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ORIGINAL ARTICLE OPEN ACCESS

Incorporation of Biologic Variables Into the Staging for Canine Cutaneous and Subcutaneous Mast Cell Tumours: Proposal of the UBo pTNM System

Laura Marconato[1] | Eugenio Faroni[1] | Emiliano Battisti[1] | Riccardo Zaccone[1] | Damiano Stefanello[2] | Silvia Sabattini[1]

[1]Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia (Bologna), Italy | [2]Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy

Correspondence: Laura Marconato (laura.marconato@unibo.it)

Received: 22 January 2024 | **Revised:** 5 June 2024 | **Accepted:** 21 July 2024

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

Keywords: dogs | grading | mastocytoma | prognosis | pTMN | staging | survival

ABSTRACT

Canine cutaneous mast cell tumours (MCTs) are currently staged based on the World Health Organization (WHO) classification, which has remained unchanged since its initial formulation. Our study aimed to assess the reliability of a novel pTNM staging system, which incorporates tumour extent (T), lymph node involvement (N), presence of distant metastases (M) and the two-tier histologic grade. We analysed medical records of dogs with one or more cutaneous/subcutaneous completely staged MCT, undergoing tumour excision with lymphadenectomy, unless distant metastases were present, in which cases, medical therapy was administered. Dogs were categorized into three stages: I (T1-2N0M0), II (T1-2N1M0) and III (distant metastases). Stages I and II were further divided based on histologic grade into 'low' and 'high'. Substage b was defined as the presence of tumour diameter of ≥3 cm and/or ulceration. Of 226 dogs, 87 (38.5%) were in Stage I (I-low, $n = 75$; I-high, $n = 12$), 107 (47.3%) in Stage II (II-low, $n = 59$; II-high, $n = 48$), and 32 (14.2%) in Stage III. The newly proposed staging system was able to significantly stratify the population for both time to progression and tumour-specific survival. Compared to Stage I-low, the risk of progression increased significantly for Stage I-high (18.3 times), Stage II-low (8.5 times), Stage II-high (41.5 times) and Stage III (110.3 times). The staging system was highly prognostic for both cutaneous and subcutaneous MCTs. Prospective validation studies are essential to compare this new system with the current WHO staging and further validate its accuracy and clinical utility.

1 | Introduction

Staging systems have been developed to enhance the understanding of the clinical behaviour of specific malignancies, determine prognosis and facilitate the comparison of outcomes among similar groups of patients.

Canine cutaneous mast cell tumours (cMCTs) have traditionally been categorized into four clinical stages based on the World Health Organization (WHO) classification, supposedly reflecting increasing biological aggressiveness [1]. The WHO stages rely mostly on macroscopic characteristics of the primary tumour, the presence of multiple MCTs, nodal involvement and distant metastasis [1].

The WHO staging system has remained unchanged since its initial formulation. However, recent advancements in knowledge challenge the prognostic value of this system, necessitating an update [2–8]. Notably, the current staging system is purely clinical and overlooks essential histopathologic characteristics of

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the disease, such as histologic grade and different nodal stages, which bear distinct prognostic significance [9, 10].

In the past decade, the variable biologic behaviour of MCT, renowned for its diversity, has been further elucidated through the two-tier histologic classification proposed by Kiupel et al. [9] to delineate the degree of malignancy. Furthermore, proposed standardized histologic criteria aim to consistently characterize nodal involvement, proving to be more accurate, reliable and reproducible compared to cytologic evaluation alone [10, 11]. Additionally, the diagnostic, prognostic and therapeutic role of lymphadenectomy has recently been confirmed [2–5, 12]. This approach, coupled with histologic examination, allows for a more precise identification of lymph node metastasis, facilitating more accurate prognostication and the determination of optimal treatment strategies [3, 10].

Finally, the category of subcutaneous MCTs (scMCTs), long presumed to exhibit indolent biological behaviour [13], has garnered renewed attention. Recent studies have revealed variable biologic behaviour within this category, predictable through the application of the two-tier histologic grading [14–18].

In light of these considerations, this retrospective study aims to evaluate the reliability of an alternative staging system for canine cMCT and scMCT. This system incorporates both clinical and histologic parameters to provide a more precise characterization of the neoplasm's biological behaviour and extent.

2 | Methods

2.1 | Study Design

Medical records of the University of Bologna were retrospectively searched for dogs with one or more cMCTs or scMCTs undergoing oncologic consultation from January 2018 to May 2023.

