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Patterns of nodal metastases, biological behavior and prognosis of canine mast cell tumors of the pinna: a multi-institutional retrospective study

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Conflict of interest statement

The authors do not have any potential conflicts of interest to declare

Abstract

Canine cutaneous mast cell tumors (cMCTs) of the pinna have been associated with an aggressive biological behavior, although data remain scarce. The knowledge acquired over the past years on histologic gradings, and the value of lymph node (LN) staging, may help in better characterizing this anatomical presentation.

The first aim was to describe the frequency, location, and histologic appearance of LN metastases in cMCT of the pinna. A second aim was to evaluate prognosis.
Medical records of dogs with cMCT of the pinna, that underwent tumor and sentinel (SLN) or regional LN (RLN) excision, were reviewed. The influence of potential prognostic variables on time to progression (TTP) and tumor-specific survival (TSS) was investigated.
Thirty-nine dogs were included: 19 (48.7%) had Kiupel high-grade (K-HG) and 20 (51.3%) had low-grade (K-LG) MCTs. Eighteen (46.1%) dogs underwent SLN mapping: the superficial cervical LN was at least one of SLN in 17 (94.4%) cases.
Twenty-two (56.4%) dogs had LN metastases; the superficial cervical LN was always involved.
On multivariable analysis, only K-HG was associated with increased risk of progression ($p=0.043$) and tumor-related death ($p=0.021$). Median TTP and TSS were 270 and 370 days in K-HG, respectively; these were not reached in dogs with K-LG tumors ($p<0.01$).
cMCTs of the pinna are often K-HG and are also associated with a higher frequency of LN metastasis; however, we confirmed the independent prognostic value of histologic grading. A multimodal treatment may lead to favorable long-term outcome. Moreover, the superficial cervical LN is most often the SLN.

Keywords: dog, pinnal, mastocytoma, nodal, metastasis, outcome

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Introduction

The biological behavior of canine cutaneous mast cell tumors (cMCT) is variable. Tumors exhibit a range of malignancy, with higher-grade tumors carrying a high risk for recurrence and metastasis. [1-4]

Anatomic location has been proposed as a simple predictor of aggressive behavior, with inguinal, perineal and orofacial sites being the most frequently cited. [1,2,3, 5-10]

Biologic behavior is predicted more broadly by histologic grade and lymph node (LN) status, independent of the site of origin. [3,7,8,11,12] But, an association between high tumor grade and inguinal, axillary or scrotal location does exist, with Kiupel high-grade (K-HG) cMCT representing 73.3%, 63.6% and 57.1% of the samples, respectively. [11] These numbers contrast with the overall prevalence of K-HG designation among cMCTs, which tends not to exceed 20%. [13] cMCT located on the muzzle have also been reported to exhibit a higher risk of LN metastasis when compared to other cutaneous sites, occurring in 58% of cases. [3]

Focusing on pinnal cMCT, Schwab and colleagues reported a percentage of Patnaik grade III (P-GIII) cMCT twice greater than Patnaik grade I (P-GI cMCT; 28.6% vs 14.3%, respectively), while in more than 50% of cases the primary cMCT was histologically classified as Patnaik grade II (P-GII). Dogs with P-GIII cMCT of the pinna had significantly shorter survival when compared to dogs with P-GI or P-GII cMCT (10 months vs not reached, respectively). LN metastases at the time of diagnosis were not identified in any of the dogs, but LN assessment was not performed in most (92.8%) cases. [12] Overall, data on pinnal cMCT remain scarce and outdated when considering the knowledge acquired over the past years on histologic tumor and LN gradings, and the value of LN staging. [10,12, 14-17]

According to the World Health Organization (WHO) clinical staging system, stage II cMCT is defined as a primary single tumor confined to the dermis with regional LN (RLN) metastasis. [18] Standardized histologic criteria have been proposed to more consistently characterize nodal involvement, and 4 histologic patterns have been identified: HN0, non-metastatic LN; HN1, pre-metastatic LN; HN2, early metastasis and HN3, overt metastasis. [19]

Based on anatomic studies, most of the lymph vessels of the pinna drain to the superficial cervical LN. A small number drain to the lateral retropharyngeal and medial retropharyngeal LNs, as well as to the parotid LN. [12-22.] The sentinel lymph node (SLN) is defined as the first LN receiving drainage from a primary tumor and is expected to be the first site of metastasis. Recent studies suggest that SLN and RLN do not match in 28-63% of cases, highlighting the importance of SLN mapping during the staging work-up. [14, 17, 23] However, there are no reports specifically describing the patterns of nodal metastases of pinnal cMCTs.

