ORIGINAL ARTICLE

Lymphadenectomy improves outcome in dogs with resected Kiupel high-grade cutaneous mast cell tumours and overtly metastatic regional lymph nodes

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Introduction: Historically, the prognosis for dogs with stage II Kiupel high-grade cutaneous mast cell tumours has been considered poor.

Objectives: The aim of this study was to explore the impact of lymphadenectomy on outcome in dogs with Kiupel high-grade cutaneous mast cell tumours and overt regional lymph node metastasis.

Material and Methods: Data from dogs with completely staged Kiupel high-grade cutaneous mast cell tumours with overt and/or certain regional lymph node metastasis undergoing excision of the primary tumours and adjuvant medical treatment were extracted. Dogs with a cytological diagnosis of regional lymph node metastasis that did not undergo lymphadenectomy were compared with dogs that underwent lymphadenectomy and had a histological diagnosis of overt lymph node metastasis.

Results: Forty-nine dogs were included, 18 did not undergo lymphadenectomy while 31 underwent lymphadenectomy. Median time to progression was significantly shorter in dogs that did not undergo lymphadenectomy (150 days, 95% confidence interval: 129 to 170) compared to the other dogs (229 days, 95% confidence interval: 191 to 266). Median survival time was also shorter in dogs that did not undergo lymphadenectomy (250 days, 95% confidence interval: 191 to 308) compared to dogs that underwent lymphadenectomy (371 days, 95% confidence interval: 311 to 430). On multivariable analysis, lack of lymphadenectomy was associated with higher risk of overall tumour progression (hazard ratio: 2.05, 95% confidence interval: 1.02 to 4.13), nodal progression (hazard ratio: 3.4, 95% confidence interval: 1.65 to 7.02) and tumour-related death (hazard ratio 3.63, 95% confidence interval: 1.72 to 7.66), whereas tumour size was associated with higher risk of local recurrence (hazard ratio: 3.61, 95% confidence interval: 1.06 to 13).

Clinical significance: Regional lymphadenectomy may improve outcome in dogs with biologically aggressive cutaneous mast cell tumours.

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INTRODUCTION

Treatment recommendations and prognosis for canine cutaneous mast cell tumours (cMCTs) are based on the combination of clinical staging and histologic grade (Patnaik *et al.* 1984, Kiupel *et al.* 2011, Blackwood *et al.* 2012, Weishaar *et al.* 2014, Lejeune *et al.* 2015, Miller *et al.* 2016, Horta *et al.* 2018, Marconato *et al.* 2018, 2020, Pizzoni *et al.* 2018).

High-grade [Kiupel high-grade (K-HG) and Patnaik grade 3 (P-G3)] cMCTs have a poorer prognosis than low-grade [Kiupel low-grade (K-LG) and Patnaik grade 1] cMCTs, due to the higher rate of recurrence and metastasis, with regional lymph nodes (RLNs) being the most commonly reported site for metastasis, occurring in 30% to 60% of dogs (Krick *et al.* 2009, Hume *et al.* 2011, Kiupel *et al.* 2011, Donnelly *et al.* 2015, Stefanello *et al.* 2015, Horta *et al.* 2018).

Current treatment recommendations for dogs with high-grade cMCTs, with or without RLN metastasis, include surgical excision of the primary tumour, with or without radiation therapy (RT), followed by systemic chemotherapy (Hayes *et al.* 2007; Hume *et al.* 2011; Blackwood *et al.* 2012; Mendez *et al.* 2020). According to the World Health Organization (WHO) clinical staging system, stage II MCT is defined as a primary single tumour confined to the dermis with nodal metastasis (Owen 1980). The prognosis for dogs with stage II, P-G3 cMCTs treated with surgical excision of the primary tumour and adjuvant systemic chemotherapy is relatively poor, with reported median survival time (mST) ranging from 142 to 194 days (Hayes *et al.* 2007; Hume *et al.* 2011).

It has been recently shown that the removal of metastatic RLNs is associated with a better outcome in canine cMCTs (Hume *et al.* 2011; Baginski *et al.* 2014; Marconato *et al.* 2018; Mendez *et al.* 2020;). Hume *et al.* (2011) showed that adequate treatment of metastatic RLN (either with surgery or RT) significantly improved survival in dogs with stage II, P-G3 MCTs, with a median survival time of 240 days.

A previous study by Marconato *et al.* (2018) reported that surgical extirpation of a metastatic lymph node [LN; early -HN2- or overt -HN3- LN metastasis according to Weishaar *et al.* 2014] alongside the resection of the primary cMCT significantly improved outcome. In the aforementioned study, dogs with both high-grade and low-grade cMCTs treated with adjuvant chemotherapy had a mean time to progression (TTP) of 1461 days and a median tumour-specific survival of 2213 days (Marconato *et al.* 2018). However, most dogs that underwent lymphadenectomy had K-LG cMCTs, and stratification according to histologic grade was not performed in the survival analysis; therefore, no further information could be specifically provided for dogs with K-HG cMCTs (Marconato *et al.* 2018).