To be eligible for inclusion, dogs had to undergo a complete work-up, including physical examination, thoracic radiographs, abdominal ultrasound (or total-body computed tomography) and liver and spleen fine-needle aspiration for cytologic examination regardless of the ultrasonographic appearance [19]. Surgical excision of the primary tumour with concurrent removal of regional or sentinel lymph node(s) was also required, unless distant metastasis were present. Techniques for sentinel lymph node mapping consisted of preoperative radiographic indirect lymphography or computed tomographic lymphography, followed by intraoperative peritumoral methylene blue injection [20, 21]. In case of cytologically confirmed distant metastasis at presentation, administration of medical therapy was deemed necessary for inclusion. For dogs undergoing surgery, adjuvant chemotherapy was also an inclusion criterion in case of high-grade MCT and/or overt nodal metastasis (HN3) [22–24]. A follow-up period of ≥180 days was required for inclusion, unless MCT-related death was registered. Regardless of treatment, it was consistently recommended to recheck dogs with low-grade MCTs through clinical examinations and imaging every 3 months for the first year, and every 6 months thereafter. For dogs with high-grade MCTs and/or HN3 metastasis, it was advised to conduct rechecks once monthly for the initial 3 months post-chemotherapy, followed by assessments every 3 months thereafter.

Dogs with recurrent or multiple concurrent MCTs non-amenable to surgical excision and regional/sentinel lymphadenectomy, MCTs arising in locations other than cutis and/or subcutis, and those with second cancer were excluded. Additionally, dogs with comorbidities that limited life expectancy to less than 6 months were not included in the study. Neoadjuvant chemotherapy was not considered ground for exclusion.

Collected information included patient details (i.e., gender, breed, age and weight), tumour characteristics (i.e., location, maximum diameter and ulceration), presence and site(s) of distant metastasis (if any), histologic grade of the primary MCT, histologic status of margins and lymph nodes (for dogs undergoing surgery), chemotherapy protocol administered (if any), development of disease progression, survival time and cause of death (if applicable). In case neoadjuvant chemotherapy was administered, the maximum diameter was recorded on the day of admission.

Spleen and/or liver involvement was defined by the presence of overt metastasis criteria according to Pecceu et al. [6]. For the purposes of the study, both cMCTs and scMCTs were graded according to the two-tier system [9, 18]. The nodal status was classified according to Weishaar et al. [10]. All microscopic evaluations were conducted by a single board-certified pathologist (SS).

2.2 | UBo Staging System

Once the necessary information was obtained, each dog was assigned a stage using a new integrated pathologic TNM (pTNM) classification developed by the authors (UBo staging system). This method is based on the evaluation of primary tumour (T) characteristics (i.e., diameter and ulceration), presence of histologic overt (HN3) lymph node metastasis (N), presence of cytologically confirmed distant metastasis (M) and two-tier histologic grade of the primary tumour (Table 1).

Distant metastasis was defined as the cytologically confirmed presence of the tumour in any extra-nodal visceral site.

A cut-off of 3 cm in the diameter of the primary tumour and presence of ulceration were used to stratify T subcategories based on previous studies [3, 6–8, 23, 25–28].

The findings related to T, N, M and the two-tier grade were integrated to form stage and substage as presented in Table 1.

In case of multiple concurrent MCTs, removed with regional/sentinel lymph node(s), the dog was assigned to the highest stage considering all tumours' clinical and histologic characteristics.

2.3 | Statistical Analysis

Patient and tumour characteristics were summarized using descriptive statistics. When appropriate, data were subjected to

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TABLE 1 | Proposed UBo pTNM staging system for canine cutaneous and subcutaneous MCTs.

T—Primary tumour	
T1	Maximum tumour diameter <3 cm
nu	Not ulcerated
u	Ulcerated
T2	Maximum tumour diameter ≥3 cm
nu	Not ulcerated
u	Ulcerated
N—Regional/sentinel lymph node status	
N_0	HN0/HN1/HN2 lymph node status
N_1	HN3 lymph node status
M—Distant metastasis	
M_0	No distant metastasis
M_1	Cytologically confirmed distant metastasis
G—Two-tier histologic grade	
G_0	Low-grade MCT
G_1	High-grade MCT
Stage	
I-*low*	$T_{1–2}$, N_0, M_0, G_0
I-*high*	$T_{1–2}$, N_0, M_0, G_1
II-*low*	$T_{1–2}$, N_1, M_0, G_0
II-*high*	$T_{1–2}$, N_1, M_0, G_1
III	Any T, any N, M_1, any G
Substage	
a	T_1*nu*
b	T_1*u* or T_2

Abbreviations: HN, histologic node; MCT, mast cell tumour.

normality tests using the D'Agostino and Pearson test. None of the numeric variables had a normal distribution and, therefore, the median and range were used as summary statistics.

