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ORIGINAL ARTICLE

The impact of extirpation of non-palpable/normal-sized regional lymph nodes on staging of canine cutaneous mast cell tumours: A multicentric retrospective study

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Metastasis to regional lymph nodes (RLNs) in dogs with cutaneous mast cell tumour (cMCT) has been correlated with shortened survival time and higher risk of spread to distant sites. In the present study, extirpation of non-palpable or normal-sized RLNs was included in the surgical management of cMCT in dogs. Correlations between histological nodal status (HN0-3) and tumour variables were analysed. Ninety-three dogs with single cMCT without distant metastasis that underwent wide surgical excision of the primary tumour and extirpation of non-palpable or normal-sized RLN were included. The association between HN (HN0 vs HN > 0; HN0-1 vs HN2-3) and tumour variables (site, longest diameter, ulceration, 3-tier and 2-tier histological grades) was analysed by a generalized linear model with multinomial error. Then, 33 (35.5%) RLNs were HN0, 14 (15%) were HN1, 26 (28%) were HN2 and 20 (21.5%) were HN3. The presence of positive (HN > 0) RLN was significantly associated with cMCT larger than 3 cm. No other association was statistically significant. Non-palpable/normal-sized RLN in dogs with cMCT can harbour histologically detectable metastatic disease in nearly half of the cases. Extirpation of the RLN should always perfomed to obtain a correct staging of the disease, even in the absence of clinical suspicion of metastasis. Further studies should evaluate the possible therapeutical effect of the tumour burden reduction obtained by exrtipartion of a positive RLN.

KEYWORDS

dogs, lymph node excision, lymphatic metastasis, mastocytoma, neoplasm staging

1 | INTRODUCTION

Lymph node (LN) metastasis is a well-known negative prognostic indicator in canine cutaneous mast cell tumours (cMCTs).[1–9] The presence of LN metastasis implies a higher risk of distant spread and the need for adjuvant chemotherapy, regardless of the characteristics of the primary tumour, such as histological grade and proliferation indexes.[9] Needless to say, an early detection of nodal metastasis is crucial for prompt and adequate therapeutic proposal, as well as for a correct staging and prognostication. It is accepted that palpation has a limited value in predicting LN metastasis in cMCT[10–12]; similarly, cytology has been associated with a high proportion of both false positive and negative results.[13] Furthermore, not all regional lymph nodes (RLNs) are feasible for immediate fine-needle aspiration because of their anatomical location or size.[14–16] Histopathology remains the gold standard for the diagnosis of RLN metastasis,[10] but the role of lymphadenectomy of non-palpable or normal-sized LNs in increasing diagnostic accuracy and delineating prognosis in canine cMCT has not been reported yet. Notably, some authors have recently explored the utility of some diagnostic and surgical procedures in an attempt to remove regional or sentinel LNs that were not clinically suspected for metastasis in cMCTs and other canine malignancies in order to obtain an early detection. [15,17–20]

Due to inconsistency in LN sampling inside the enrolled population, selection of different inclusion criteria for the study population (eg, high-risk cMCT or Patnaik grade II cMCT only) and different sampling methods (cytology vs histology) within and among studies, the exact rate of metastatic nodal involvement in canine cMCT is difficult to state based on the current literature.[3,11,14–16,21–23] In a recent paper, the reported rate of nodal metastasis for canine cMCT at first

presentation confirmed by means of cytology was 18.1%[23]; this rate increased to 61% in the study by Baginski et al. that included 90 dogs with grade II MCTs, of which 55 had an enlarged RLN.[11]

One of the major concerns encountered in the histological diagnosis of nodal metastasis in cMCTs is the interpretation of individual mast cells or small aggregates within the LN.[8,15] Recently, standardized histological criteria have been proposed to describe nodal involvement, and 4 histological patterns have been identified, based on the number and distribution of mast cells within LNs: HN0, Non-metastatic LN; HN1, Pre-metastatic LN; HN2, Early metastasis and HN3, Overt metastasis. The disease-free interval has been reported to be significantly prolonged for dogs with RLN classified as HN0 and HN1, when compared with dogs with HN2 and HN3 LNs.[24] Based on this novel categorization,[24] the purpose of the current study was to assess the metastatic rate of non-palpable or normal-sized, surgically removed, RLNs in canine cMCT. It was hypothesized that non-palpable or normal-sized RLNs may often harbour histopathologically detectable metastatic disease. The RLN status was then correlated with tumour variables recorded at the time of admission to surgery, including both histopathological grading systems,[25,26] in an attempt to find a possible predictive association.