In a more recent study, RLN removal with or without RLN bed irradiation resulted in a significant prolongation of progression-free survival (PFS) and overall survival (OS) in dogs with stage II high-grade cMCTs, with a median PFS and OS of 125 and 330 days, respectively (Mendez *et al.* 2020).

Collectively, the above data support the beneficial effect of lymphadenectomy on the outcome of dogs with stage II high-grade cMCTs; however, none of these studies has specifically focused on dogs with K-HG cMCTs and HN3 LNs.

The aim of this retrospective study was to explore the impact of lymphadenectomy as part of the primary tumour surgery on TTP and ST in dogs with K-HG cMCT and overt (HN3)/certain RLN metastasis while also receiving adjuvant medical treatment as part of their treatment.

MATERIAL AND METHODS

Study design and study population

We performed a multi-institutional retrospective cohort study. The electronic medical records of four European institutions (University of bologna (Italy); Department of small animal clinical science, university of liverpool (UK); Centro veterinario torinese, Turin (Italy); Clinica veterinaria tibaldi, Milan (Italy)) were searched retrospectively to identify dogs with firstly occurring, treatment-naïve, histologically confirmed K-HG cMCT with certain and/or overt RLN metastasis confirmed either by cytology (Krick *et al.* 2009) or histology (Weishaar *et al.* 2014), between July 1, 2014 and July 21, 2021. Medical records were searched by four operators independently doing the same investigation. Searched terms used included "dog", "cutaneous MCTs", "Kiupel high-grade", "nodal or LN metastasis", "certain nodal or LN metastasis", "lymphadenectomy, and "overt/HN3 nodal or LN metastasis".

The RLN was defined as the LN draining the anatomical region surrounding the cMCT, and was identified by palpation, ultrasound or surgical exploration.

Inclusion criteria

For this study, dogs were only included if histopathology or cytology confirmed overt (HN3;) or certain RLN metastasis, respectively, of at least one RLN (Krick *et al.* 2009, Weishaar *et al.* 2014). Overt nodal metastasis (HN3) was histologically defined as the disruption or effacement of normal nodal architecture by discrete foci, nodules, sheets of overt masses of mast cells (Weishaar *et al.* 2014); whereas cytologically certain metastasis was defined as the effacement of lymphoid tissue by mast cells, and/or the presence of aggregated, poorly differentiated mast cells with pleomorphism, anisocytosis, anisokaryosis and/or decreased or variable granulation, and/or greater than five aggregated for more than three mast cells (Krick *et al.* 2009). Primary cMCTs and LNs were histologically evaluated by multiple board-certified pathologists and slides were not reviewed.

In addition, dogs were eligible for inclusion if they underwent complete clinical staging, surgical excision of the primary cMCT and adjuvant medical treatment. Furthermore, a follow-up of at least 4 months from surgery had to be available. Dogs that had disease progression or were dead due to tumour-related causes within 4 months from surgery were included in the analysis. Follow-up information was collected from the clinical records of each institution.

Dogs with subcutaneous or multiple MCT/s, and/or with stage IV disease at the time of diagnosis, were excluded from the study. Dogs treated with radiotherapy were also excluded.

Staging and treatment

Clinical staging included haematological and biochemical analysis, cytological evaluation of the primary cMCT and RLN; thoracic radiographs (three views), abdominal ultrasound and fine-needle aspiration (FNA) of liver and spleen regardless of their sonographic appearance.

Adjuvant medical treatment consisted of vinblastine [(Velbe; EuroGenerici) 2 to 3 mg/m^2 intravenously (iv) every 2 weeks for a total of eight doses] and prednisolone [(Prednicortone; Dechra) 1 mg/kg orally once daily for the duration of the protocol], toceranib phosphate [(Palladia; Zoetis) 2.4 to 2.8 mg/kg orally on Monday, Wednesday, Friday schedule for 6 months] or both [vinblastine (1.6 mg/m^2 iv every 2 weeks for a total of eight doses) and toceranib phosphate (2.4 to 2.8 mg/kg orally on Monday, Wednesday, Friday for the duration of the course)]. Dogs also received additional medications during their treatment for prophylactic management of paraneoplastic conditions associated with cMCTs, consisting of chlorpheniramine [(Chlorphenamine; Crescent) 0.2 to 0.5 mg/kg orally twice daily] and ranitidine [(Zantadine; CEVA) 2 to 4 mg/kg orally twice daily].

For MCTs located on either the trunk, proximal part of the limb, inguinal/perineal region, head and neck and mammary region, excision of the primary tumour included at least 2 cm of macroscopically normal tissue around the tumour and at least one deep fascial plan; for MCTs located on the distal region of the limb, a maximum of a deep subcutaneous tissue was removed en bloc with the mass and when possible a reconstructive surgery (*e.g.* axial flap) was performed to close large lateral skin defects. Finally, for digit MCTs, digit amputation was performed.