Hence, the first aim of this multi-institutional retrospective study was to describe the frequency, location and histologic stage of nodal metastases among dogs with cMCT of the pinna that underwent concurrent lymphadenectomy. The second aim was to evaluate prognosis.

Materials and Methods

Study design and inclusion criteria:

Medical records of eight (University of Bologna [n= 14 cases], University of Liverpool [n=8 cases], Royal Veterinary College [n=6 cases], Pronto Soccorso Veterinario Laudense [n=3 cases], University of Glasgow [n=2 cases], University of Turin [n=2 cases], North Downs Specialist referrals [n=2 cases], University of Cambridge [n=2 cases]) institutions were retrospectively reviewed to identify dogs with a single, treatment-naïve cMCT of the pinna that underwent surgical excision of the primary tumor and concurrent lymphadenectomy of SLN/s or RLN/s. To be eligible for inclusion, dogs had to undergo a staging work-up, including hematologic and biochemical analysis, thoracic imaging (radiography or computed tomography; CT), abdominal ultrasound and fine-needle aspiration of liver and spleen. Data of additional tests (e.g., sentinel LN mapping, cytologic evaluation of RLN vs SLN, CT-total body) were collected, if available, for review.

Dogs with multiple head and neck MCTs at the time of diagnosis were excluded from the analysis.

Among dogs that underwent SLN mapping, SLNs were identified pre-operatively by means of CT-lymphangiography, using peritumoral administration of water-soluble iodine-based contrast agents, as previously reported. [24] We considered the SLN to be the first LN/s (station 1 nodes) receiving contrast from neoplastic lesion, by the same or different lymphatic pathways. All other nodes were presumed to be afflicted subsequently and were not included in the analysis. [24,25]

The primary tumor was graded into Kiupel low-grade (K-LG) and K-HG. [26] All LNs were classified according to Weishaar criteria. [19] Primary MCTs and LNs were evaluated by multiple pathologists and slides were not available for review.

Data on adjuvant medical oncologic treatments were retrieved from the records; additional medical therapies were allowed and recorded.

For each case the following data were recorded: signalment (breed, age, sex and weight), clinical substage (i.e., asymptomatic [a] or symptomatic [b]), primary tumor description (presence of ulceration, largest diameter, location [right or left pinnae]), SLN-mapping (yes or no), SLN location, number of SLN/s or RLN/s removed, LN status (HN0, HN1, HN2, HN3), clinical stage (e.g., stage I [HN0/HN1], stage II [HN2/HN3]), type of surgery (marginal [nodulectomy] or wide [pinnectomy]), histologic grade (K-LG or K-HG), histologic margins (complete or incomplete), adherence to underlying cartilage (yes or no) based on histopathology reports, and adjuvant medical treatment (e.g. traditional chemotherapy, tyrosine kinase inhibitors [TKIs], none).

In order to assess outcome, the following information was also retrieved: local recurrence (cMCT relapse at or within 2 cm of the surgical scar, confirmed by cytology or histopathology), nodal progression (presence of new metastatic LN/s), distant progression (occurrence of cytologically confirmed metastasis at distant organs), date of death or last follow-up examination, and cause of death. [27] Dogs receiving chemotherapy were reassessed at every treatment session, then every 2-3 months for the first year, and every 3-6 months thereafter. Dogs undergoing surgery alone were reassessed every 2-3 months for the

first year, and every 3-6 months thereafter. Imaging including abdominal ultrasound, was carried out at the clinicians' discretion, depending on owners' compliance and evidence of progressive disease.

