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THE IMPACT ON STAGING OF EXTIRPATION OF NON-PALPABLE/NORMAL SIZED REGIONAL LYMPH NODE IN CANINE CUTANEOUS MAST CELL TUMORS: A MULTICENTRIC RETROSPECTIVE STUDY

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THE IMPACT ON STAGING OF EXTIRPATION OF NON-PALPABLE/NORMAL-SIZED REGIONAL LYMPH NODE IN CANINE CUTANEOUS MAST CELL TUMOURS: A MULTICENTRIC RETROSPECTIVE STUDY

ABSTRACT

Metastasis to regional lymph nodes (RLN) in cutaneous mast cell tumour (cMCT) in dogs have been correlated with shorted of survival time and higher risk of spread to distant sites. In the present study, extirpation of no-palpable or normal-sized regional RLNs was included in the surgical management of cMCT in dogs. Correlations between histological nodal status (HN0-3) and tumour variables were analized.

Ninety-three dogs with single cMCT without distant metastasis that underwent wide surgical excision of the primary tumour and extirpation of no-palpable or normal-sized RLN were included. The association between HN (HN0 vs HN>0; HN0-1 vs HN2-3) and tumour variables (site, dimension, ulceration, 3-tier and 2-tier histological grades) was analysed by a generalized linear model with multinomial error.

Thirty-three (35.5%) RLN 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. Mean and median follow-up time were 695 and 504 days, respectively (range, 10-2429). Seven dogs developed metastatic spread to other lymph nodes and/or other organs.

Non-palpable/normal-sized RLN in dogs with cMCT can harbor histologically detectable metastatic disease in nearly half of the cases. Further studies should evaluate the possible therapeutical effect of the tumour burden reduction obtained by exrtipartion of a positive RLN.

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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 lymph node metastasis in cMCT[10-12]; cytology as well has been associated with a high proportion of both false positive and negative results.[13] Furthermore, not all regional lymph nodes (RLN) are feasible for immediate fine-needle aspiration due to 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 lymph nodes 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 population enrolled, selection of different inclusion criteria for the study population (e.g. high-risk cMCT or Patnaik grade II cMCT only) and different sampling methods (cytology vs. histology) within and among studies, it is difficult to extrapolate from the literature the exact rate of metastatic nodal involvement in canine cMCT.[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 Baginsky

and colleagues (2014) that included 90 dogs with grade 2 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 described to document nodal involvement, consisting of 4 histological patterns that correlated with outcome.[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 harbor histopathologically detectable metastatic disease. The RLN status was then correlated with tumour variables, including both histopathological grading systems,[25,26] in an attempt to find a possible predictive association.

Materials and Methods

Case selection and data collection

Medical records of client-owned dogs with a single cMCT referred to the XXX, YYY and ZZZ 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 from 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, thoracic radiography (3 views), complete blood cell count and biochemistry evaluation, 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] All histopathological samples had to be available for review in order to apply the 3-tier and 2-tier histological grading systems on the primary MCT[25-26], and the Weishaar histological classification on the RLN.[24]

Additional retrieved information included breed, age, weight, sex, presentation (first vs recurrence), anatomic location of cMCT, maximum diameter of cMCT, presence of ulceration, histological margin status (infiltrated vs not infiltrated), RLN location and adjuvant treatment, if performed.

All dogs were re-checked (physical examination, fine-needle aspiration of new lesions) every 3 months during the first year, and every 6 months thereafter. A re-staging was always performed in the case of new or recurrent cMCT or LN metastasis. For dogs undergoing adjuvant medical therapy, clinical evaluation was repeated at every scheduled administration, or once a month in the case of continuous oral administration.

Local recurrence was defined as the occurrence of a cMCT located less than 2 cm from the previous scar. Loco-regional progression was defined as the presence of metastatic disease to LNs other than the RLN, assessed via cytology and/or histology. Distant progression was defined as the development of distant metastatic disease to any organ with the exception of LNs, assessed via cytology and/or histology. Time between RLN extirpation and loco-regional or distant progression was calculated. Overall survival was defined as the time from surgery to death. In case of death, the cause (related or not to cMCT) was retrieved.