For descriptive purposes, Shar Pei, American Staffordshire Terrier, Weimaraner, Rottweiler and Shih Tzu were considered breed predisposed to the development of biologically aggressive MCTs [26, 29–31]. Head and neck, digits, inguinal and perineal regions were considered as locations associated with biologically aggressive tumours [8, 26, 30, 32–34].

Regarding outcome, local recurrence (LR) was defined as the cytologic evidence of a recurrent MCT within 2 cm from the surgical scar, in case of previously removed MCT(s). Local progression (LP) was defined as a 20% increase of maximum tumour diameter in case of macroscopic tumour(s) [35]. Nodal progression (NP) was defined as the evidence of newly diagnosed cytologically confirmed metastatic lymph node(s) or a 20% increase of short-axis dimension in case of previously cytologically confirmed and unremoved metastatic lymph node(s) [35]. Distant progression (DP) was defined as the occurrence of cytologically confirmed distant metastasis or worsening of clinical conditions in dogs already diagnosed with distant metastasis.

For Stage I or II dogs, time to progression (TTP) was calculated from the date of surgery to the occurrence of one or more of LR, NP or DP. For Stage III dogs, TTP was calculated from the cytological confirmation of distant metastasis to the occurrence of one or more of LP, NP or DP. Dogs that did not develop disease progression were censored at the date of the last visit or death.

The development of one or more newly appearing MCTs during the follow-up time was also recorded but not considered as a criterion of disease progression.

Tumour-specific survival (TSS) was calculated from the date of surgical intervention (for Stage I or II dogs) or the cytologic confirmation of distant metastasis (for Stage III dogs) to the date of death or the last visit. Only MCT-related deaths were recorded as events.

To confirm their prognostic significance, individual parameters (i.e., MCT diameter ≥ 3 cm, tumour ulceration, histologic high-grade, HN3 nodal status and distant metastasis at admission) of UBo staging system were tested for prognostic independence through a multivariable Cox regression analysis. MCT diameter of ≥3 cm and tumour ulceration were further tested for prognostic independence with UBo stage.

Univariable Cox regression analysis was applied to test the prognostic relevance of the UBo staging system in dogs with scMCTs as a separate category.

The risk of disease progression and tumour-related death across different stages was then assessed through univariable Cox regression analysis using Stage I-low as the reference category. In case no events were registered in one group (complete separation), stages were compared using log-rank test only.

Survival estimates for each group defined by UBo staging system were presented as medians with corresponding 95% confidence intervals (95% CI). Survival curves obtained using the Kaplan–Meier method were compared with the log-rank test. Survival rates at 6 months, 1 year and 2 years were also calculated for each stage.

Data were analysed using a commercial software (SPSS Statistics v. 26, IBM, Somers, NY). p Values ≤0.05 were considered statistically significant.

3 | Cell Line Validation Statement

No cell lines were used in the current study.

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4 | Results

4.1 | Dogs' Characteristics

A total of 226 dogs fulfilled the inclusion criteria and were ultimately included in the study. One hundred fourteen (50.4%) dogs were males, of which 41 (36.0%) were neutered, while 112 (49.6%) were females, of which 87 (77.7%) were spayed. Sixty-two (27.4%) dogs were mixed breed, while 164 (72.6%) were purebred, with Labrador Retriever ($n = 27$; 11.9%), French Bouledogue ($n = 20$; 8.8%), Boxer ($n = 17$; 7.5%), Golden Retriever ($n = 11$; 4.9%) and English Setter ($n = 9$; 4.0%) being the most frequent breeds. The median age was 9 years (range, 2–16), and the median weight was 24.5 kg (range, 1.9–58.7). One hundred and ninety-eight (87.6%) dogs had a single MCT, 22 (9.7%) dogs had two MCTs and 6 (2.7%) dogs had three MCTs. The median tumour diameter was 2.0 cm (range, 0.2–20.0).

4.2 | UBo Staging System

According to UBo staging system, 139 (65.3%) dogs were classified as T_1 and 74 (34.7%) as T_2. In the remaining 13 dogs, the diameter of the primary tumour was not known, but they were still included due to the presence of distant metastasis (Stage III).

Forty-one (18.5%) dogs had an ulcerated MCT (subcategory *u*), while 181 (81.5%) had a non-ulcerated MCT (subcategory *nu*); in the remaining 4 cases, the ulceration status was not known, but they were still included due to the presence of distant metastasis (Stage III).

Eighty-seven (41.2%) and 124 (58.8%) cases fell into categories N_0 and N_1, respectively; in the remaining 15 dogs, the lymph node status was not known, but they were still included due to the presence of distant metastasis (Stage III).