To evaluate the impact of lymphadenectomy on outcome, the following categories of dogs were created: dogs that had no lymphadenectomy but underwent FNA of the RLN/s with a cytological diagnosis of metastasis; dogs that underwent lymphadenectomy and had a histological diagnosis of HN3 LN. The decision on whether to perform lymphadenectomy of the metastatic RLN was made at the personal discretion of each clinician, as well as the number of LNs sampled or excised when more than one LN was assessed by cytology or histology, respectively.

Data extracted

For each case, the following data were recorded: breed, sex, age and weight at presentation, clinical substage (a or b); cMCT anatomic site, size and presence of ulceration; size (recorded as either normal or enlarged, based on physical examination or diagnostic imaging findings), site and number of evaluated RLNs; histologic or cytological results of all excised or sampled LNs, respectively; date of surgery; intra- and postoperative severe complications (severe complications were defined as those that required additional medical treatment and/or surgical revision to resolve; only for dogs in that underwent lymphadenectomy), histopathologic evaluation of surgical margins [complete, clean but close (tumour cells extending within 1 mm of any cut margins) incomplete]; Ki-67 index/ KIT pattern/c-kit mutational status (if performed); adjuvant medical treatment [cytotoxic chemotherapy; tyrosine kinase inhibitors (TKIs) or both].

To evaluate the impact of lymphadenectomy on TTP and ST, the following information was also retrieved: local recurrence (defined as cMCT relapse at or within 2 cm of the surgical scar, confirmed by cytology), nodal progression (defined as nodal progressive disease according to RECIST criteria for dogs in which lymphadenectomy was not performed; Nguyen *et al.* 2015, or the presence of new metastatic LNs for dogs that undergo lymphadenectomy); distant progression (defined as the occurrence of cytologically confirmed metastasis at distant organs); date of death or last follow-up examination and cause of death.

Statistical analysis

Descriptive statistic was used in the analysis of dogs and tumour characteristics. Data were tested for normality by use of Shapiro–Wilk normality test. All tested values were not normally distributed and therefore were expressed as median (range).

The chi-squared test/Fisher exact probability test (categorical variables), and the Mann-Whitney U test (continuous variables) were applied to evaluate differences in demographic features and possible prognostic factors between groups A and B. The considered variables included breed [predisposition to biologically aggressive MCTs (*i.e.*, Labrador retriever, golden retriever, Shar pei) *versus* others; Dobson & Scase 2007], sex (male *versus* female), age, bodyweight, anatomic location of the primary cMCT (sites associated with a worse prognosis, *i.e.*, head and neck, inguinal/perineal region, scrotal, digital, mammary *versus* sites associated with a better prognosis, *i.e.*, trunk, limbs excluding digital; Blackwood *et al.* 2012; Pizzoni *et al.* 2018), macroscopic tumour longest diameter (>3 *versus* ≤3 cm; Mendez *et al.* 2020), ulceration (yes *versus* no), substage (a *versus* b), Patnaik grading (P-G2 *versus* P-G3). For age and weight, the median was used as a cut-off value.

The influence of potential prognostic variables on TTP and ST was investigated with univariable and multivariable Cox's regression analyses. All variables associated with outcome with a P-value ≤0.1 at univariable analysis were selected for multivariable analysis.

Outcome was reported as time to local recurrence, calculated from the date of surgery to the date of local recurrence; time to nodal progression, calculated from the date of surgery to the date of nodal progression; time to distant progression, calculated from the date of surgery to the date of diagnosis of distant metastasis; TTP, calculated from the date of surgery to the first occurrence of at least one of the following: local recurrence, nodal progression or distant metastasis; ST, calculated from the date of surgery to the date of death or to the date of the last visit if death did not occur. Only dogs deceased for cMCT-related causes were considered as events. Dogs with no disease progression, still alive or dead for MCT-unrelated causes at the time of data closure were censored from the respective statistical analysis.

Survival plots were generated according to the Kaplan–Meier product-limit method. Survival estimates were presented as medians with the corresponding 95% confidence intervals (95% CIs). TTP and ST of both groups obtained with the Kaplan–Meier method were compared by use of the log-rank test.

Statistical analysis was performed with SPSS Statistics v.25 (IBM). Significance was set at P<0.05.

RESULTS

Patient data and tumour characteristics

The electronic medical records search identified 60 dogs potentially suitable for the study. Six dogs were excluded as they had multiple MCTs at presentation and five were excluded due to lack of follow-up information.

A total of 49 dogs were eventually included in the study: 18 dogs did not undergo lymphadenectomy and 31 underwent lymphadenectomy. No significant difference was found among the two groups with respect to demographic features and possible outcome variables, apart from medical treatment (Table 1), as dogs that did not undergo lymphadenectomy were treated more often with TKIs with or without systemic cytotoxic chemotherapy.