Statistical analysis

The association between histopathological node (HN) category (Weishaar et al, 2014) and clinicopathological variables was evaluated by generalized linear models with binomial error. Two separate analysis 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] Maximum tumour diameter was included in its original measurement scale and also considered as categorical variable, coded as 0 if < 3 cm and 1 if > 3 cm.[23]

Firstly, 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: maximum 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 odds ratios 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 stastistics. As odds ratio is a measure of the association that is not of a direct clinical interpretation, the risk ratio corresponding to the odds ratio was also provided for the comparison discussed into results section.[32,33]

Analysis of outcome was explored. Time to event was calculated as the time elapsed from surgery to the date of distant or loco-regional progression or death (in absence of previous disease progression). For dogs being alive at the end of the study (censured data), time was calculated from the date of surgery to the one of the last clinical examination. Survival and event-free probabilities were estimated by the Kaplan-Meier method. The correct application of log-rank test was investigated by examining the relative shape of Kaplan-Meier estimated curves. In the case of crossing curves, log-rank is not an adequate test. Specific modelling techniques based on the adjustment of log-rank weights are available but they are not suitable in the case of low number of events. Thus, in the presence of crossing hazard, an approach based on Landmarking was considered for explorative aim[34].

Follow-up time was partitioned in intervals of 25 days and for each interval the hazard ratio was estimated from a Cox model on subjects at risk to the beginning of the interval. The approach allowed to show the time dependent pattern of HN prognostic impact.

Median, first and third quartile for follow-up time were estimated by the reverse Kaplan-Meier method.[35]

All analyses were performed with a software package (R-Software; www.r-project.org) and a $p \leq 0.05$ was considered significant.

Results

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 was 7.5 and 7 years, respectively (range 1-14 years). Mean and median weight was 23.8 and 25.6 kg, respectively (range 2.9-47 kg).

Ninety (96,8%) cMCT represented a first presentation, whereas 3 cMCT (3.2%) were a cMCT recurrence after 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 knee joints), 5 (5.4%) on the digit and 17 (18.3%) in the genital region (scrotal, perineal, perivulva, prepuzial,

inguinal region). Mean and median dimension 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 (Table 1).

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

Results of univariate analysis are summarized in Table 2. Only dimension of the primary tumour was associated with RLN status: dogs with cMCT bigger than or equal to 3 cm had a higher probability to have HN>0 LN if compared to dogs with smaller tumours (risk ratio=1.42).

Despite no statistically significant Patnaik grade II and III cMCT tended to have a greater probability of HN>0 compared to 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 to Patnaik grade I/Kiupel low-grade tumour. (risk ratio= 1.60 and risk ratio=1.40 respectively) Unexpectedly, Kiupel high-grade MCTs had a risk of having a RLN HN>0 about a quarter lower than that of Kiupel low-grade cMCT (risk ratio=0.76).

By multivariate analysis, dimension remained a significant prognostic variable for HN>0 (risk ratio=1.43, Table 3).

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

Results of univariate analysis are summarized in Table 4. 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 5). A HN2-HN3 RLN tended to be more likely for cMCTs > 3 cm (risk ratio =1.40).

Outcome

Forty-nine dogs (52.7%) did not receive any adjuvant therapy. The remaining 44 (47,3%) dogs received adjuvant chemotherapy: 29 (31.2%) received vinblastine-prednisone, 6 (6.5%) vinblastine-prednisone in association with tyrosine kinase inhibitors (TKI), 6 (6.5%) TKI only, and 3 (3.2%) received other chemotherapeutic agents (n=2 chlorambucil, and n=1 lomustine). The LN status of these 44 dogs included 6 HN0, 6 HN1, 15 HN2 and 17 HN3.