Statistical analysis

Descriptive statistics were used in the analysis of dogs and tumor characteristics. When appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus normality test. No data had normal distribution and were therefore expressed as median (range).

Time to progression (TTP) was calculated from the date of surgery to the first occurrence of at least one of the following events: local recurrence, nodal progression or distant progression.

Tumor-specific survival (TSS) was calculated from the date of surgery to the date of death due to tumor-related causes.

If tumor progression or death for tumor-related causes did not occur, dogs were censored for the respective statistical analysis.

The influence of potential prognostic variables on TTP and TSS was investigated with univariable and multivariable Cox's regression analyses. The following variables were analyzed: breed (predisposition to biologically aggressive MCTs [i.e., Shar pei, American Staffordshire, Weimaraner, Rottweiler, Shih-Tzu] or others), [13,11,28] sex (male or female), age, body weight, macroscopic tumor longest diameter (> 3 cm or $\leq$ 3 cm), [30,31] ulceration (yes or no), substage (a or b), type of surgery (marginal or wide), grading (K-LG or K-HG), margins (complete or incomplete), adherence to cartilage (yes or no), LN status (HN3 or other), clinical stage (I or other), adjuvant medical treatment (yes or no). For age and body weight, the median was used as a cut-off value.

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). Survival curves obtained with the Kaplan-Meier method were compared with the log-rank test.

The impact of histologic grade (K-LG or K-HG) on the presence of LN metastasis at admission was assessed by binary logistic regression analysis.

Statistical analysis was performed using SPSS Statistic v.25 (IBM, Armonk, NY, United States). Significance was set at $p<0.05$.

Cell Line Validation Statement:

No cell lines were used in the current study.

Results

Patient data and tumor characteristics:

The electronic search between 2014 and 2022 identified 74 dogs potentially suitable for the study. Twenty-nine dogs were excluded as information regarding the SLN/RLN location and/or status was not available, and 6 were excluded as they had multiple head and neck cMCTs at presentation.

A total of 39 dogs with cMCTs of the pinnae were ultimately included. There were mixed breeds (n=13; 33.4%), Labrador retriever (n=5; 12.8%), golden retriever (n=3; 7.7%), Boxer (n=3; 7.7%), and one (2.5%) each of American Staffordshire, cane Corso, Bernese mountain dog, Maltese, miniature Schnauzer, American bulldog, Beagle, French bouledogue, Brittany, Chihuahua, Shih-Tzu, English springer spaniel, English setter and Vizsla.

There were 23 (58.9%) females (of which 18 spayed) and 16 (41.1%) males (of which 8 castrated). The median age was 8 years (range, 1-15 years), and the median weight was 22 kg (range, 7-45.5 kg).

Six (15.4%) dogs were symptomatic (n=3 pruritus, n=2 inappetence, n=1 lethargy).

Twenty-one (53.8%) tumors were located on the left pinna, while 18 (46.2%) on the right pinna. Median tumor diameter was 2 cm (range, 0.3-5.5 cm); 8 (20.5%) tumors were ulcerated.

Information regarding RLN size was available for 15/21 (71.4%) dogs: 11 (73.3%) dogs had subjectively enlarged RLN/s, whereas 4 (26.7%) had normal-sized RLN/s. Among dogs that underwent SLN mapping, information regarding SLN size was available in 13/18 (72.2%) cases: 9 (69.2%) dogs had subjectively normal-sized SLN/s, whereas 4 (30.8%) had enlarged SLN/s.

Eighteen (46.2%) dogs underwent SLN mapping by means of CT-lymphangiography: in 9 (50%) cases the superficial cervical LN was the only SLN, and in 8 (44.4%) cases it was at least one of the SLNs (Table 1).

Information regarding cytologic evaluation of LN/s was available for 29/39 (74.3%) dogs: 10 (34.5%) LN aspirates were interpreted as normal, 5 (17.2%) as reactive, 4 (13.8%) as possible metastasis, 5 (17.2%) as probable metastasis, and 5 (17.2%) as certain metastasis according to Krick's cytological evaluation. [32]

Thirty-four (87.2%) dogs underwent pinnectomy, while 5 (12.8%) dogs underwent nodulectomy.