2 | MATERIALS AND METHODS

2.1 | Case selection and data collection

Medical records of client-owned dogs with a single cMCT referred to the veterinary teaching hospitals of Università degli Studi di Milano (Italy) and Università degli Studi di Torino (Italy), and to Centro Oncologico Veterinario (Sasso Marconi, Italy) were reviewed. Dogs with multiple concurrent or subcutaneous MCTs were excluded. To be eligible for inclusion, all dogs had to be staged negative at admission for distant metastasis, and the primary tumour and the RLN had to be surgically removed. The excision of the primary tumour included 2 to 3 cm of normal tissue around the palpable edge of the mass and at least 1 deep fascial plane. Dogs were included if the RLN identified as the anatomically closest LN to the primary cMCT was non-palpable or normal-sized (not clinically enlarged, and equal to the contralateral). To exclude distant metastasis, complete blood cell count and biochemistry evaluation, thoracic radiography (3 views), ultrasound-guided cytology of spleen and liver regardless of their ultrasonographic appearance, with or without bone marrow cytologic evaluation were performed, as previously described.[27–29] Original histopathological reports always included the 3-tier and 2-tier histological grading systems on the primary MCT[25,26] assigned by 3 pathologists (CG, SI, VG) who reached an optimal consensus, and the histological classification of the RLN status according to Weishaar et al.,[24] with the exception of cases that were dated before 2014. In these latter cases, histological slides of the extirpated RLNs were reviewed in order to apply the appropriate score HN0-HN3 (Table 1).[24]

Additional retrieved information included breed, age (at the time of surgical procedures), weight (at the time of surgical procedures), sex, presentation (first vs recurrence), anatomic location of cMCT, longest diameter of cMCT, presence of ulceration, histological margin status (infiltrated vs not infiltrated) and RLN location.

TABLE 1 Classification system for histopathological evaluation of node metastasis proposed by Weishaar et al.,[24]

Classification	Histopathological criteria	Proposed interpretation
HN0	None to rare (0-3), scattered, individualized (isolated) mast cells in sinuses (subcapsular, paracortical or medullary) and/or parenchyma per ×400 field (0-3 mast cells per ×400 field), or does not meet criteria for any other classification below	Non-metastatic
HN1	Greater than 3 individualized (isolated) mast cells in sinuses (subcapsular, paracortical or medullary) and/or parenchyma in a minimum of 4 ×400 fields (unless otherwise stated, at least 4 ×400 fields each, which contain more than 3 mast cells)	Pre-metastatic
HN2	Aggregates (clusters) of mast cells (≥3 associated cells) in sinuses (subcapsular, paracortical or medullary) and/or parenchymal, or sinusoidal sheets of mast cells	Early metastasis
HN3	Disruption or effacement of normal nodal architecture by discrete foci, nodules, sheets or overt masses composed of mast cells	Overt metastasis

2.2 | Statistical analysis

The association between histopathological node (HN) category[24] and clinicopathological variables was evaluated by generalized linear models with binomial error. Two separate analyses were performed: the first for HN0 vs HN > 0 and the second for HN0-HN1 vs HN2-HN3. Model response was the HN category, coded as 0 if HN0 and 1 if HN > 0 for the first analysis, and coded as 0 if HN0-HN1 and 1 if HN2-HN3 for the second analysis. Explanatory variables were both categorical and continuous. Categorical variables (location, ulceration, Patnaik grade and Kiupel grade) were considered as dummy variables, thus for a categorical variable with K categories, K-1 dummy variables were included into the regression model and one of the categories was considered as reference one. The variable "location" was categorized in 2 groups: sites historically associated with worse prognosis (head and neck, genital [including inguinal, scrotal, perivulvar and perineal] and digit) vs sites historically associated with better prognosis (lateral thorax and abdomen, and limb, excluding digits).[30] Longest tumour diameter was included in its original continuous measurement scale (interval of 1 cm) and also considered as categorical variable, coded as 0 if < 3 cm and 1 if >3 cm.[23] First, univariate analysis was performed for each of the above-mentioned variables, and then multivariate analysis was performed to evaluate the joint role of the variables. To obtain reliable results in the multivariate analysis, the maximum number of explicative variables was decided according to the rule suggesting a ratio of at least 10 between the number of subjects with model response coded as 1, and the number of regressors.[31] To reach this aim, the following variables, considered as related to each other, were evaluated in the multivariate analysis: longest tumour diameter, location and Kiupel grade.