Seven (7.5%) dogs were lost to follow-up at a mean and median time of 458 and 650 days, respectively (one of which with metastatic disease at 302 days, the remaining

had no sign of disease). Overall median follow-up was 596 days, 25% of cases were observed for a period longer than 1188 days, and 75% of cases were observed for a period longer than 266 days.

Local recurrence was detected in 2 dogs after 29 and 337 days from surgery, respectively. Seven dogs experienced metastatic disease. Loco-regional progression with a positive LN was detected in 5 cases after a range of 52 to 1071 days from surgery (Table 6). Distant progression to spleen and liver was identified in 5 dogs after a range of 52 to 1071 days (3 out of this 5 dogs had also simultaneous loco-regional relapse) (Table 6). The RLN status of dogs with loco-regional and/or distant relapse included 4 HN3, 1 HN2, 1 HN1, and 1 HN0 (Table 6).

Sixty-eight dogs were still alive at the end of the study, 13 dogs were dead for causes unrelated to cMCT, and 5 dogs were dead due to cMCT. The survival probability at 730 and at 1460 days was 0.856 (95% confidence interval: 0.776-0.945) and 0.591 (95% confidence interval: 0.434-0.803), respectively. Considering the first event (loco-regional or distant metastasis or death in absence of disease progression) analysis, 20 cases with events were observed (13 cases were dead without loco-regional or distant progression, 5 cases were dead after loco-regional or distant progression, and 2 cases were alive despite distant or loco-regional progression). The probability of remaining free from event was 0.833 (95% confidence interval: 0.750-0.925) at 730 days, and 0.577 (95% confidence interval: 0.424- 0.785) at 1460 days.

The prognosis for HN0-HN1 cases was better than the prognosis for HN2-HN3 cases up to 1000 days; however, after this follow-up time a reverse pattern was observed (Figure 1). The probability of remaining free from event at 730 days was 0.902 (95% confidence interval: 0.801-1.000) and 0.766 (95% confidence interval: 0.6422- 0.915)

for HN0-HN1 and HN2-HN3, respectively, whereas the probability of remaining free from event at 1460 days was 0.434 (95% CI:0.226-0.834) and 0.719 (95%CI: 0.5781 -0.893) for HN0-HN1 and HN2-HN3, respectively (Figure 1). Because of the wide confidence intervals of the event free survival curve, this reverse pattern should be considered with caution. The event-free survival curves of HN0-HN1 vs HN2-HN3 were not statistically significant. The landmarking approach suggested a risk of event for dogs with HN2-HN3 higher than that for dogs with HN0-HN1 in the early period (before 175 days the estimated HRs decreased from 1.2 to 1.1); following which a reverse pattern was estimated and the risk of event was higher for dogs with HN0-HN1 (the estimated HRs decreased from 0.987 to 0.170 at 825 days).

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 (2012) in a smaller case-series, in which 12 out of 19 cases had a positive sentinel LN, even if the histopatological categorization of nodal metastasis was not available at that time.[15] Based on the documented prognostic value of HN2 and HN3 reported by Weishaar and colleagues (2014),[24] our results have a significant clinical impact, because in the absence of histopathological evaluation of the RLN, 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.[8,36] Surprisingly, only a small proportion of dogs with HN2 and HN3 had a Kiupel high-grade (n=5; 5.3%) or Patnaik grade III cMCT (n=3; 3.2%). Consequently,

in a big proportion of dogs with Kiupel low grade (n= 41; 44.1%) and Patnaik grade I (n= 3; 3.2%), or Patnaik grade II cMCT (n=40; 43%) a systemic adjuvant treatment would have not been offered if the LN was not removed.

The association between clinicopathological 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 (lymph node extirpation versus 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.[24,36] Therefore, two different analyses were performed by including HN1 cases with HN2-HN3 and with HN0.