Distant metastasis (M_1) was observed at diagnosis in 32 (14.2%) dogs. Cytologically confirmed metastatic sites included liver and spleen ($n = 17$); liver ($n = 5$); spleen ($n = 3$); liver, spleen and bone marrow ($n = 3$); spleen and bone marrow ($n = 2$); liver, spleen and peritoneum ($n = 1$); and liver, spleen and lungs ($n = 1$).

One hundred forty-one (65.3%) and 75 (34.7%) dogs had histologically low grade (G_0) and high grade (G_1) MCTs, respectively; in the remaining 10 cases, the histologic grade of the primary tumour was not known, but they were still included due to the presence of distant metastasis (Stage III).

Overall, 87 (36.1%) dogs were in Stage I (I-low, $n = 75$; I-high, $n = 12$), 107 (44.4%) were in Stage II (II-low, $n = 59$; II-high, $n = 48$) and 32 (13.3%) in Stage III. There were 17 (22.7%) Substage b dogs in Stage I-low, 5 (41.7%) in Stage I-high, 30 (50.8%) in Stage II-low, 33 (68.8%) in Stage II-high and 12 of 19 (63.2%) in Stage III. The main demographic and tumour characteristics stratified by UBo stage are presented in Table 2.

4.3 | Treatment and Outcome

Stage I and II dogs underwent surgical excision of 228 MCTs (145 cutaneous and 83 subcutaneous) and simultaneous lymphadenectomy of regional or sentinel lymph nodes in 142 (62.3%) and 86 (37.7%) tumours, respectively. Margins were histologically complete and incomplete in 188 (82.5%) and 40 (17.5%) cases, respectively. Twelve (6.2%) dogs received one to two doses (median, 1) of neoadjuvant chemotherapy (vinblastine and prednisolone) prior to surgery. Stage I-high and Stage II dogs also received adjuvant chemotherapy with vinblastine ($n = 95$), toceranib ($n = 18$), vinblastine and toceranib ($n = 4$) or lomustine ($n = 2$). Stage III dogs exclusively received medical therapy with toceranib ($n = 18$); vinblastine and toceranib ($n = 7$); vinblastine ($n = 4$); vinblastine and lomustine ($n = 2$); and vinblastine, lomustine and toceranib ($n = 1$).

During follow-up, 32 (14.2%), 48 (21.2%) and 53 (23.5%) dogs developed LR/LPs, NPs and DPs, respectively. Overall, disease progression was registered in 86 (38.1%) dogs. Thirty-three (14.6%) dogs developed one or more new MCTs. At data analysis closure, 78 (34.5%) and 19 (8.4%) dogs were dead of tumour-related and -unrelated causes, respectively. Causes of MCT-related death included DP ($n = 47$; 60.3%); NP ($n = 6$; 7.7%); DP and NP ($n = 8$; 10.3%); LP and NP ($n = 7$; 8.9%); LP ($n = 6$; 7.7%); LP and DP ($n = 3$; 3.8%); and LP, NP and DP ($n = 1$; 1.3%).

The remaining 129 (57.1%) dogs were still alive, after a median follow-up time of 582 days (range, 180–2576). The 6-month, 1-year and 2-year survival rates were 85.0%, 67.2% and 47.9%, respectively. Overall, median TTP and TSS could not be estimated since the survival curves did not fall below 0.5. Information regarding the outcome, stratified by UBo stage, is presented in Table 3.

MCT diameter of ≥3 cm, tumour ulceration, histologic high-grade, HN3 nodal status and visceral metastasis at admission were all independently associated with increased risk of tumour progression and tumour-related death in the whole population (Table 4). MCT diameter of ≥3 cm and tumour ulceration were then included in a multivariable model with UBo stage, and all were found to be significantly associated with increased risk of both tumour progression and tumour-related death (Table 5).

Considering Stage I-low dogs as reference category, the risk of progression was 18.3 times higher for Stage I-high dogs (95% CI, 3.7–91.2; $p < 0.001$), 8.5 times for Stage II-low dogs (95% CI, 1.9–37.8; $p = 0.005$), 41.5 times for Stage II-high dogs (95% CI, 9.9–173.7; $p < 0.001$) and 110.3 times for Stage III dogs (95% CI, 25.4–478.8; $p < 0.001$). As no MCT-related death was recorded in Stage I-low dogs (complete separation), the log-rank test only was used to compare TSS among different UBo stages. Kaplan–Meier survival estimates of both TTP and TSS for each UBo stage are presented in Figures 1 and 2 with the relative log-rank tests' results.

When considering dogs with scMCTs as a separate category, 48 (55.8%) were in Stage I-low, 5 (5.8%) were in Stage I-high, 17 (19.8%) were in Stage II-low, 8 (9.3%) were in Stage II-high and 8 (9.3%) were in Stage III (Table 2). Overall, 20 (23.2%) dogs with scMCT experienced disease progression and 19 (22.1%) died

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TABLE 2 | Dogs and tumour characteristics in 226 dogs with cutaneous or subcutaneous mast cell tumour (MCT).