Results of the regression model were reported as odds ratio (OR) with corresponding 95% confidence intervals. The odds is the ratio between the proportion of subjects with HN > 0 (or HN2-HN3) and the proportion of subjects with HN = 0 (or HN0-HN1). For each categorical variable with K categories K-1 ORs are reported, each one representing the ratio between the odds for the category and the odds for the reference category. If OR > 1, the estimated proportion of subject with HN > 0 (or HN2-HN3) in the category is greater than that in the reference category (and vice versa). In the absence of association between a variable and HN, OR is expected to be 1. The null hypothesis of OR = 1 was tested by Wald statistics. As OR is a measure of the association that is not of a direct clinical interpretation, the risk ratio corresponding to the OR was also provided for the comparison discussed in the Section 3.[32,33]

All analyses were performed with a software package (R-Software; www.r-project.org) and a $P \leq .05$ was considered significant.

3 | RESULTS

3.1 | Patient population

Ninety-three dogs fulfilled the inclusion criteria. There were 21 (22.6%) mixed-breed dogs, 25 (26.9%) Retrievers, 11 (11.8%) Boxers, 4 (4.3%) Shar-pei and 32 (34.4%) dogs belonging to other pure breeds (from 1 to 3 dogs for each breed). Thirty-six (38.7%) dogs were males (10 neutered), and 57 (61.3%) were females (41 neutered). Mean and median age were 7.5 and 7 years, respectively (range 1-14 years). Mean and median weight were 23.8 and 25.6 kg, respectively (range 2.9-47 kg).

Ninety (96.8%) cMCT occurred for the first time, whereas 3 cMCT (3.2%) represented a recurrence after a previous surgery. Eleven (11.8%) cMCT were ulcerated. Twenty-two (23.7%) cMCTs were located on the head, 4 (4.3%) on the neck, 25 (26.8%) on the trunk (including above knee and elbow joint, lateral thorax and lateral abdomen), 20 (21.5%) on the distal limb (distal to elbow and stifle joints), 5 (5.4%) on the digit and 17 (18.3%) in the genital region (scrotal, perineal, perivulvar, preputial, inguinal region). Mean and median longest diameters were 1.83 and 1.5 cm, respectively (range 0.2-5.3 cm).

Histologically, there were 7 (7.5%) Patnaik grade I cMCTs, 81 (87.1%) Patnaik grade II and 5 (5.4%) Patnaik grade III cMCTs; using the 2-tier grading system, 83 (89.3%) cases were low-grade cMCTs, and 10 (10.7%) were high-grade tumours. All Patnaik grade I cMCTs were Kiupel low grade, and all Patnaik grade III cMCTs were Kiupel high grade. Seventy-six of the 81 (93.8%) Patnaik grade II cMCTs were Kiupel low grade, while 5 (6.2%) Patnaik grade II cMCTs were Kiupel high-grade tumours. In 24 (25.8%) cases, the margins were infiltrated (all Patnaik grade II; 23 Kiupel low grade and 1 Kiupel high grade).

The extirpated RLN included 24 (25.8%) mandibular nodes, 20 (21.5%) prescapular nodes, 28 (30.1%) popliteal nodes, 18 (19.3%) superficial inguinal nodes, 2 (2.2%) axillary nodes and 1 (1.1%) axillary accessory node. Histologically, 33 (35.5%) LNs were classified as HN0, 14 (15%) as HN1, 26 (28%) as HN2 and 20 (21.5%) as HN3. Distribution of each HN status among site, longest diameter, ulceration, Patnaik and Kiupel histologic grades are summarized in Table 2.

3.2 | Association between clinicopathological variables and HN category (HN0 vs HN > 0)

Results of univariate analysis are summarized in Table 3. Only the longest diameter of the primary tumour was associated with RLN status: dogs with cMCT with the longest diameter greater than or equal to 3 cm had a higher probability to have HN > 0 LN when compared with dogs with smaller tumours (risk ratio = 1.42).