Based on histopathology, there were 20 (51.3%) K-LG cMCTs and 19 (48.7%) K-HG cMCTs.

Information regarding cartilage involvement was available for 29 (74.3%) dogs: in 19 (65.5%) cases there was no cartilage involvement. Surgical margins were complete in 33 (84.6%) cases and incomplete in 6 (15.4%); among dogs with incomplete margins, 4 dogs underwent nodulectomy and 2 dogs underwent pinnectomy. Mutational analysis was available for 6 (15.4%) cMCTs: 3 had ITD on exon 11, 1 had ITD on exon 8, and 2 were wild type.

Twenty-one (56.4%) dogs underwent regional lymphadenectomy, while 18 (46.2%) underwent SLN/s removal.

Overall, 27 SLNs (n=17 superficial cervical; n=6 mandibular; n= 3 parotid; n=1 medial retropharyngeal) and 31 RLNs (n=18 mandibular; n=11 superficial cervical; n=2 medial retropharyngeal) were surgically removed. Based on histopathology, 18 LNs were HN0, 12 HN1, 14 HN2, and 14 HN3. HN3 metastatic LNs included 12 superficial cervical and 2 medial retropharyngeal LNs, while HN2 metastatic LNs

included 10 superficial cervical, 2 mandibular and 2 parotid LNs. In all dogs with metastatic MCT the superficial cervical LN was involved alone (n=18) or in addition to other LNs (n=4).

Seventeen (43.5%) dogs had stage I disease and 22 (56.5%) dogs had stage II disease. None had distant metastasis.

Among dogs with K-LG cMCTs, 13 (65%) had stage I (HN0/HN1) disease and 7 (35%) had stage II (n=6 HN2; n=1 HN3) disease. Among dogs with K-HG cMCTs, 4 (21.1%) had stage I disease and 15 (78.9%) had stage II (n=11 HN3; n=4 HN2) disease.

Dogs with K-HG cMCTs had a 6.96 fold increased risk of having LN metastasis (HN2/HN3; 95% CI: 1.66-29.26; p=0.008) and a 26.13 fold increased risk of having overt (HN3) LN metastasis (95% CI: 2.67-237.55; p=0.004).

Overall, 22 (88%) dogs were treated with traditional chemotherapy consisting of vinblastine and prednisolone (n= 20) or lomustine and prednisolone (n=2), and 3 (12%) received TKIs (n=2 masitinib; n=1 toceranib); all dogs with K-HG cMCTs and 6 (30%) dogs with K-LG cMCTs received adjuvant medical treatment. All dogs with K-LG cMCTs treated with adjuvant medical treatment had nodal metastasis (n=5 HN2; n=1 HN3).

Outcome and prognostic variables:

Fourteen (35.9%) dogs experienced disease progression after a median of 116 days (range, 21-499 days); among them 13 (92.8%) had K-HG cMCTs and 1 (7.2%) had a K-LG cMCT.

Eight (20.5%) dogs experienced local recurrence after a median of 147 days (range, 21-320 days), all had K-HG cMCTs; 3 out of these 8 dogs underwent nodulectomy and had their cMCT removed with incomplete margins.

Twelve (30.7%) dogs experienced nodal progression after a median of 116 days (range, 21-499 days); among them, 11 (91.7%) had K-HG cMCTs.

Six (15.4%) dogs had distant progression after a median of 335 days (range, 90-500 days); all had K-HG cMCTs.

At data analysis closure, 23 (59%) dogs were alive with a median follow-up of 569 days (range, 150-1493 days), while 16 (41%) dogs had died because of tumor-related (n=13; 81%) or unrelated (n=3; 19%) causes. In the latter, causes of death were appendicular osteosarcoma, congestive heart failure and chronic kidney disease. Among dogs that had died due to tumor-related causes, 12 (92.3%) had K-HG cMCTs and one had a K-LG cMCT.

