doi: 10.1111/vco.12425

Therapeutic impact of regional lymphadenectomy in canine stage II cutaneous mast cell tumors

Abstract

Lymph node (LN) metastasis in canine cutaneous mast cell tumors (cMCTs) is a well-known negative prognostic factor. The role of lymphadenectomy in the treatment of stage II disease remains controversial because of its uncertain therapeutic benefit. Aim of this retrospective study was to investigate the impact of lymphadenectomy on tumor control and survival for dogs with stage II cMCTs. Dogs with firstly-occurring, histologically-confirmed cMCT with LN metastasis undergoing resection of the primary tumor and medical treatment thereafter were retrospectively enrolled. Dogs were classified into two groups: LN sampling (LNS; diagnosis of metastasis obtained by cytology) and regional LN dissection (LND; diagnosis obtained by histopathology). To determine the therapeutic value of lymphadenectomy, the characteristics of recurrence (local, nodal, distant) and survival were compared between groups. Evaluated outcome variables included signalment, anatomic location, diameter, ulceration, substage, surgical margins, Patnaik grading, Kiupel grading, and medical treatment. Overall, 152 dogs were included: 81 underwent LND as part of primary surgery, and 71 LNS. The median follow-up time was 409 days for LND group and 620 days for LNS group. On univariable analysis, the risk of developing local, nodal or distant relapse was significantly higher in the LNS group compared with LND ($P < 0.001$). On multivariable analysis, the risk of tumor progression and tumor related-death were 5.47 and 3.61 times higher in the LNS group, respectively ($P < 0.001$). Regional lymphadenectomy may have therapeutic value and improve prognosis

in dogs with stage II cMCTs undergoing surgical removal of the primary tumor and medical treatment.

Keywords

Mast cell tumor, lymph node metastasis, stage II, lymphadenectomy, prognosis, dog

Introduction

The benefit of surgical extirpation of metastatic lymph nodes (LNs) in the surgical management of dogs with solid cancer is unclear.

Approximately 20% of dogs with cutaneous mast cell tumors (cMCTs) have nodal metastasis (stage II) at initial diagnosis.[1] LN metastasis has been associated with decreased survival time (ST) in several studies.[2-5] Given the prognostic significance of nodal metastases, assessment of the regional LNs by cytology and/or histology is a fundamental diagnostic step in dogs with cMCT.[3,5,6]

Currently, the primary standard treatment for dogs with stage II cMCT comprises surgical excision of the primary tumor with or without radiation therapy (RT) and adjuvant medical treatment.[7-9] In this context, it has to be emphasized that the role of elective lymphadenectomy has historically been related to surgical staging for recognizing the true disease extent by detecting overt metastasis as well as pre-metastatic lesions.[3] However, the benefits of lymphadenectomy may extend beyond merely staging the burden of disease. If cancer morbidity is a function of the burden of disease in the primary tumor site and the locoregional LNs, successful removal of the primary tumor and metastatic LN would be expected to confer a significant survival advantage. Yet, to the authors' knowledge, the

therapeutic role of metastatic LN dissection has received relatively little attention, and only one retrospective study has suggested a favorable impact of lymphadenectomy on tumor specific survival (TSS) time in dogs with stage II cMCTs.[10] The authors hypothesized that the resection of the regional LN along with the primary tumor might ensure eradication of a reservoir for mast cell proliferation, thereby improving long-term outcome.[10] In a later study by Weishaar, dogs with a more extensive nodal involvement (HN2/HN3) had shorter disease-free interval and ST when compared to dogs with a less advanced nodal involvement (HN0/HN1) when evaluated with Gehan-Breslow-Wilcoxon test.[3] However, in that study, the population of included dogs was small and medical treatment was not administered to all patients, thereby biasing the outcome results.

The aim of the current retrospective study was to explore the impact of lymphadenectomy on tumor control and TSS for dogs with stage II cMCTs.

Material and methods

Case selection

Members of xxxx were invited to review their records for dogs with treatment-naive, firstly occurring, histologically confirmed cMCT with regional LN metastasis, confirmed either by cytology or histology. For the purpose of this study, stage II refers to dogs with LN metastasis regardless of the dimension of the primary cMCT, to avoid the confusions and ambiguities in the classification of WHO stage III disease. No time limits were defined for case enrollment and no minimum follow-up time was established.

To be eligible for recruitment, dogs had to undergo wide surgical excision of the primary MCT and medical treatment (consisting of cytotoxic chemotherapy, tyrosine kinase

inhibitors [TKI] or both) thereafter. Wide surgical excision was defined as a lateral margin of 2 -3 cm and a deep margin of one facial plane, depending on tumor size and location.