Despite not being statistically significant, Patnaik grades II and III cMCT tended to have a greater probability of RLN scored as HN > 0 compared with Patnaik grade I tumours (risk ratio = 1.56 and risk ratio = 1.40, respectively), and the same consideration held true for Patnaik grade II/Kiupel low grade and Patnaik grade III/Kiupel high-grade cMCT if compared with Patnaik grade I/Kiupel low-grade tumour. (risk ratio = 1.60 and risk ratio = 1.40 respectively) Unexpectedly, Kiupel high-grade cMCTs had a risk of having an RLN HN > 0 about a quarter lower than that of Kiupel low-grade (risk ratio = 0.76).

By multivariate analysis, the longest diameter remained a significant prognostic variable for HN > 0 (risk ratio = 1.43, Table 4).

3.3 | Association between clinicopathological variables and HN category (HN0-1 vs HN2-HN3)

Results of univariate analysis are summarized in Table 5. Despite the absence of statistical significance for all variables, cMCT bigger than 3 cm, ulcerated or of Patnaik grade III tended to have a higher risk for RLN categorized as HN2-3 (risk ratio = 1.28, risk ratio = 1.34 and risk ratio = 1.40, respectively).

No significant statistical association was found by multivariate analyses (Table 6). A HN2-HN3 RLN tended to be more likely for cMCTs >3 cm (risk ratio = 1.40).

4 | DISCUSSION

In the present study, 93 dogs with a single cMCT and non-palpable or normal-sized RLNs underwent LN extirpation. Surprisingly, half of the RLNs were documented as metastatic, based on histopathology (HN2 and HN3). When including the pre-metastatic status, this percentage increased to 65%. These data are similar to those reported by Worley in a smaller case series, in which 12 out of 19 cases had a positive sentinel LN, even if the histopathological categorization of nodal metastasis was not available at that time.[15] Based on the documented prognostic value of HN2 and HN3 reported by Weishaar et al.,[24] our results have a significant clinical implication, because in the absence of RLN extirpation and subsequent histological evaluation, all these cases would have been incorrectly staged, possibly overestimating prognosis and undertreating dogs. Actually, the histological grading of the primary cMCT is considered one of the most important prognostic factors guiding treatment.[8,34] If non-palpable/normal-sized LNs had not been surgically removed, the diagnosis of HN2/HN3 LN involvement would have been missed in 46 of 93 dogs in the present series. Notably, the majority of these 46 dogs had a Patnaik grade I (n = 3; 3.2%), Patnaik grade II (n = 40; 43%) or Kiupel low grade (n = 41; 44.1%) cMCTs, further emphasizing the role of clinical staging in

TABLE 2 Distribution of histological lymph node (LN) status among tumour variables

cMCT variables	Total cases 93	HN0 33	HN1 14	HN2 26	HN3 20
Site					
Not associated with worse prognosis	46 (49.5%)	16 (34.8%)	7 (15.2%)	16 (34.8%)	7 (15.2%)
Associated with worse prognosis[a]	47 (50.5%)	17 (36.2%)	7 (14.9%)	10 (21.3%)	13 (27.6%)
Longest diameter					
Median (cm)	1.5	1.3	1.9	1.7	1.75
3 cm cut-off					
<3 cm	74 (79.6%)	30 (40.5%)	10 (13.5%)	18 (24.4%)	16 (21.6%)
> = 3 cm	19 (20.4%)	3 (15.8%)	4 (21%)	8 (42.1%)	4 (21%)
Ulceration					
Yes	11 (11.8%)	3 (27.3%)	1 (9.1%)	2 (18.2%)	5 (45.5%)
No	82 (88.2%)	30 (36.5%)	13 (15.9%)	24 (29.3%)	15 (18.3%)
Patnaik					
I	7 (7.5%)	4 (57.1%)	0 (0%)	1 (14.3%)	2 (28.6%)
II	81 (87,1%)	27 (33.3%)	14 (17.3%)	23 (28.4%)	17 (21%)
III	5 (5.4%)	2 (40%)	0 (0%)	2 (40%)	1 (10%)
Kiupel					
Low grade	83 (89.2%)	28 (33.7%)	14 (16.9%)	23 (27.7%)	18 (21.7%)
High grade	10 (10.8%)	5 (50%)	0 (0%)	3 (30%)	2 (20%)
Patnaik-Kiupel					
I-low grade	7 (7.5%)	4 (57.1%)	0 (0%)	1 (14.3%)	2 (28.6%)
II-low grade	76 (81.7%)	24 (31.6%)	14 (18.4%)	22 (28.9%)	16 (21.1%)
II-high grade	5 (5.4%)	3 (60%)	0 (0%)	1 (20%)	1 (20%)
III-high grade	5 (5.4%)	2 (40%)	0 (0%)	2 (40%)	1 (20%)

[a] Head and neck, genital (including inguinal, scrotal, perivulvar and perineal) and digit location.