Dogs that did not undergo lymphadenectomy

Among the 18 dogs that did not undergo lymphadenectomy, there were nine (50%) females (of which six spayed), and nine (50%) males (of which seven were neutered). At the time of diagnosis, the median age was 10 years (range, 0.5 to 13), and the median weight was 22.8 kg (range, 2.5 to 39). Represented breeds included: mixed breed (n=7; 38.9%), Labrador retriever (n=4; 22.2%), golden retriever (n=2; 11.2%), boxer (n=2; 11.2%) and one (5.5%) each of Dobermann, American Staffordshire terrier and West Highland White terrier.

All dogs were asymptomatic at presentation (substage a).

The most common primary tumour location was trunk (n=6; 33.3%), followed by limbs (n=4; 22.2%), inguinal/perineal region (n=3; 16.7%), head and neck (n=2; 11.1%), digital (n=2; 11.1%) and mammary region (n=1; 5.6%).

Data on tumour diameter were available for 17 dogs. Median tumour diameter was 2 cm (range, 1 to 4.5). At presentation, six (33.3%) tumours were ulcerated.

Four (22%) dogs had normal-sized RLNs, whereas 14 (78%) dogs had an enlarged RLN.

Metastatic RLNs included inguinal (n=6; 33.3%), popliteal (n=4; 22.2%), superficial cervical (n=3; 16.7%), axillary (n=3; 16.7%) and mandibular (n=2; 11.1%) LN.

Based on histopathology reports, there were 11 (61.1%) K-HG/P-G3 cMCTs, and seven (38.9%) K-HG/P-G2 cMCTs. Surgical margins were complete in 11 (61.1%) cMCTs, clean but close in three (16.7%) cases and incomplete in four (22.2%) cases.

Ki67 immunohistochemistry was available for five (27.8%) cases. Ki67 score ranged from 2% to 23%. KIT staining pattern

Table 1. Demographic information and distribution of variables potentially associated with prognosis in 49 dogs with Kiupel high-grade mast cell tumours and metastatic regional lymph nodes. Differences in data distribution were assessed with chi-squared test/Fisher's exact test (categorical variables) or Mann-Whitney U test (continuous variables)

Variable	Dogs that did not undergo lymphadenectomy (n=18)	Dogs that underwent lymphadenectomy (n=31)	P value
Breeds predisposed to biologically aggressive cMCT			
Yes	6	4	0.087
No	12	27	
Sex			
Male	9	17	0.744
Female	9	14	
Age (years)			
Median (range)	10 (1 to 13)	10 (5 to 19)	0.875
Weight (kg)			
Median (range)	22.8 (2.5 to 39.0)	23.0 (4.9 to 55.0)	0.852
Substage			
a	18	27	0.112
b	0	4	
Anatomic location			
Trunk, limbs	10	18	0.864
Others	8	13	
Diameter			
Less than 3 cm	11	20	0.990
At least 3 cm	6	11	
Ulceration			
Yes	6	12	0.707
No	12	19	
Patnaik grading			
2	7	8	0.338
3	11	23	
Surgical margins			
Complete	14	27	0.395
Incomplete	4	4	
Treatment			
Chemotherapy	13	30	0.034*
Chemotherapy + TKI	3	1	
TKI	2	0	

cMCT cutaneous mast cell tumour, TKI tyrosine kinase inhibitors
*Significant

was available for six (33.3%) cases: two cMCTs had pattern III, two had pattern II and two had pattern I. Mutational analysis was available for 12 (66.7%) cMCTs: two had an ITD on exon 11, one had ITD on exon 8, and nine were wild type.

Dogs that underwent lymphadenectomy

Among the 31 dogs undergoing lymphadenectomy, there were 17 males (of which nine were neutered) and 14 females (of which 11 were spayed). At the time of diagnosis, the median age was 10 years (range, 5 to 15), and the median weight was 23 kg (range, 4.9 to 55). Represented breeds included: mixed breed (n=10; 32.2%), miniature Pinscher (n=4; 12.9%), cane corso (n=3; 9.7%), golden retriever (n=2; 6.45%) and one (3.2%) each of Labrador retriever, Shar pei, American Staffordshire terrier, bichon, Bernese Mountain dog, Dobermann, German shepherd, Jack Russell terrier, Weimaraner, Maltese terrier and Griffon.

Four (12.9%) dogs showed clinical signs (n=2 pruritus, n=1 vomiting, n=1 diarrhoea; substage b) at presentation. The most common primary tumour location was limb (n=11; 35.5%), followed by trunk (n=8; 25.8%), inguinal/perineal region (n=6; 19.3%), head and neck (n=3; 9.7%) and digits (n=3; 9.7%). Median tumour diameter was 3 cm (range, 2 to 4.2). At presentation, 12 (38.7%) tumours were ulcerated.

Six (19.4%) dogs had normal-sized RLNs, whereas 25 (80.6%) dogs had an enlarged RLN.

A total of 52 RLNs were removed, including inguinal (n=16; 30.8%), superficial cervical (n=14; 26.9%), axillary (n=10; 19.2%), popliteal (n=6; 11.5%), mandibular (n=3; 5.9%), retropharyngeal (n=2; 3.8%) and medial iliac (n=1; 1.9%) LN. In 14 (57.9%) dogs, one LN was removed; in 15 (31.6%) dogs, two LNs were removed; and in two (7.9%) dogs, four LNs were removed.