Information on clinical stage was obtained by means of the following: hematological and biochemical analysis; cytological evaluation of the cutaneous nodule and regional LN; thoracic radiographs; abdominal ultrasound, and fine-needle aspirates of liver and spleen.

The regional LN was defined as the closest LN in the expected lymphatic drainage, and was identified either by palpation or by ultrasound.

Dogs were classified into two groups: LN sampling (LNS; diagnosis of regional LN metastasis was made by cytology with no subsequent lymphadenectomy) and LN dissection (LND; dogs undergoing both excision of the cMCT and regional lymphadenectomy and thus whose diagnosis was obtained by histopathology). Decisions regarding whether to perform LNS or LND were made according to each clinician's discretion.

Dogs were enrolled in the LNS group if LN cytology yielded a certain diagnosis of metastasis according to Krick's criteria,[5] whereas enrollment in the LND group was possible only following histopathological confirmation of early (HN2) or overt (HN3) nodal metastasis according to Weishaar.[3]

Dogs with concurrent multiple or subcutaneous MCTs, and those with stage IV disease were excluded from the study. Dogs with nodal pre-metastatic disease on histology (HN1 based on Weishaar)[3] were also excluded.

Background information recorded for each dog included: signalment; primary tumor description (location, size, presence of ulceration); clinical substage; site of nodal involvement; LN clinical characteristics (normal size and consistency or abnormal [increased in size or with a firm consistency compared to the contralateral]; mobile or fixed); histopathological evaluation of surgical margins (clean, clean but close [presence of

neoplastic cells within 1 mm from the surgical margin], incomplete); histologic grade of the primary cMCT according to Patnaik and Kiupel classification systems;[11,12] Ki67-index (expressed in percentage by counting a total of 1000 cells in 10 high power field);[13] Kit-pattern;[14] c-kit mutational status; date of surgery; medical treatment (cytotoxic chemotherapy, TKIs or both); use of post-operative RT; local recurrence (defined as the cytological evidence of a recurrent MCT within 2 cm from previous scar); nodal relapse (defined for the LNS group as nodal progressive disease with a more than 20% increase in size or presence of new metastatic LNs, and for the LND group as presence of new metastatic LNs); distant relapse (defined as the occurrence of visceral metastasis); date of death or last follow-up examination, and cause of death.

To determine the therapeutic value of lymphadenectomy, the characteristics of relapse (local, nodal and distant) and the survival impact were compared between the LNS and LND groups.

While under medical treatment, dogs were monitored every 2-4 weeks. Afterwards, dogs were followed-up every 1-3 month, depending on clinicians' discretion and owners' compliance.

Statistical analysis

Descriptive statistics were used in the analysis of dogs and tumour characteristics. When appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus normality test. Values were expressed as mean ± standard deviation in case of normal distribution, or as median with a range in case of non-normal distribution.

The distribution of demographic features and possible outcome variables between the LNS and LND groups where assessed with Student's T-test (numerical, parametric variables), the Mann Whitney U test (numerical, non-parametric variables) or the Chi-squared test (categorical variables).

The considered variables included breed (predisposition to biologically aggressive MCTs, i.e. Shar-pei, Labrador retriever and Golden retriever)[15], age, body weight, sex, anatomic location of the primary cMCT (head and neck, trunk [including tail], limbs [excluding digital tumors], inguinal region [including perineal and scrotal], mammary region, and digits), macroscopic tumor diameter, ulceration, substage, surgical margins, Patnaik grading (P-G1, P-G2 or P-G3), Kiupel grading (K-LG or K-HG), Ki67-index, Kit staining pattern, c-Kit mutational status, medical treatment (cytotoxic chemotherapy, TKI or both) and the use of post-operative RT. For age, weight and tumor diameter, the median was used as cut-off value.

Time to local recurrence (TLR) was calculated from the date of surgery to the date of local recurrence. Time to nodal relapse (TNR) was calculated from the date of surgery to the date of nodal recurrence for LND or nodal progression for LNS. Time to distant relapse (TDR) was calculated from the date of surgery to the date of diagnosis of visceral metastases. Time to progression (TTP) was calculated from the date of surgery to the first occurrence of one or more of local recurrence, nodal or distant relapse. Dogs with no recurrence or disease progression at the date of the last visit or death were censored.

TSS was 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 MCT-related causes were considered as events.

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% CI). The influence of potential prognostic variables on tumor progression and tumor-specific survival was investigated with univariable and multivariable Cox's regression analyses.

Data were analyzed by use of commercial software programs (SPSS Statistics v. 19, IBM, Somers, NY, and Prism v. 5.0, GraphPad, San Diego, CA). *P* values ≤ 0.05 were considered significant.