TABLE 3 Association between cMCT clinicopathological variables and HN category (HN0 vs HN > 0): univariate analysis

cMCT variables	Odds ratio	95% CI	P	Risk ratio
SITE				
Not associated vs associated with worse prognosis	1.06	0.45-2.49	0.89	1.02
LONGEST DIAMETER				
Increasing of 1 cm[a]	1.30	0.88-1.93	0.19	
> 3 cm vs <= 3 cm	3.64	0.97-13.58	0.05*	1.42
ULCERATION				
Yes vs no	1.54	0.38-6.25	0.55	1.15
PATNAIK				
II vs I	2.67	0.56-12.78	0.22	1.56
III vs I	2.00	0.19-20.62	0.56	1.40
KIUPEL				
High vs low grade	0.51	0.14-1.91	0.32	0.76
HISTOLOGICAL GRADE				
II-low grade vs I-low grade	2.89	0.60-13.93	0.19	1.60
II-high grade vs I-low grade	0.89	0.09-9.16	0.92	0.93
III-high grade vs I-low grade	2.00	0.19-20.62	0.56	1.40

Odds ratio, ratio between odds HN > 0 of each category and odds HN > 0 of reference category; 95% CI, 95% confidence interval of odds ratio; *P*, *P* value for Wald statistics; risk ratio, ratio between proportion of HN > 0 of each category and proportion HN > 0 of reference category. *P* value ≤.05 was considered significant (*).

[a] ODDS ratio represents the increase in odds for the increase of each cm in tumour longest diameter.

anticipating prognosis and dictating therapy. Indeed, chemotherapy should be recommended in the case of metastatic (HN2/HN3) LNs.

The association between clinical and pathological variables of cMCTs and the histological LN status[24] was analysed as an initial step for a possible prediction model for non-palpable/normal-sized LN metastasis, possibly dictating surgical decisions (LN extirpation vs no lymphadenectomy). Unfortunately, the low number of dogs included in each category precluded the possibility to analyse each group separately. The role of the pre-metastatic HN1 LNs is still under debate.[24,35] Therefore, 2 different analyses were performed by including HN1 cases with HN2-HN3 and with HN0.

TABLE 4 Association between cMCT clinicopathological variables and HN category (HN0 vs HN > 0): multivariate analysis

cMCT variables	Odds ratio	95% CI	*P*	Risk ratio
SITE				
Not associated vs associated with worse prognosis	0.69	0.27-1779	0.45	0.88
LONGEST DIAMETER				
> 3 cm vs <= 3 cm	4.28	1.07-17.21	0.04*	1.46
KIUPEL				
High vs low grade	0.43	0.11-1.75	0.24	0.66

Odds ratio, ratio between odds HN > 0 of each category and odds HN > 0 of reference category; 95% CI, 95% confidence interval of odds ratio; *P*, *P* value for Wald statistics; risk ratio, ratio between proportion of HN > 0 of each category and proportion HN > 0 of reference category. *P* value ≤.05 was considered significant (*).

The statistical analysis failed to associate the RLN status with other clinical and pathological variables, including both histological grading systems. Only tumours larger than 3 cm were statistically correlated with a higher probability of RLN classified as HN > 0. However, this significant correlation was not confirmed when the pre-metastatic status (HN1) was considered combined with HN0. Nevertheless, some aspects must be underlined. Although there is no general agreement for evaluating OR in terms of strength of association, some authors reported an OR greater than 1.6 and lower than 3.0 as moderate association for epidemiologic studies.[36] Considering the number of dogs included in the present study, such estimates cannot result as "statistically significant," because a sample of about 354 cases, equally subdivided in the 4 categories of histological node status, would have been required to obtain a 90% power of the test. Taking into consideration the aforementioned statement, further studies should be designed to better explain the negative prognostic correlation between Patnaik grades II and III cMCT and nodal metastasis, and the low rate of nodal metastasis for Kiupel high-grade tumours reported in the present work. Notably, the application of both grading systems simultaneously also failed to clarify the prognostic role on non-palpable and normal-sized RLN metastasis detection.[23,35] These results highlight the complexity of the relationship and maybe the independency between staging and grading in cMCT in dogs.