Concerning the HN3 LNs, 30 dogs had one RLN classified as HN3, while one dog had two RLNs classified as HN3. Among the remaining 20 extirpated LNs, there were eight HN2, seven HN1 and five HN0.

Based on histopathology reports, there were 23 (74.2%) K-HG/P-G3 cMCTs, and eight (25.8%) K-HG/P-G2 cMCTs. Surgical margins were complete in 22 (71%) cMCTs, clean but close margins in five (16.1%) cases, and incomplete in four (12.9%) cases.

Ki67 immunohistochemistry was available for four (12.9%) cases. Ki67 score ranged from 9% to 29%. KIT staining pattern was available for four (12.9%) cases: three had pattern II, and one case had pattern I. Mutational analysis was available for 13 (41.9%) cMCTs: six had an ITD on exon 11, one had ITD on exon 8 and six were wild type.

Treatment and outcome

Dogs that did not undergo lymphadenectomy

All dogs received adjuvant medical treatment. Among them, 13 (72.2%) were treated with systemic chemotherapy consisting of vinblastine and prednisone, three (16.7%) with toceranib alone and two (15.4) with both. Among the five dogs treated with toceranib alone or in combination with vinblastine, two had an ITD mutation on exon 11 and one dog had an ITD mutation on exon 8.

All dogs developed disease progression. Of those, eight (44.5%) experienced local recurrence after a median of 170 days (range, 60 to 511); three of these eight dogs had their cMCT removed with incomplete surgical margins. All (100%) dogs experienced nodal progression after a median of 148 days (range, 30 to 511) and seven (39%) dogs developed distant metastasis after a median of 180 days (range, 72 to 205).

Median TTP was 150 days (95% CI, 129 to 170 days; Fig 1). Four (22%) dogs received additional medical treatment at the time of disease progression: one dog received lomustine ([Lomustine; medac] 70 mg/m^2 orally every 4 weeks) and prednisolone, and three dogs received toceranib.

At the end of the study, all dogs had died because of cancer-related (n=17; 92%) or unrelated (n=1; 8%) causes. The latter dog died due to gastric dilation volvulus after 140 days.

Median ST was 250 days (95% CI, 311 to 430 days; Fig 2).

Dogs that underwent lymphadenectomy

Lymphadenectomy was well tolerated in all cases and no major complications were reported. Thirty (97%) dogs were treated with systemic chemotherapy consisting of vinblastine and prednisolone, while one dog (3%) was treated with vinblastine and toceranib. The latter dog had an ITD mutation on exon 11. Overall, 17 (54.8%) dogs developed progressive disease. Of those, eight (25.8%) dogs experienced local recurrence after a median of 218 days (range, 160 to 536); two of these eight dogs had their cMCT removed with incomplete surgical margins. Thirteen (41.9%) dogs experienced nodal relapse after a median of 228 days (range, 97 to 287) and 12 (38.7%) dogs developed distant metastasis after a median of 267 days (range, 120 to 371). Median TTP was 229 days (95% CI, 191 to 266 days; Fig 1).

Six (35%) dogs received additional medical treatment at the time of disease progression: two dogs received lomustine (60 mg/

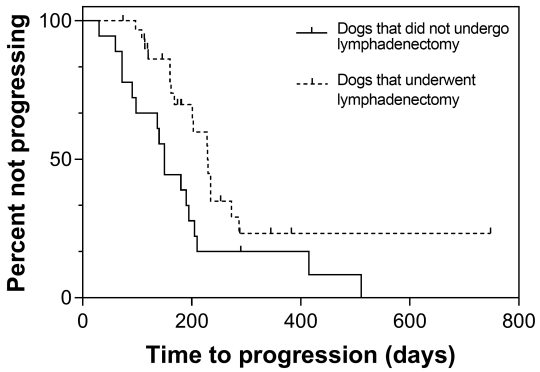

FIG 1. Time to progression for dogs with Kiupel high-grade cutaneous mast cell tumours treated by surgical excision of the primary tumour with or without lymphadenectomy, and adjuvant medical treatment. Median time to progression for dogs that underwent lymphadenectomy was significantly longer than median time to progression for dogs that did not undergo lymphadenectomy (229 days *versus* 150 days, respectively; P<0.001)

m^2 orally every 4 weeks and 70 mg/m^2 orally every 4 weeks, respectively) and prednisolone and four dogs received toceranib.

At data analysis closure, 13 (41.9%) dogs were alive, with a median follow-up of 180 days (range, 123 to 594), while 18 (58.1%) dogs had died because of cancer-related (n=15; 48.4%) or unrelated (n=3; 9.7%) causes. Two dogs died due to acute pancreatitis, and one dog due to heart failure.

Median ST was 371 days (95% CI, 311 to 430 days; Fig 2).