	Stage I-*low* (*n* = 75)	Stage I-*high* (*n* = 12)	Stage II-*low* (*n* = 59)	Stage II-*high* (*n* = 48)	Stage III (*n* = 32)
Sex					
Male	44 (58.7%)	7 (58.3%)	22 (37.3%)	25 (52.1%)	16 (50.0%)
Female	31 (41.3%)	5 (41.7%)	37 (62.7%)	23 (47.9%)	16 (50.0%)
Breeds with known predisposition to biologically aggressive MCT [19, 25–27]	4 (5.3%)	0 (0%)	2 (3.4%)	3 (6.3%)	3 (9.4%)
Median age (range, years)	8 (2–16)	9 (4–13)	8 (3–14)	10 (3–15)	10 (4–15)
Median weight (range, kg)	25.0 (4.5–58.7)	28.9 (5.2–38.1)	24.0 (1.9–44.5)	23.2 (4.9–50.0)	26.6 (2.9–47.0)
Tumour site					
Head and neck	10 (13.3%)	2 (16.7%)	10 (16.9%)	11 (22.9%)	9 (28.1%)
Trunk and tail	22 (29.3%)	3 (25.0%)	3 (8.5%)	3 (6.2%)	8 (25.0%)
Legs excluding digits	29 (38.7%)	3 (25.0%)	25 (42.4%)	16 (33.3%)	9 (28.1%)
Digits	3 (4.0%)	0 (0%)	2 (3.4%)	2 (4.2%)	1 (3.1%)
Mammary	6 (8.0%)	0 (0%)	8 (13.6%)	4 (8.4%)	0 (0%)
Inguinal/perineal	5 (6.7%)	4 (33.3%)	9 (15.2%)	12 (25.0%)	5 (15.7%)
Location associated with biologically aggressive MCT [19, 26, 28–30]	18 (24.0%)	6 (50.0%)	21 (35.6%)	25 (52.1%)	15 (46.9%)
MCT diameter >3 cm	16 (21.3%)	4 (33.3%)	21 (35.6%)	22 (45.8%)	11 (55.6%)[a]
Tumour ulceration	1 (1.3%)	3 (25.0%)	12 (20.3%)	20 (41.7%)	5 (21.7%)[a]
Tumour location					
Cutaneous	27 (36.0%)	7 (58.3%)	42 (71.2%)	40 (83.3%)	24 (75.0%)
Subcutaneous	48 (64.0%)	5 (41.7%)	17 (28.8%)	8 (16.7%)	8 (25.0%)
Neoadjuvant chemotherapy	4 (5.3%)	1 (8.3%)	4 (6.8%)	3 (6.3%)	NA
Incomplete surgical margins	6 (8.0%)	3 (25.0%)	12 (20.3%)	15 (31.3%)	NA

Abbreviation: NA, not applicable.
[a]Not available for all cases.

from tumour-related causes. No dog in Stage I-low experienced a tumour-related event. The UBo staging system, applied solely to scMCTs, was significantly associated with both tumour progression and tumour-related death (Table 6).

5 | Discussion

The current WHO staging system for canine MCTs presents several limitations, primarily due to the lack of integration of prognostic variables that have gained relevance over the years. Specifically, the WHO system does not take into account either the histologic grade or the nodal status, both of which have demonstrated pivotal roles in the formulation of prognosis and the informed guidance of therapeutic decisions [9, 10, 17, 19, 24, 25, 36]. Furthermore, the WHO staging includes dogs with multiple MCTs, which have an uncertain biological behaviour and are usually not submitted to surgery, thus having unknown histologic grade and nodal status [25, 37].

The UBo staging system was conceived to provide an integrated clinical-pathologic approach, aiming to obtain the most useful information for prognosis determination. The proposed staging was tested on a canine population characterized by homogeneity in terms of clinical management and histopathologic examination, as all dogs were managed by the same oncology unit and surgical samples were analysed by the same pathologist.

The system proposed in this study incorporates the diameter of the primary tumour, the presence of ulceration, histologic grade and nodal stage, variables that, both in prior studies and in the current analysis, have independently demonstrated an

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TABLE 3 | Outcome information of 226 dogs with cutaneous or subcutaneous MCT, stratified by UBo stage.