The statistical analysis failed to associate the RLN status with other clinicopathological variables, including both histological grading systems. Only tumours bigger 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 odds ratio in terms of strength of association, some authors reported an *odds ratio* greater than 1.6 and lower than 3.0 as moderate association for epidemiologic studies.[37] 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 grade 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 system simultaneously also failed to clarify the prognostic role on non-palpable and normal-sized RLN metastasis detection.[23,38] These results highlight the complexity 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 lymph node metastasis with contralateral dissemination.[18] In the study of Worley (2012), 8 out 19 dogs with MCTs had a sentinel LN recognized by lymphoscintigraphy that differed from the anatomically identified RLN.[15] Nonetheless, due to 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.

The analysis of outcome was not a primary aim of the study due to its retrospective nature and the heterogeneity of treatment and follow-up examinations. However, some results are of interest and should be further explored. First of all, the number of cases with metastatic progression (n= 7) was low if considering the high number of metastatic LN at admission (n=46; 49.5%); also, it was lower than what reported by Weishaar and colleagues (2014).[24] It is possible that the inclusion of non-palpable or normal-sized RLN in the current study may have selected “early” cases, thus carrying

a better prognosis than dogs with clinically enlarged RLNs. Baginsky and colleagues (2014) also hypothesized that the reduction of tumour burden by means of extirpation of metastatic LNs in Patnaik grade II cMCT may prolong survival, and this may be emphasized in the case of early micrometastasis.[11] Whether chemotherapy should be administered in dogs with metastatic non-palpable or normal-sized lymph node remains to be elucidated. However, based on the current results and in agreement with the Weishaar's study,[24] medical antitumour treatment should be offered and undertaken in the case of HN2-HN3 LNs, regardless of their size. Dogs with HN0-HN1 nodes tended to progress at a later stage compared to dogs with HN2-HN3 nodes. The progression rate was too low to reach definitive evidence, and further studies should verify a possible diversification of time to progression between the different categories of histopathological nodal metastasis. At the same time, the relative high rate of progression for dogs with HN3 RLN (4 out of 7) compared to dogs with HN2 RLN (1 out of 7), prompts to verify the role of each histological node category.

Even if the collaboration of 3 veterinary referrals permitted to collect almost 100 cases, this value was still low prcluding 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[24,36] prevented to achieve a correct results interpretation. 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 permit to draw conclusion on 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 clinicopathological 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 Statement

The authors declare no conflicts of interest.

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Tables

Table 1. Distribution of histological lymph node status among tumour variables.

cMCT variables	HN0	HN1	HN2	HN3
Site				
Not associated with worse prognosis	16	7	16	7
Associated with worse prognosis	17	7	10	13
Dimension				
Median (cm)	1.3	1.9	1.7	1.75
3 cm cut-off				
<3cm	30	10	18	16
>=3cm	3	4	8	4
Ulceration				
Yes	3	1	2	5
No	30	13	24	15
Patnaik				
I	4	0	1	2
II	27	14	23	17
III	2	0	2	1
Kiupel				
Low grade	28	14	23	18
High grade	5	0	3	2
Patnaik-Kiupel				
I-low grade	4	0	1	2
II-low grade	24	14	22	16
II-high grade	3	0	1	1
III-high grade	2	0	2	1

Table 2. Association between cMCT clinicopathological variables and HN category (HN0 vs. HN>0): Univariate analysis.

cMCT variables	Odds Ratio	95% C.I.	Z	p	Risk Ratio
SITE					
Not associated vs. associated with worse prognosis	1.06	0.45-2.49	0.14	0.89	1.02
DIMENSION					
Increasing of 1 cm	1.30	0.88-1.93	1.33	0.19	
> 3 cm vs. <= 3cm	3.64	0.97-13.58	1.92	0.05	1.42
ULCERATION					
Yes vs. no	1.54	0.38-6.25	0.60	0.55	1.15
PATNAIK					
II vs. I	2.67	0.56-12.78	1.23	0.22	1.56
III vs. I	2.00	0.19-20.62	0.58	0.56	1.40
KIUPEL					
High vs. low grade	0.51	0.14-1.91	-1.00	0.32	0.76
HISTOLOGICAL GRADE					
II-low grade vs. I-low grade	2.89	0.60-13.93	1.32	0.19	1.60
II-high grade vs. I-low grade	0.89	0.09-9.16	-0.10	0.92	0.93
III-high grade vs. I-low grade	2.00	0.19-20.62	0.58	0.56	1.40

Legend: Odds Ratio = ratio between Odds HN>0 of each category and Odds HN>0 of reference category; 95% C.I. = 95% confidence interval of Odds Ratio; Z= Wald Statistics; Risk Ratio = ratio between proportion of HN>0 of each category and proportion HN>0 of reference category.

