and 21.9% local recurrence), which is higher than the historically reported 8–9% rates (Thompson et al. 2011; Newman et al. 2007) and more in line with Marconato et al. (2023) (18.6%) and Cherzan et al. (2023) (28.8%) despite multimodal treatment. The reduced recurrence rate observed in the study by Marconato et al. (2023) could be attributed to the study's prospective design, which involved achieving clean histological margins and regional lymphadenectomy in all cases and systematic follow-up treatment. Notably, Treggiari et al. (2023) reported a 15% disease recurrence in their study, adding further context to the observed variations in recurrence rates.

The presence of affected margins did not show an association with tumour recurrence in our study, which is consistent with previous reports. (Thompson et al. 2011; Cherzan et al. 2023; Treggiari et al. 2023) In Gill et al. (2020), the local recurrence rate in dogs with incomplete surgical margins was 21%, and in Cherzan et al. (2023), it was up to 27.2%. Within the scope of our study, 2 out of 8 (25%) incompletely excised ScMCTs experienced local recurrence. While the significance of recurrence in relation to incomplete excision may not be definitively established, considering scar revision as a follow-up treatment in cases with affected margins is noteworthy. Nonetheless, this warrants further prospective evaluation. Adjuvant RT and ECT may also play a role in this scenario, as neither of the two dogs in our cohort with affected margins and treated with adjuvant RT and ECT experienced recurrence. A recent publication involving 302 dogs strongly supports the use of adjuvant RT for the long-term control of incompletely or narrowly excised cutaneous and ScMCTs in dogs. (Mason et al. 2021) However, the data supporting the use of ECT in this setting remains limited. (Spugnini et al. 2006, 2011)

Several histopathological features have been associated with more aggressive biological behaviour in ScMCTs, such as multinucleation, infiltrative growth pattern, and MC >4 in 10 HPF. Infiltrative behaviour and multinucleation (referred to as atypia in this study) did not show an association with poorer prognosis. Patients with MC >4 in 10 HPF were found to have a shorter DSST, although the association with DFI was not statistically significant. Marconato et al. (2023) demonstrated a significant correlation between MC greater than 4 in 10 HPF and local, nodal, and distant relapse, as well as tumour-related death. Our study's lack of significant findings could be attributed to a type II error, potentially due to the relatively low sample size.

The cytologic grading system described by Camus et al. (2016) is a useful predictor for treatment planning and prognostication in cMCTs and may also be reliable for the Sc subset, according to Marconato et al. (2023). Ideally, future research should consider a prospective evaluation of the Camus et al. criteria with a larger population or the development of specific cytological criteria for

ScMCTs. Such criteria could serve as a valuable tool for predicting more aggressive behaviour in ScMCTs.

To the best of the author's knowledge at the time of publication, this is the first report to robustly correlate the presence of Darier's sign, as described above, with disease recurrence and tumour-related death in ScMCTs treated with a curative-intent approach. The presence of Darier's sign may indicate a more aggressive biological behaviour and should alert clinicians to monitor the patient more closely. Additionally, it could prompt consideration of adjunctive medical therapies after surgery, particularly when combined with other negative prognostic factors such as a high mitotic count or nodal metastatic disease.

Patients in clinical stages II and III had an increased risk of tumour progression and ScMCT-related death in this study, highlighting the potential value of the clinical stage as a straightforward tool for prognostic anticipation in this disease. Staging for this study was conducted according to the WHO system, with a slight modification classifying patients with multiple unrelated ScMCTs at presentation and without nodal involvement as stage I. These findings might suggest that such patients, when treated with a curative-intent approach, could have a better prognosis compared to stage III patients with large infiltrative tumours. Multiple ScMCTs at presentation are uncommon, occurring in approximately 5% of cases and 3.1% in this study, indicating that further research is needed to evaluate the potential need for modifying the WHO system for the ScMCT subset. Additionally, patients who received chemotherapy after surgery were associated with poorer outcomes. However, this association could be attributed to a selection bias favouring dogs with more aggressive tumours. Prospective evaluation is necessary to determine the potential benefits of post-surgery follow-up chemotherapy in dogs with ScMCTs.