Analysis of outcome and prognostic variables

Median TTP for dogs that underwent lymphadenectomy (229 days, 95% CI 191 to 266 days) was significantly longer than median TTP for dogs in which lymphadenectomy was not performed (150 days, 95% CI 129 to 170 days, P<0.001; Fig 1).

Median ST for dogs that underwent lymphadenectomy (371 days, 95% CI 311 to 430 days) was significantly longer than median ST for dogs in which lymphadenectomy was not performed (250 days, 95% CI, 191 to 308 days, P=0.001, Fig 2).

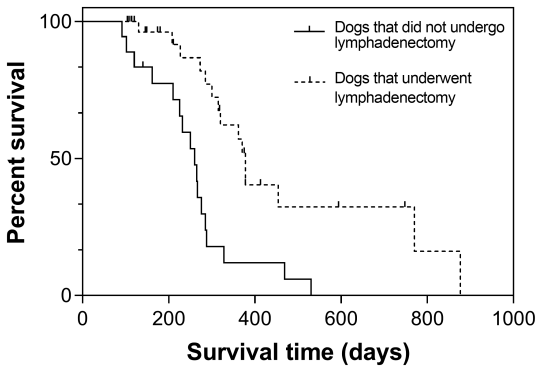

FIG 2. Survival time for dogs with Kiupel high-grade cutaneous mast cell tumours treated by surgical excision of the primary tumour with or without lymphadenectomy, and adjuvant medical treatment. Median survival time for dogs that underwent lymphadenectomy was significantly longer than median survival time for dogs that did not undergo lymphadenectomy (371 days *versus* 250 days, respectively; P=0.001)

Lack of lymphadenectomy was the only variable associated with a higher risk of overall tumour progression both in univariable [hazard ratio (HR): 2.19, 95% CI: 1.11 to 4.33; P=0.024] and multivariable (HR: 2.05, 95% CI: 1.02 to 4.13; P=0.043) analyses (Tables 2 and 3).

When recurrence/progression characteristics were evaluated separately, tumour diameter of more than 3 cm (HR: 5.53, 95% CI: 1.73 to 17.72; P=0.004) and incomplete surgical margins (HR: 3.99, 95% CI: 1.38 to 11.57; P=0.011) were associated with a higher risk of local recurrence on univariable analysis (Table 2). Lack of lymphadenectomy was the only variable significantly associated with a higher risk of nodal progression (HR: 3.40, 95% CI: 1.65 to 7.02; P<0.001), while none of the evaluated prognostic variables was associated with an increased risk of distant progression (Table 2).

On multivariable analysis, only tumour diameter of more than 3 cm remained significant for local recurrence (HR: 3.61, 95% CI: 1.06 to 13; P=0.041; Table 4).

Lack of lymphadenectomy was the only variable associated with a higher risk of tumour-related death both in univariable (HR: 3.57, 95% CI: 1.70 to 7.48; P=0.001) and multivariable (HR: 3.63, 95% CI: 1.72 to 7.66; P=0.001) analyses (Tables 5 and 6).

DISCUSSION

In the current study, it was documented that dogs with K-HG cMCTs undergoing lymphadenectomy of HN3 LN as part of their primary surgery and adjuvant medical treatment had a significant improvement in TTP and ST compared with those in which the metastatic LN was not excised. These findings further

Table 2. Univariable Cox regression analysis of variables potentially associated with increased risk of tumour progression, local recurrence, nodal progression and distant progression in 49 dogs with Kiupel high-grade mast cell tumours and metastatic regional lymph nodes

Variable	Tumour progression		Local recurrence		Nodal progression		Distant progression	
	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P	Hazard Ratio (95% CI)	P value	Hazard Ratio (95% CI)	P value
Breed predisposed to biologically aggressive cMCT	1.93 (0.89 to 4.19)	0.098	1.36 (0.44 to 4.27)	0.595	1.33 (0.56 to 3.15)	0.513	1.93 (0.65 to 5.78)	0.238
Female sex	0.96 (0.49 to 1.91)	0.900	1.23 (0.46 to 3.30)	0.683	1.05 (0.52 to 2.13)	0.897	0.76 (0.30 to 1.95)	0.569
Weight, atleast 23 kg	1.18 (0.59 to 2.38)	0.642	1.07 (0.40 to 2.86)	0.900	0.71 (0.35 to 1.45)	0.345	0.70 (0.28 to 1.74)	0.437
Age, at least 10 years	1.11 (0.55 to 2.24)	0.773	1.61 (0.54 to 4.83)	0.393	0.02 (0.49 to 2.13)	0.956	0.56 (0.22 to 1.42)	0.226
Substage b	0.85 (0.26 to 2.80)	0.785	1.09 (0.24 to 4.89)	0.915	1.05 (0.32 to 3.47)	0.940	0.85 (0.20 to 3.68)	0.824
Biologically aggressive anatomic location	1.10 (0.56 to 2.19)	0.778	0.84 (0.30 to 2.33)	0.738	0.90 (0.44 to 1.85)	0.779	0.82 (0.32 to 2.08)	0.670
Tumour diameter, more than 3 cm	1.75 (0.88 to 3.48)	0.113	5.53 (1.73 to 17.72)	0.004*	1.05 (0.50 to 2.20)	0.895	1.35 (0.54 to 3.34)	0.522
Ulceration	1.50 (0.76 to 2.96)	0.244	1.52 (0.55 to 4.20)	0.422	1.03 (0.50 to 2.13)	0.939	1.16 (0.47 to 2.88)	0.754
Incomplete surgical margins	2.27 (0.95 to 5.44)	0.066	3.99 (1.38 to 11.57)	0.011*	1.98 (0.79 to 4.95)	0.145	0.75 (0.17 to 3.36)	0.706
Patnaik grade 3	1.40 (0.65 to 3.03)	0.395	3.92 (0.89 to 17.27)	0.071	0.87 (0.40 to 1.87)	0.721	1.70 (0.56 to 5.14)	0.350
Lack of regional lymphadenectomy	2.19 (1.11 to 4.33)	0.024*	2.67 (0.96 to 7.42)	0.060	3.40 (1.65 to 7.02)	<0.001*	1.25 (0.49 to 3.21)	0.640