Which LN should be removed is currently based on its anatomical proximity to the tumour rather than on the assessment of the lymphatic drainage pathway with sentinel LN mapping methods. A recent study considering different malignancies on the head (including 3 cMCTs) found a high frequency of medial retropharyngeal LN metastasis with contralateral dissemination.[18] In the study of Worley, 8 out 19 dogs with MCTs had a sentinel LN recognized by lymphoscintigraphy that differed from the anatomically identified RLN.[15] Nonetheless, as a result of the high rate of nodal involvement retrieved in the present study, it may be hypothesized that the detection of draining LNs with mapping techniques matches quite well with the anatomical selection. Further studies on the application of sentinel LN mapping techniques should be performed to elucidate the real advantages of this extra diagnostic procedure and the possible error related to the anatomical detection.

TABLE 5 Association between cMCT clinicopathological variables and HN category (HN0-1 vs HN2-3): univariate analysis

cMCT variables	Odds ratio	95% CI	*P*	Risk ratio
SITE				
Not associated vs associated with worse prognosis	1.04	0.46-2.35	0.92	1.02
LONGEST DIAMETER				
Increasing of 1 cm[a]	1.14	0.81-1.62	0.46	
> 3 cm vs <= 3 cm	2.02	0.72-5.70	0.19	1.38
ULCERATION				
Yes vs no	1.93	0.52-7.10	0.32	1.34
PATNAIK				
II vs I	1.30	0.27-6.18	0.74	1.15
III vs I	2.00	0.19-20.61	0.56	1.40
KIUPEL				
High vs low grade	1.02	0.28-3.81	0.97	1.01
HISTOLOGICAL GRADE				
II-low grade vs I-low grade	1.33	0.28-6.36	0.72	1.17
II-high grade vs I-low grade	0.89	0.09-9.16	0.92	0.93
III-high grade vs I-low grade	2.00	0.19-20.62	0.56	1.40

Odds ratio, ratio between odds HN > 1 of each category and odds HN > 1 of reference category; 95% CI, 95% confidence interval of odds ratio; *P*, *P* value for Wald statistics; risk ratio, ratio between proportion of HN > 1 of each category and proportion HN > 1 of reference category. *P* value ≤.05 was considered significant.

[a] ODDS ratio represents the increase in odds for the increase of each cm in tumour longest diameter.

TABLE 6 Association between cMCT clinicopathological variables and HN category (HN0-1 vs HN2-3): multivariate analysis

cMCT variables	Odds ratio	95% CI	*P*	Risk ratio
SITE				
Not associated vs associated with worse prognosis	0.87	0.36-2.10	0.76	0.93
LONGEST DIAMETER				
> 3 cm vs < = 3 cm	2.13	0.71-6.33	0.18	1.40
KIUPEL				
High vs low grade	0.98	0.25-3.80	0.98	0.99

Odds ratio, ratio between Odds HN > 1 of each category and Odds HN > 1 of reference category; 95% CI, 95% confidence interval of odds ratio; *P*, *P* value for Wald statistics; risk ratio, ratio between proportion of HN > 1 of each category and proportion HN > 1 of reference category. *P* value ≤.05 was considered significant.

Even if the collaboration of 3 veterinary referrals permitted to collect almost 100 cases, this value was still low precluding the possibility to analyse each HN category as a unique variable. In addition, the relative high number of dogs with HN1 and its unclear prognostic role[24,34] prevented the achievement of a more comprehensive interpretation of the results. Further studies should focus on the prognostic role of RLN status. The different post-surgical treatment approach and the influence of owner's decision did not allow to analyse the possible therapeutic role of metastatic non-palpable or normal-sized RLN extirpation. Finally, the identification of the RLN by means of anatomical evaluation rather than sentinel LN mapping techniques may have led to selection bias and limited the number of dogs enrolled, as only cases in which the RLN was recognizable and removable were included in the current study.

In conclusion, non-palpable or normal-sized RLN may harbour occult metastatic disease in dogs with cMCT, regardless of the histological grade of the primary cMCT. The extirpation of non-palpable or normal-sized RLNs permitted an early detection of nodal metastasis and a more accurate tumour staging. Even if size of the primary tumour tended to correlate with a positive node, no significant correlation with clinical-pathological variables was found. Further prospective studies are needed to elucidate the therapeutic role of lymphadenectomy of metastatic non-palpable or normal-sized RLN.

Conflict of interest

The authors declare no conflict of interest.

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