The study provides valuable insights into ScMCTs; however, there are several limitations to consider. The study's retrospective nature lacks standardisation due to heterogeneity in clinical management, including variations in treatment and staging procedures. Furthermore, relying on data from a single institution may introduce selection bias. The small sample size also limits the generalisability and relevance of our findings. Moreover, the histopathological slides were not evaluated by a single pathologist, which may introduce potential bias. Lastly, the MC should have been performed using a 2.37 mm^2 area rather than 10 HPF, as the latter is not standardised. To address these limitations, further research involving larger, multi-institutional cohorts and prospective study designs could enhance our understanding of the prognostic factors, treatment, and outcomes for ScMCTs in dogs.

The assertion that ScMCTs are more benign than cMCTs can be misleading if negative prognostic factors such as grade, MC, or stage are not specified. Recent publications indicate a shift towards more aggressive behaviour in ScMCTs, so our study contributes further evidence to this evolving perspective. The relatively high rate of nodal metastasis (37.5%) observed in our study suggests that these tumours might be more prone to spread than previously understood. These findings have substantial implications for diagnosis, staging, and therapeutic approaches.

Identifying nodal metastasis, especially when overt, highlights the importance of a comprehensive approach involving various diagnostic modalities and collaborative efforts among clinicians. Relying solely on surgical excision might not be sufficient, and including systematic LN assessment could enhance the accuracy of prognosis and guide treatment decisions.

Author Contributions

Conception and design: Xavier Escoda, Eduard Anadón and Ignasi Rodríguez. *Acquisition of data*: Xavier Escoda, Eduard Anadón and Ignacio Rodríguez. *Analysis and interpretation of data*: Xavier Escoda, Núria Llorens and Ignacio Rodriguez. *Drafting of article*: Xavier Escoda.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a retrospective study on client-owned dogs, treatment was undertaken in the context of advanced veterinary medicine and written-informed consent was obtained from the owners.

Data Availability Statement

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

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/vms3.70291.

References

Blackwood, L., S. Murphy, P. Buracco, et al. 2012. "European Consensus Document on Mast Cell Tumours in Dogs and Cats." *Veterinary and Comparitive Oncology* 10, no. 3: e1–e29. https://doi.org/10.1111/j.1476-5829.2012.00341.x.

Camus, M. S., H. L. Priest, J. W. Koehler, et al. 2016. "Cytologic Criteria for Mast Cell Tumor Grading in Dogs with Evaluation of Clinical Outcome." *Veterinary Pathology* 53, no. 6: 1117–1123. https://doi.org/10.1177/0300985816638721.

Cherzan, N. L., K. Fryer, B. Burke, and J. Farrelly. 2023. "Factors Affecting Prognosis in Canine Subcutaneous Mast Cell Tumors: 45 Cases." *Veterinary Surgery* 52, no. 4: 531–537. https://doi.org/10.1111/vsu.13944.

Ferrari, R., L. E. Chiti, M. Manfredi, et al. 2020. "Biopsy of Sentinel Lymph Nodes After Injection of Methylene Blue and Lymphoscintigraphic Guidance in 30 Dogs With Mast Cell Tumors." *Veterinary Surgery* 49, no. 6: 1099–1108. https://doi.org/10.1111/vsu.13483.

Ferrari, R., L. Marconato, P. Buracco, et al. 2018. "The Impact of Extirpation of Non-Palpable/Normal-Sized Regional Lymph Nodes on Staging of Canine Cutaneous Mast Cell Tumours: A Multicentric Retrospective Study." *Veterinary and Comparative Oncology* 16, no. 4: 505–510. https://doi.org/10.1111/vco.12408.

Gill, V., N. Leibman, S. Monette, D. M. Craft, and P. J. Bergman. 2020. "Prognostic Indicators and Clinical Outcome in Dogs With Subcutaneous Mast Cell Tumors Treated With Surgery Alone: 43 Cases." *Journal of the American Animal Hospital Association* 56, no. 4: 215–225. https://doi.org/10.5326/JAAHA-MS-6960.

Kiupel, M., J. D. Webster, K. L. Bailey, et al. 2011. "Proposal of a 2-Tier Histologic Grading System for Canine Cutaneous Mast Cell Tumors to More Accurately Predict Biological Behavior." *Veterinary Pathology* 48, no. 1: 147–155. https://doi.org/10.1177/0300985810386469.

Krick, E. L., A. P. Billings, F. S. Shofer, S. Watanabe, and K. U. Sorenmo. 2009. "Cytological Lymph Node Evaluation in Dogs With Mast Cell Tumours: Association With Grade and Survival *." *Veterinary and Comparative Oncology* 7, no. 2: 130–138. https://doi.org/10.1111/j.1476-5829.2009.00185.x.