Table 3. Association between cMCT clinicopathological variables and HN category (HN0 vs. HN>0): Multivariate analysis.

cMCT variables	Odds Ratio	95% C.I.	Z	p	Risk Ratio
SITE **Not associated vs. associated with worse prognosis**	0.69	0.27-1779	-0.76	0.45	0.88
DIMENSION **> 3 cm vs. <= 3cm**	4.28	1.07-17.21	2.05	0.04	1.46
KIUPEL **High vs. low grade**	0.43	0.11-1.75	-1.18	0.24	0.66

Legend:Odds Ratio = ratio between Odds HN>0 of each category and Odds HN>0 of reference category; 95% C.I. = 95% confidence interval of Odds Ratio; Z= Wald Statistics; Risk Ratio = ratio between proportion of HN>0 of each category and proportion HN>0 of reference category.

Table 4 . Association between cMCT clinicopathological variables and HN category (HN0-1 vs. HN2-3): Univariate analysis.

cMCT variables	Odds Ratio	95% C.I.	Z	p	Risk Ratio
SITE **Not associated vs. associated with worse prognosis**	1.04	0.46-2.35	0.10	0.92	1.02
DIMENSION **Increasing of 1 cm** **> 3 cm vs. <= 3cm**	1.14 2.02	0.81-1.62 0.72-5.70	0.74 1.32	0.46 0.19	 1.38
ULCERATION **Yes vs. no**	1.93	0.52-7.10	0.99	0.32	1.34
PATNAIK **II vs. I** **III vs. I**	1.30 2.00	0.27-6.18 0.19-20.61	0.33 0.58	0.74 0.56	1.15 1.40
KIUPEL **High vs. low grade**	1.02	0.28-3.81	0.04	0.97	1.01
HISTOLOGICAL GRADE **II-low grade vs. I-low grade** **II-high grade vs. I-low grade** **III-high grade vs. I-low grade**	1.33 0.89 2.00	0.28-6.36 0.09-9.16 0.19-20.62	0.36 -0.10 0.58	0.72 0.92 0.56	1.17 0.93 1.40

Legend:Odds Ratio = ratio between Odds HN>1 of each category and Odds HN>1 of reference category; 95% C.I. = 95% confidence interval of Odds Ratio; Z= Wald Statistics; Risk Ratio = ratio between proportion of HN>1 of each category and proportion HN>1 of reference category.

Table 5. Association between cMCT clinicopathological variables and HN category (HN0-1 vs. HN2-3): Multivariate analysis.

cMCT variables	Odds ratio	95% C.I.	Z	p	Risk Ratio
SITE **Not associated vs associated with worse prognosis**	0.87	0.36-2.10	-0.31	0.76	0.93
DIMENSION **> 3 cm vs <= 3cm**	2.13	0.71-6.33	1.36	0.18	1.40
KIUPEL **High vs low grade**	0.98	0.25-3.80	-0.03	0.98	0.99

Legend: Odds Ratio = ratio between Odds HN>1 of each category and Odds HN>1 of reference category; 95% C.I. = 95% confidence interval of Odds Ratio; Z= Wald Statistics; Risk Ratio = ratio between proportion of HN>1 of each category and proportion HN>1 of reference category.