	Stage I-*low* (*n* = 75)	Stage I-*high* (*n* = 12)	Stage II-*low* (*n* = 59)	Stage II-*high* (*n* = 48)	Stage III (*n* = 32)
Median follow-up time (range, days)	530 (181–2156)	829 (537–1070)	797 (180–2818)	461 (190–1089)	NA
Local recurrence/ progression	2 (2.7%)	3 (25.0%)	5 (8.5%)	16 (33.3%)	6 (18.8%)
Nodal progression	0 (0%)	4 (33.3%)	9 (15.3%)	20 (41.7%)	15 (46.9%)
Distant progression	0 (0%)	2 (16.7%)	4 (6.8%)	15 (31.3%)	32 (100%)
New MCT development	14 (18.7%)	1 (8.3%)	14 (23.7%)	4 (8.3%)	0 (0%)
MCT-related death	0 (0%)	4 (33.3%)	10 (16.9%)	32 (66.7%)	32 (100%)
6-month survival rate	100%	83%	95%	79%	41%
1-year survival rate	100%	75%	87%	42%	16%
2-year survival rate	100%	67%	72%	24%	0%
Median TTP (95% CI, days)	Not reached	603 (range not estimable)	Not reached	230 (196–264)	113 (67–159)
Median TSS (95% CI, days)	Not reached	Not reached	Not reached	321 (243–399)	150 (127–173)

Abbreviations: MCT, mast cell tumour; NA, not applicable; TTP, time to progression; TSS, tumour-specific survival.

TABLE 4 | Multivariable analysis for risk of tumour progression and tumour-related death in 226 dogs with cutaneous or subcutaneous MCT.

	Tumour progression		Tumour-related death	
	HR (95% CI)	*p*	HR (95% CI)	*p*
MCT diameter ≥3 cm	1.8 (1.1–3.0)	0.027*	2.4 (1.4–4.2)	0.002*
Tumour ulceration	1.8 (1.1–3.0)	0.033*	2.2 (1.2–3.9)	0.008*
Histologic high grade	5.0 (2.8–9.0)	<0.001*	5.7 (2.9–11.1)	<0.001*
HN3 nodal status	3.1 (1.4–6.7)	0.004*	4.6 (1.6–13.2)	0.004*
Distant metastasis at admission	9.5 (4.0–22.2)	<0.001*	15.4 (6.0–39.4)	<0.001*

Abbreviations: CI, confidence interval; HN, histologic node; HR, hazard ratio; MCT, mast cell tumour.
*Significant.

TABLE 5 | Multivariable analysis model assessing the prognostic significance of MCT diameter and tumour ulceration in comparison to the UBo staging system.

	Tumour progression		Tumour-related death	
	HR (95% CI)	*p*	HR (95% CI)	*p*
MCT diameter ≥3 cm	1.9 (1.2–3.0)	0.0110*	2.3 (1.4–3.8)	0.002*
Tumour ulceration	1.8 (1.1–2.9)	0.021*	2.1 (1.2–3.6)	0.005*
UBo stage	3.0 (2.3–3.9)	<0.001*	3.8 (2.8–5.3)	<0.001*

Abbreviations: CI, confidence interval; HR, hazard ratio; MCT, mast cell tumour.
*Significant.

association with tumour progression and tumour-related death [4, 5, 24, 25]. Given its solid tumour nature, the authors believe that a TNM system assessing anatomic extent of the tumour (T), lymph node spread (N) and presence of distant metastases (M) would be better suited for staging canine MCT. Moreover, recent literature strongly emphasizes the importance of histologically evaluating the regional, ideally the sentinel, lymph node(s) rather than relying solely on cytology to define the nodal status and guide treatment [3, 12, 23, 27, 38–40]. In this regard, standardized histologic criteria have been proposed to ensure a more consistent characterization of nodal involvement [10]. Although the original study by Weishaar et al. [10] identified two categories of nodal metastasis (early or HN2 and overt or HN3), recent literature suggests that

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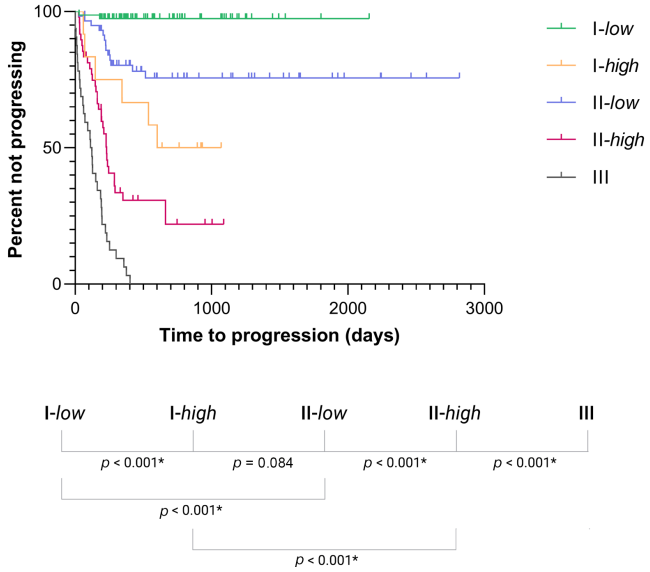

FIGURE 1 | Kaplan–Meier survival estimate of time to progression (TTP) for each UBo stage with relative log-rank tests' results.