LeBlanc, A. K., M. Atherton, R. T. Bentley, et al. 2021. "Veterinary Cooperative Oncology Group—Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2) Following Investigational Therapy in Dogs and Cats." *Veterinary and Comparative Oncology* 19, no. 2: 311–352. https://doi.org/10.1111/vco.12677.

Marconato, L., D. Stefanello, F. Solari Basano, et al. 2023. "Subcutaneous Mast Cell Tumours: A Prospective Multi-Institutional Clinicopathological and Prognostic Study of 43 Dogs." *The Veterinary Record* 193, no. 1: e2991. https://doi.org/10.1002/vetr.2991.

Mason, S. L., C. Pittaway, B. P. Gil, et al. 2021. "Outcomes of Adjunctive Radiation Therapy for the Treatment of Mast Cell Tumors in Dogs and Assessment of Toxicity: A Multicenter Observational Study of 300 Dogs." *Journal of Veterinary Internal Medicine* 35, no. 6: 2853–2864. https://doi.org/10.1111/jvim.16264.

Meuten, D. J., ed. 2017. *Tumors in Domestic Animals*. 5th ed. Wiley/Blackwell.

Newman, S. J., L. Mrkonjich, K. K. Walker, and B. W. Rohrbach. 2007. "Canine Subcutaneous Mast Cell Tumour: Diagnosis and Prognosis." *Journal of Comparative Pathology* 136, no. 4: 231–239. https://doi.org/10.1016/j.jcpa.2007.02.003.

Nguyen, S. M., D. H. Thamm, D. M. Vail, and C. A. London. 2015. "Response Evaluation Criteria for Solid Tumours in Dogs (v1.0): A Veterinary Cooperative Oncology Group (VCOG) Consensus Document." *Veterinary and Comparative Oncology* 13, no. 3: 176–183. https://doi.org/10.1111/vco.12032.

Patnaik, A. K., W. J. Ehler, and E. G. MacEwen. 1984. "Canine Cutaneous Mast Cell Tumor: Morphologic Grading and Survival Time in 83 Dogs." *Veterinary Pathology* 21, no. 5: 469–474. https://doi.org/10.1177/030098588402100503.

Ribeiro, P. R., M. V. Bianchi, M. B. Bandinelli, et al. 2022. "Pathological Aspects of Cutaneous Mast Cell Tumors With Metastases in 49 Dogs." *Veterinary Pathology* 59, no. 6: 922–930. https://doi.org/10.1177/03009858221114468.

Spugnini, E. P., B. Vincenzi, F. Baldi, G. Citro, and A. Baldi. 2006. "Adjuvant Electrochemotherapy for the Treatment of Incompletely Resected Canine Mast Cell Tumors." *Anticancer Research* 26, no. 6: 4585–4589. https://doi.org/10.1186/1479-5876-5-48.

Spugnini, E. P., B. Vincenzi, G. Citro, I. Dotsinsky, T. Mudrov, and A. Baldi. 2011. "Evaluation of Cisplatin as an Electrochemotherapy Agent for the Treatment of Incompletely Excised Mast Cell Tumors in Dogs." *Journal of Veterinary Internal Medicine* 25, no. 2: 407–411. https://doi.org/10.1111/j.1939-1676.2011.0678.x.

Thompson, >J. J., D. L. Pearl, J. A. Yager, S. J. Best, B. L. Coomber, and R. A. Foster. 2011. "Canine Subcutaneous Mast Cell Tumor: Characterization and Prognostic Indices." *Veterinary Pathology* 48, no. 1: 156–168. https://doi.org/10.1177/0300985810387446.

Treggiari, >E., P. Valenti, I. Porcellato, G. Maresca, and G. Romanelli. 2023. "Retrospective Analysis of Outcome and Prognostic Factors of Subcutaneous Mast Cell Tumours in Dogs Undergoing Surgery With or Without Adjuvant Treatment." *Veterinary and Comparative Oncology* 21, no. 3: 437–446. https://doi.org/10.1111/vco.12902.

Weishaar, K. M., D. H. Thamm, D. R. Worley, and D. A. Kamstock. 2014. "Correlation of Nodal Mast Cells With Clinical Outcome in Dogs With Mast Cell Tumour and a Proposed Classification System for the Evaluation of Node Metastasis." *Journal of Comparative Pathology* 151, no. 4: 329–338. https://doi.org/10.1016/j.jcpa.2014.07.004.