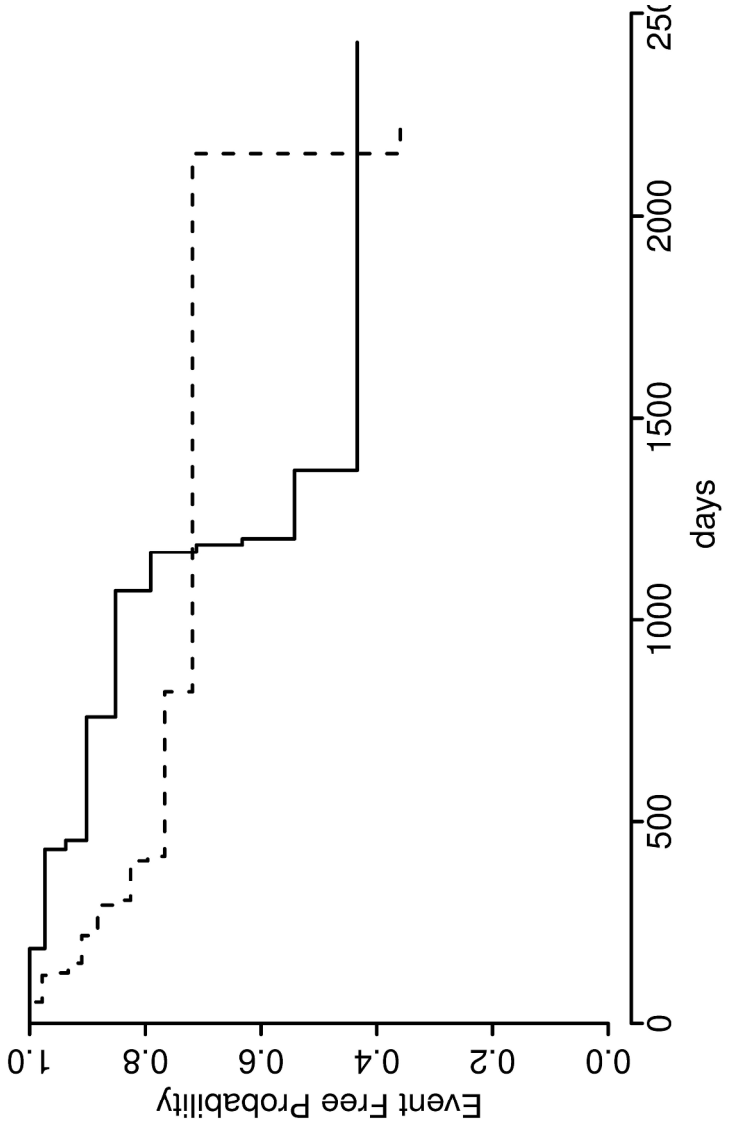
Table 6. Cases with loco-regional and/or distant metastatic progression.

Case	MCT size (cm)	MCT site	RLN	3-tier grading	2-tier grading	HN	Chemotherapy	MCT progression (days)	Survival
1	5	neck	mandibular	II	low	3	Vinblastine + prednisone	LRP (52) DP (52)	Death due to cMCT
2	1,5	inguinal	inguinal	III	high	3	Vinblastine + prednisone + TKI	LRP (415)	Death due to cMCT
3	2,2	head	mandibular	II	low	3	Vinblastine + prednisone + TKI	LRP (126)	Alive
4	3,2	trunk	prescapular	II	low	2	TKI	DP (218)	Death due to cMCT
5	1,5	head	mandibular	II	low	1	no	DP (759)	Death due to cMCT
6	1,2	head	mandibular	II	high	3	Vinblastine + prednisone + TKI	LRP (293) DP (302)	Alive
7	1,5	perineal	inguinal	II	low	0	no	LRP (1071) DP (1071)	Death due to cMCT

Legend: TKI= Tirosin-kinase inhibitor; LRP= loco-regional progression; DP= distant progression.

Figure legends

Figure 1. Kaplan-Meier curves described the probability to be free of event (loco-regional progression or distant progression or death without progression) for dogs with lymph node status HN0-HN1 (solid line) and for dogs with lymph node status HN2-HN3 (dotted line). Vertical lines are censured data (case alive at the end of the study or lost to follow-up in absence of disease progression).



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