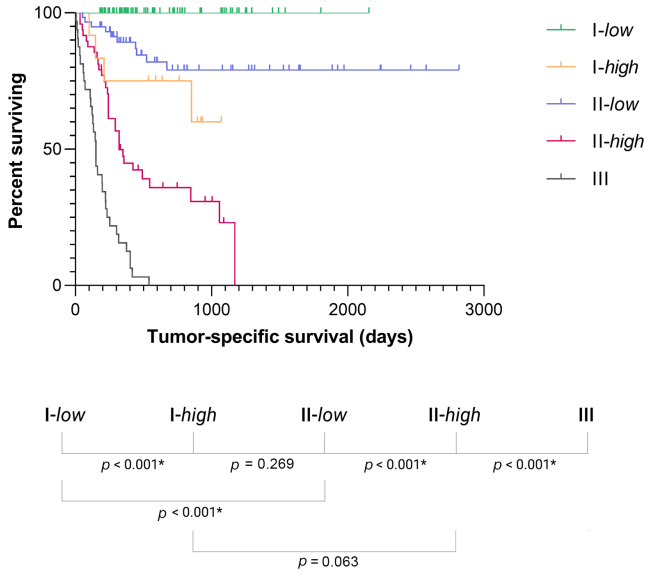

FIGURE 2 | Kaplan–Meier survival estimate of tumour-specific survival (TSS) for each UBo stage with the relative log-rank tests' results.

only HN3 dogs necessitate adjuvant medical therapy following surgery [12, 24]. For this reason, only MCTs with overt nodal metastasis have been classified as Stage II in the UBo stage. Finally, the histologic tumour grade, assessed according to Kiupel, demonstrated a robust correlation with prognosis and currently represents a straightforward and cost-effective method to differentiate between low-risk dogs curable solely through surgery and those requiring additional adjuvant therapy [9, 26]. The integration of histologic grade into the TNM system (pTNM) could thus hold clinical significance.

A novel aspect of the UBo staging system is also its applicability to both cMCT and scMCT. The latter account for a substantial subset of all canine MCTs, and several studies have underscored their variable biologic behaviour which does not always result in favourable prognoses [14–18], contrary to previous assertions [13]. Moreover, as recently demonstrated, the two-tier grading system also proves valuable in predicting the outcome of scMCTs [18]. Taken together, this provides the rationale for staging scMCTs using the same criteria applied to cMCTs.

In this study, 23% of dogs with scMCTs experienced progression, and 22% died from tumour-related causes. This finding further confirms the variable behaviour of scMCTs [16]. Despite a higher percentage of low-grade Stage 1 scMCTs compared to cMCTs, nearly half were high-grade and/or metastatic, with almost half of these progressing unfavourably. The staging proposed here is therefore effective in identifying cases with more aggressive behaviour.

Following the application of the UBo stage, not unexpectedly, dogs with a cutaneous or subcutaneous low-grade MCT and HN0–HN2 lymph node (Stage I-low) had the most favourable outcome, with a 100% survival rate at 2 years post-surgical excision. At the opposite extreme, none of the Stage III patients were alive 2 years from diagnosis, consistent with previous studies [6, 34, 41, 42].

Both in Stage I and II, the histologic grade has effectively stratified dogs into two subgroups with different outcomes. In fact, Stage I-high and Stage II-high dogs had statistically worse TTP and TSS compared to Stage I-low and Stage II-low patients, respectively. The prognostic impact of the two-tier histologic grading system in dogs with HN3 nodal status has been previously reported [24]. Overall, the prognosis for dogs in Stage II-low remained favourable despite HN3 nodal status, with survival rates at 24 months exceeding 70%, confirming the effectiveness of lymphadenectomy followed by adjuvant chemotherapy in disease control. No significant differences were observed between the outcomes of Stage I-high and Stage II-low dogs. The absence of distinction might truly reflect a similarity in the biologic behaviour of these two stages or could be attributed to the limited number of Stage I-high dogs included. Additionally, the small sample size might also account for the lack of statistical difference in TTP between Stage I-high and Stage II-high dogs, despite the significant difference in TSS.