CI confidence interval, cMCT cutaneous mast cell tumour
*Significant

Table 3. Multivariable Cox regression analysis for risk of tumour progression in 49 dogs with Kiupel high-grade mast cell tumours and metastatic regional lymph nodes. Variables with a significance level of P≤0.1 at univariable analysis were included in the model

Variable	Tumour progression	
	Hazard ratio (95% CI)	P
Breed predisposed to biologically aggressive cMCT	2.02 (0.91 to 4.47)	0.083
Incomplete surgical margins	1.97 (0.80 to 4.80)	0.139
Lack of regional lymphadenectomy	2.05 (1.02 to 4.13)	0.043*

CI confidence interval, cMCT cutaneous mast cell tumour
*Significant

Table 4. Multivariable Cox regression analysis for risk of local recurrence in 49 dogs with Kiupel high-grade mast cell tumours and metastatic regional lymph nodes. Variables with a significance level of P≤0.1 at univariable analysis were included in the model

Variable	Local recurrence	
	Hazard ratio (95% CI)	P value
Tumour diameter, more than 3 cm	3.61 (1.06 to 13.00)	0.041*
Incomplete surgical margins	3.01 (0.96 to 9.46)	0.060
Patnaik grade 3	1.80 (0.34 to 9.64)	0.490
Lack of regional lymphadenectomy	2.13 (0.70 to 6.43)	0.181

CI confidence interval
*Significant

Table 5. Univariable Cox regression analysis of variables potentially associated with increased risk of tumour-related death in 49 dogs with Kiupel high-grade mast cell tumours and metastatic regional lymph nodes

Variable	Tumour-related death	
	Hazard ratio (95% CI)	P value
Breed predisposed to biologically aggressive cMCT	1.23 (0.52 to 2.93)	0.638
Female sex	0.74 (0.35 to 1.56)	0.430
Weight, at least 23 kg	0.80 (0.39 to 1.65)	0.540
Age, at least 10 years	0.52 (0.24 to 1-11)	0.091
Substage b	0.94 (0.28 to 3.15)	0.925
Biologically aggressive anatomic location	0.88 (0.43 to 1.79)	0.717
Tumour diameter, more than 3 cm	1.39 (0.67 to 2.90)	0.376
Ulceration	1.23 (0.60 to 2.54)	0.576
Incomplete surgical margins	1.90 (0.80 to 4.53)	0.149
Patnaik grade 3	1.06 (0.48 to 2.31)	0.892
Lack of regional lymphadenectomy	3.57 (1.70 to 7.48)	0.001*

*Significant
CI confidence interval, cMCT cutaneous mast cell tumour

Table 6. Multivariable Cox regression analysis for risk of tumour-related death in 49 dogs with Kiupel high-grade mast cell tumours and metastatic regional lymph nodes. Variables with a significance level of P≤0.1 at univariable analysis were included in the model

Variable	Tumour-related death	
	Hazard ratio (95% CI)	P value
Age, at least 10 years	0.50 (0.23 to 1.09)	0.083
Lack of regional lymphadenectomy	3.63 (1.72 to 7.66)	0.001*

CI confidence interval
*Significant

support the therapeutic benefit of lymphadenectomy, also in the face of biologically aggressive cMCTs.

It is widely accepted that canine cMCTs metastasise in a stepwise manner from the primary tumour to the draining LN/s and then systemically to distant sites (Warland *et al.* 2014). Accordingly, the LN involvement is of prognostic importance not only because it indicates a more aggressive tumour behaviour, but also because persistent neoplastic cells in LN/s can be the source of subsequent metastases, as proposed by the "Halstedian" theory (Halsted 1907). Considering the above, a reasonable explanation for the beneficial effects of metastatic LN dissection includes the reduction of tumour burden and the elimination of a potential source of neoplastic cells which could result in further spread and fatal outcome (Halsted 1907, Kawada & Taketo 2011).