On univariable analysis, substage b (HR: 7.11; p=0.001), marginal surgery (HR: 4.30; p=0.015), K-HG (HR: 25.35; p=0.002), incomplete histological margins (HR: 5.02; p=0.005) and the presence of HN3 LNs (HR: 6.6; p<0.001) were significantly associated with higher risk of tumor progression (Table 2).

On multivariable analysis, K-HG (HR: 10.38; p=0.043) remained significant (Table 3).

On univariable analysis, substage b (HR: 9.00; p<0.001), marginal surgery (HR: 4.56; p=0.017), K-HG (HR: 25.82; p=0.002), incomplete histological margins (HR: 5.53; p=0.004) and the presence of HN3 LNs (HR: 12.03; p<0.001) and were significantly associated with higher risk of tumor-related death (Table 2).

K-HG was the only variable significantly associated with a higher risk of tumor-related death in multivariable analysis (HR: 14.15; P=0.021; Table 4).

Median TTP (mTTP) for dogs with K-HG cMCTs (270 days; 95% CI: 35-505) was significantly shorter than mTTP for dogs with K-LG cMCTs (not reached; 95% CI: 150-2230; p<0.01; Figure 1).

Median TSS (mTSS) for dogs with K-HG cMCTs (370 days; 95% CI: 280-460) was significantly shorter than mTSS for dogs with K-LG cMCTs (not reached; 95% CI: 150-1992; p<0.01; Figure 2).

Discussion

In agreement with previous studies, [14-16] the results of the current work suggest that the superficial cervical LN is most often the SLN in cMCTs of the pinna, as in approximately 95% of cases the superficial cervical LN was at least one of, if not the only SLN.

Overall, LN metastasis was detected in 56% dogs at the time of diagnosis, and the superficial cervical LN was always involved in case of metastatic disease, further proving to be the most likely first lymphatic drainage site in dogs with MCT of the pinna. The rate of LN metastasis observed in our study is higher when compared to a previous study, in which nodal metastasis was not identified in any of the included dogs with cMCTs of the pinna. [12] It is important to note that in Schwab's study only 2/28 (7%) dogs underwent cytologic evaluation of the mandibular LN and that no lymphadenectomy was performed. [12] Additionally, the superficial cervical LN was never investigated, possibly missing metastatic foci. Our results confirm in a larger population the findings of De Bonis and colleagues, where one out of two dogs with cMCTs of the pinna had SLN metastasis (superficial cervical LN; HN3). [15]

Interestingly, approximately 50% of cMCTs of the pinna in this study were histologically K-HG. These results suggest that cMCTs located on the pinnae may be associated with higher histologic grade as compared to cMCTs of other locations. In Schwab's study, 28.6% of MCTs of the pinna were histologically classified as Patnaik P-GIII, while 57.1% and 14.3% tumors were classified as P-GII and P-GI, respectively. [12] Although it is difficult to compare our results with the aforementioned study due to the different grading systems, it is plausible that a portion of P-GII tumors were K-HG cMCTs. [12, 35] This discrepancy may be due to the involvement of referral oncology centers in the current study, which deal more frequently with aggressive tumors, leading to a possible selection bias.

In agreement with a previous study, [30] we found a significant correlation between the histologic grade of the primary tumor and LN status, since dogs with K-HG cMCTs had a higher risk of developing nodal metastasis when compared to dogs with K-LG tumors.

Furthermore, the current study confirmed the independent prognostic value of histologic grading: the presence of K-HG cMCTs was significantly associated with a higher risk of both tumor progression and tumor-related death, and outclassed the impact of nodal metastasis in multivariable analysis.