Alongside histologic grade, nodal status and presence of visceral metastasis, tumour diameter of ≥3 cm and ulceration have emerged as negative prognostic factors independently associated with both TTP and TSS, consistent with previous literature [3, 6–8, 23, 25–28]. While these two factors are not directly utilized in defining the UBo stage, they contribute to characterizing the substage. As shown in Table 2, tumours at higher stages were more likely to be >3 cm and/or ulcerated, although even Stage I-low tumours could exhibit these features. The authors believe that while these variables alone may not justify the pursuit of adjuvant therapies, they should still be taken into account as they could indicate a more aggressive tumour behaviour. This should prompt the assessment of further prognostic markers (e.g., Ki67 and AgNOR) and warrant closer monitoring for dogs in lower stages (e.g., Stage I-low).

There are several limits that need to be acknowledged.

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TABLE 6 | Univariable regression analysis assessing the prognostic significance of the UBo staging system in 86 dogs with subcutaneous MCTs.

	Tumour progression		Tumour-related death	
	HR (95% CI)	***p***	**HR (95% CI)**	***p***
UBo stage	2.9 (2.0–4.1)	<0.001*	3.2 (2.2–4.8)	<0.001*

Abbreviations: CI, confidence interval; HR, hazard ratio; MCT, mast cell tumour.
*Significant.

First, the proposed staging system, excluding Stage III, is only applicable to dogs undergoing surgical removal of the regional or sentinel lymph node(s) along with the primary tumour. It is therefore not applicable in the initial presentation without the pathologic data related to the MCT being known. On the other hand, even in human medicine, the TNM clinical system (cTNM) has been integrated with the pathologic classification (pTNM), which includes additional data in light of the post-surgical histopathologic examination, requiring the removal of the primary tumour mass and corresponding lymph node(s) (only feasible if the patient is eligible). Relevant examples include gastric, pancreatic, mammary and colorectal cancers, among others [43–47]. In these cases, incorporating histologic grade into the TNM system resulted in improved prognostic categorization of the groups and more accurate individual prognostication [44–48].

The histologic grade was unknown for dogs with distant metastasis (Stage III). Once distant metastases are identified during the initial staging, it is challenging and perhaps unethical to propose to the owner to obtain a biopsy for confirmation of the histologic grade, since the prognosis remains poor regardless, as documented here and in previous studies [6, 34, 41].

Second, 62% of the dogs in Stages I and II underwent regional lymph node removal, and only 38% had sentinel lymph node(s) removed. Since the regional lymph node does not always coincide with the sentinel lymph node [21, 38, 39, 48], the removal of regional lymph nodes may have led to a potential underestimation in stage assignment and the possible persistence of a reservoir of neoplastic cells, resulting in a negative impact on patient prognosis. Another limitation to acknowledge is related to the fact that, in this study, the sentinel lymph node mapping was conducted by combining preoperative lymphography or lymph-CT with intraoperative methylene blue dye alone. Compared to lymphoscintigraphy or near-infrared fluorescence, these techniques are less effective in sentinel lymph node mapping since the tracers used in the preoperative and intraoperative phases differ [39, 42, 48, 49]. However, in clinical practice, they represent the mapping techniques that can be more frequently applied.

Third, although cases were managed by the same medical staff, treatments, schedule and modality of re-staging were subjected to variations due to the retrospective nature of the study, possibly impacting outcome data. Notably, the administration of neoadjuvant chemotherapy was not ground for exclusion, as it reflects a common practice in clinical settings. To consider the potential effects of downstaging obtained through neoadjuvant chemotherapy, the UBo stage was assessed at the time of surgery in these patients.

Fourth, upon documentation of progressive disease, most dogs underwent rescue treatments, including second surgery or radiation therapy in cases of LR and chemotherapy and/or tyrosine kinase inhibitors in cases of NP and/or DP. Unfortunately, these treatments were not standardized, potentially introducing a bias in the calculation of TSS.

Last, there was a limited number of patients included in certain stages. The limited number of dogs in Stage I-high is likely due to the fact that high-grade MCTs tend to metastasize early to lymph nodes [9, 10]; hence, it is rarer to find high-grade MCTs non-metastatic at admission [28].

In conclusion, a pathologic TNM staging system was implemented and validated, which includes parameters related to histologic grading and the histologic assessment of neoplastic infiltration at the nodal level. These changes reflect evolving insight into canine MCT arising from the results of numerous clinical studies. According to preliminary data, the proposed staging system enabled an accurate stratification of survival times and patient prognosis. To ascertain the true advantage of this staging method in clinical practice, it would be beneficial to conduct prospective validation studies, comparing the new staging system with the currently used WHO staging.

Acknowledgements

Open access publishing facilitated by Universita degli Studi di Bologna, as part of the Wiley - CRUI-CARE agreement.

Ethics Statement

In compliance with local legislation, ethical approval was not required for this study. Dogs were treated according to the current standards. All owners signed a written informed consent.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

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