To better define the impact of lymphadenectomy on TTP, we also evaluated separately recurrence/progression characteristics between the two groups. Lack of lymphadenectomy was the only variable significantly associated with a higher risk of nodal progression. These results were not surprising since dogs in which lymphadenectomy was not performed had persistent metastatic nodal disease, most likely representing the source of the subsequent nodal progression.

Tumour diameter was the only variable significantly associated with an increased risk of local recurrence on multivariable analysis. Dogs with tumour diameter of more than 3 cm had an increased risk of local recurrence regardless of histologic margins. This result is in agreement with a previous study in which dogs with P-G3 MCTs greater than 3 cm were at higher risk of local recurrence, despite complete surgical margins (Hume *et al.* 2011). It is important to note that in the aforementioned study, as well as in the current study, the exact technique of surgical trimming, as well as the number of sections of surgical margins evaluated in each case, was not reported. The impact of the specimen trimming technique on margin evaluation has been previously reported (Dores *et al.* 2017, Liptac 2020). It has been shown that tangential sectioning detected more incomplete surgical margins than radial sectioning because the former evaluates a considerably greater percentage of the total margin surface area (Dores *et al.* 2017). Moreover, it could be hypothesised that K-HG cMCTs greater than 3 cm are associated with more infiltrative growth patterns. In these cases, radial sections might be expected to have even poorer precision in detecting incomplete surgical margins (Dores *et al.* 2017). Thus, it is possible that the number of surgical margins determined to be complete in the current study as well as in the Hume study was overestimated, thereby skewing the results. Further studies are required to establish the impact of the trimming technique, tumour size and histologically free-surgical margins on local recurrence in dogs with K-HG MCTs.

None of the other evaluated variables, including lack of lymphadenectomy, was significantly associated with an increased risk of developing distant metastasis. There are some potential explanations for this result: first, since all dogs included in this study had biologically aggressive cMCTs, it is possible that, at least in some cases, the metastatic cascade had already been initiated, but

was not detectable at the time of staging. If this was the case, lymphadenectomy may have not disrupted the metastatic cascade, but it may have contributed to slowing down the metastatic progression. Secondly, although all dogs in group B underwent lymphadenectomy of at least one overtly metastatic LN, none of them underwent sentinel LN (SLN) mapping. Thus, it is possible that not all SLNs were removed, potentially leaving a source of neoplastic cells which could then spread to distant sites (Wong & Hynes 2006, Kawada & Taketo 2011). Moreover, due to the retrospective nature of this study, the number of LNs excised was not standardised. Indeed, most dogs that underwent lymphadenectomy had only one HN3 LN removed. Since lymphocentra may contain more than one LN, it is possible that some metastatic LNs were left behind and spread to distant organs (Wong & Hynes 2006; Kawada & Taketo 2011; Suami *et al.* 2013).

The current work has several limitations. First, despite performing a multi-institutional study, inclusion criteria were strict, resulting in a total population of 49 dogs only. Secondly, the retrospective nature of this study did not allow for obtaining information regarding Ki67 index, KIT-pattern and c-kit mutational status in all cases, which might have provided further relevant prognostic information.

Thirdly, although all dogs received adjuvant medical treatment, protocols were not standardised, rather the choice of the protocol and dosage were left to the primary clinician, making comparison of the effect of medical treatment on outcome more challenging. Furthermore, decisions regarding whether to perform lymphadenectomy were made according to each clinician's description or owner preferences, rather than random allocation.

Fourthly, primary cMCTs and LNs were histologically evaluated by multiple pathologists and slides were not reviewed, potentially affecting study results. Nevertheless, both Kiupel and Weishaar schemes are well described and widely used by pathologists worldwide, as they both rely on reproducible criteria. Additionally, Kiupel's grading system has been proven to have a high interobserver agreement (Kiupel *et al.* 2011).

Finally, even though lymphadenectomy was well tolerated in all cases, it must be pointed out that most dogs underwent lymphadenectomy of one peripheral LN. It is possible that the dissections of a higher number of LNs or the removal of intracavitary LNs might be associated with an increased incidence of postoperative morbidity.

In conclusion, the present study showed that lymphadenectomy along with the resection of the primary tumour and adjuvant medical treatment improves outcome for dogs with K-HG cMCTs and overt nodal metastasis. The findings of the current study provide additional support for the therapeutic role of lymphadenectomy and further insight into the management of stage II Kiupel high-grade cMCTs. Further prospective studies are warranted to explore the effect of surgical extirpation of metastatic SLN and the number of LNs removed on outcome in dogs with K-HG cMCTs.

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Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Ethics statement

Ethical approval was requested and provided by the University of Liverpool Veterinary School Research Ethics Committee (VREC1010).

Data Availability statement

As per the data sharing policy, data associated with this paper are available.

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