The prognosis for dogs with K-HG cMCTs undergoing multimodal treatment, consisting of surgical excision of the primary tumor, lymphadenectomy and adjuvant medical treatment, was not unfavorable, even in the presence of LN metastasis (15/19 cases; 78.9%), with a mTSS of 370 days. These findings support recent studies in which outcome is improved if a multimodal treatment is pursued. [31, 37] In one study, dogs with K-HG cMCTs and LN metastasis (stage II) that underwent surgical excision of the primary tumor, lymphadenectomy, with or without LN bed irradiation, had a median progression free survival and overall survival of 125 and 330 days, respectively. [31] In a more recent study, dogs with K-HG cMCTs and HN3 LN that underwent lymphadenectomy as part of their primary surgery and received adjuvant medical treatment had mTTP and survival time of 277 and 371 days, respectively. [37]

Here, mTTP and mTSS for dogs with stage II K-LG cMCTs were not reached, as few events occurred; most (6/7; 85.7%) of them had HN2 LNs. Among those, only one dog with early metastasis did not receive adjuvant medical treatment. It has been previously suggested that dogs with surgically removed K-LG cMCTs that undergo concurrent excision of an HN2 LN do not necessarily require adjuvant chemotherapy. [38] Thus, it is possible that even without adjuvant medical treatment the prognosis for these dogs would have still been favorable.

Recent studies showed that the removal of an early and/or overtly metastatic LN is associated with a better outcome in both LG and HG cMCTs. [27, 31, 37] Considering the above, clinicians should be aware that in most cases of pinnal cMCT the superficial cervical LN is the SLN, and in the authors' opinion this should always be investigated even in the absence of more sophisticated imaging techniques.

The current study has several limitations mainly due to its retrospective design. First, despite performing a multi-institutional study, inclusion criteria were strict; this, in addition to the low frequency of cMCT on the pinna, resulted in a relatively small sample size.

Second, not all dogs underwent SLN mapping, precluding a more detailed analysis of SLNs. Third, LNs were histologically evaluated by multiple pathologists and slides were not reviewed, potentially affecting results. Finally, follow-up was not standardized, potentially biasing results.

In conclusion, the present study showed that cMCTs of the pinna are often K-HG and are also associated with a higher frequency of LN metastasis. Moreover, the superficial cervical LN is most often the SLN in dogs with pinnal cMCTs. Although the pinnal anatomical location has been previously associated with more aggressive biologic behavior, a multimodal treatment may lead to favorable long-term outcome.

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Table 1. SLN/s location among 18 dogs with cMCTs of the pinna that underwent SLN mapping.

cMCT† location	Identified SLN‡
Right pinna	Right superficial cervical (n=5)
Left pinna	Left superficial cervical (n=5)
Right pinna	Right superficial cervical and right mandibular (n=2)
Left pinna	Left superficial cervical and left mandibular (n=1)
Left pinna	Left superficial cervical and left parotid (n=1)
Right pinna	Right superficial cervical and right parotid (n=1)
Left pinna	Left superficial cervical, left parotid and left mandibular (n=1)
Left pinna	Left superficial cervical and left medial retropharyngeal (n=1)
Right pinna	Right mandibular (n=1)

†cutaneous mast cell tumor; ‡ sentinel lymph node.

Table 2. Univariable Cox regression analysis of variables potentially associated with increased risk of tumor progression and tumor-related death in 39 dogs with cMCTs of the pinnae.

Variable	**Tumor progression**		**Tumor-related death**	
	Hazard Ratio (95% CI†)	*P*	Hazard Ratio (95% CI)	*P*
Breed predisposed to biologically aggressive cMCT‡	>0.99 (0.27-3.62)	0.994	1.16 (0.31-4.29)	0.828
Age ≥ 7 years	2.10 (0.69-6.46)	0.194	1.81 (0.57-5.73)	0.313
Female sex	1.55 (0-52-4.61)	0.432	1.30 (0.42-4.03)	0.652
Weight ≥ 22 kg	0.59 (0.19-1.81)	0.359	0.73 (0.23-2.31)	0.595
Tumor diameter > 3 cm	1.47 (0.49-4.38)	0.491	1.21 (0.38-3.81)	0.749
Ulceration	2.07 (0.64-6.75)	0.227	2.17 (0.65-7.23)	0.206
Substage b	7.11 (2.29-22.07)	0.001*	9.00 (2.75-29.47)	<0.001*
Marginal surgery	4.39 (1.33-14.46)	0.015*	4.56 (1.31-15.86)	0.017*
K-HG¶	24.35 (3.15-188.37)	0.002*	25.82 (3.27-204.06)	0.002*
Incomplete histological margins	5.02 (1.64-15.34)	0.005*	5.53 (1.73-17.71)	0.004*
Presence of adherence to cartilage	1.10 (0.27-4.40)	0.899	1.51 (0.36-6.38)	0.577
HN3 LN§	6..60 (2.18-20.04)	0.001*	12.03 (3.19-45.34)	<0.001*

Clinical stage>I	66.89 (0.75-5933.68)	0.066	71.78 (0.66-7773.95)	0.074
Adjuvant oncological medical treatment	0.02 (0.00-1.76)	0.086	0.02 (0-2.25)	0.104

†confidence interval; ‡ cutaneous mast cell tumor; ¶ Kiupel high grade; § lymph node; *significant.

Table 3. Multivariable Cox regression analysis for risk of tumor progression in 39 dogs with cMCTs of the pinna. Variables with p≤0.05 at univariable analysis were included in the model.

Variable	**Tumor progression**	
	Hazard Ratio (95% CI†)	*P*
Substage b	1.50 (0.40-5.61)	0.545
Marginal surgery	2.91 (0.48-17.61)	0.246
K-HG‡	10.38 (1.07-100.50)	0.043*
Incomplete histological margins	1.83 (0.39-8.61)	0.445
HN3 LN§	1.50 (0.40-5.61)	0.545

†confidence interval; ‡ Kiupel high grade; § lymph node; *significant.

Table 4. multivariable Cox regression analysis for the risk of tumor-related death in 39 dogs with cMCTs of the pinna. Variables with p≤0.05 at univariable analysis at univariable analysis were included in the model.

Variable	**Tumor-related death**	
	Hazard Ratio (95% CI†)	*P*
Substage b	2.91 (0.52-16.22)	0.224
Marginal surgery	2.53 (0.44-14.61)	0.299
K-HG‡	14.15 (1.50-133.85)	0.021*
Incomplete histological margins	2.06 (0.50-8.48)	0.318
HN3 LN§	3.78 (0.73-19.46)	0.112

†confidence interval; ‡ Kiupel high grade; § lymph node; *significant.

Figure 1. Time to progression for dogs with Kiupel high-grade vs Kiupel low-grade cutaneous mast cell tumors of the pinna. Median time to progression for dogs with Kiupel high-grade tumors was significantly shorter than median time to progression for dogs with Kiupel low-grade tumors (270 days vs not reached, respectively; p<0.01).

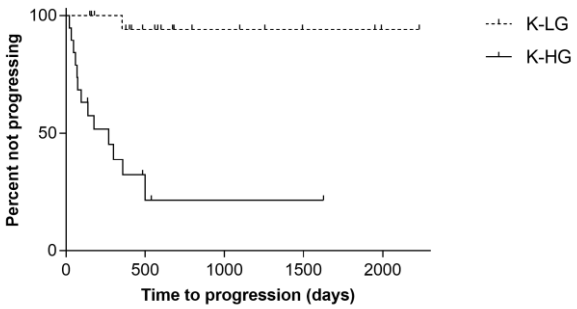
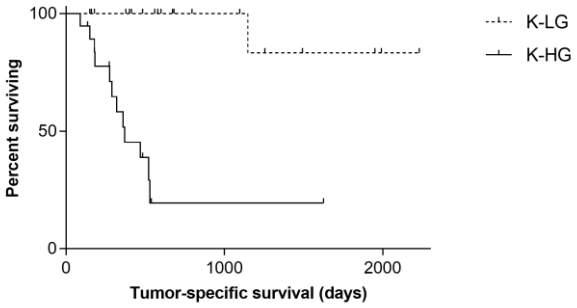
Figure 2. Tumor-specific survival for dogs with Kiupel high-grade vs Kiupel low-grade cutaneous mast cell tumors of the pinna. Median tumor-specific survival for dogs with Kiupel high-grade tumors was significantly shorter than median tumor-specific survival for dogs with Kiupel low-grade tumors (370 days vs not reached, respectively; $p<0.01$).