Results

Patient and tumor characteristics

A total of 152 dogs fulfilled the inclusion criteria. Among these dogs, 81 underwent LND as part of primary surgery, and 71 underwent LNS. There was good balance between groups regarding demographic features and possible outcome variables (Table 1). Only medical treatment differed among groups: cytotoxic chemotherapy was more often administered to dogs in the LND group, and TKI to dogs in the LNS group ($P < 0.001$).

Among dogs undergoing LND, there were 22 (27.3%) mixed breed dogs, 11 (13.7%) Labrador retrievers, 6 (7.5%) boxers, 6 (7.4%) French bulldogs, 6 (7.4%) golden retrievers, 3 (3.7%) Maltese terriers, 3 (3.7%) shar peis, 3 (3.7%) Bernese mountain dogs, 2 (2.5%) Brittany spaniels, 2 (2.5%) pugs, 2 (2.5%) pit bull terriers, 2 (2.5%) dogo Argentino and one (1.2%) each of the following: Chihuahua, Alaskan malamute, Pomeranian, English setter, Gordon setter, grand bleu de Gascogne, bull mastiff, Jack Russell terrier, dachshund, Dalmatian, poodle, cane corso, and great Dane. Mean age was 8.3 ± 3.0 years (range, 3 to 16 years), and median weight was 24.3 kg (range, 2.5 to 58.7 kg). There were 48 female dogs (of which 39 were spayed) and 33 males (of which 6 were castrated).

The tumors were located on limbs (n=33; 40.7%), head and neck (n=20; 24.7%), digits (n=11; 13.6%), inguinal region (n=7; 8.6%), mammary region (n=6; 7.5%), and trunk

(n=4; 4.9%). Tumor diameter ranged from 0.5 to 18 cm (median, 2.5 cm); 53 (65.4%) cMCTs were not ulcerated, while 28 (34.6%) were.

Seventy-six (93.8%) dogs were asymptomatic at presentation (substage a), whereas the remaining 5 (6.2%) dogs had signs of systemic effects of cMCT (vomiting, diarrhea, pruritus, and regional edema; substage b).

Based on the Patnaik grading system, there were 2 (2.5%) P-G1 cMCTs; 58 (71.6%) P-G2 cMCTs, and 21 (25.9%) P-G3 cMCTs. Based on the Kiupel grading system, there were 53 (65.5%) K-LG cMCTs and 27 (33.3%) K-HG cMCTs. The Kiupel grade was not available for 1 (1.2%) dog.

Histopathological evaluation revealed clean surgical margins in 47 (58.1%) cMCTs, clean but close margins in 1 (1.2%) case, and incomplete margins in 33 (40.7%) cases.

Ki67 immunohistochemical labeling was available for 30 (37.0%) cases. Ki67 counts ranged from 1% to 65% with a median of 7%. Kit immunolabeling was available for 28 (34.6%) cases. Perimembranous Kit labeling (pattern 1) was observed in 8 cMCTs, focal-/stippled Kit labeling (pattern 2) was present in 9; and diffuse cytoplasmic Kit labeling (pattern 3) was found in 11. Mutational analysis was available for 43 (53.1%) cMCTs: 12 cMCTs were mutated (10 had an ITD on exon 11, and 2 had an ITD on exon 8), while the remaining 31 were wild type.

The following metastatic ipsilateral LNs were removed: popliteal (n=31; 38.4%), submandibular (n=20; 24.7%), superficial cervical (n=13; 16.0%), inguinal (n=13; 16.0%), and axillary (n=4, 4.9%). Sixteen (20%) had normal size and consistency, while 65 (80%) were abnormal; 69 (85%) were mobile and 12 (15%) were fixed. Based on the Weishaar study, 28 (34.6%) LNs were classified as HN2, and 53 (65.4%) as HN3.

Among dogs undergoing LNS, there were 18 (25.4%) mixed breed dogs, 15 (21.1%) Labrador retrievers, 6 (8.5%) boxers, 6 (8.5%) golden retrievers, 4 (5.6%) American

Staffordshire terriers, 2 (2.8%) Dobermanns, 2 (2.8%) shih tzus, 2 (2.8%) pinschers, 2 (2.8%) pit bull terriers and one each of the following: Irish setter, German shepherd dog, Australian terrier, beagle, West Highland white terrier, dogue de Bordeaux, cane corso, Bernese Mountain dog, Yorkshire terrier, rottweiler, griffon, shar pei, fila San Miguel, and cavalier King Charles spaniel. Mean age was 8.9 ± 3.0 years (range, 1 to 14 years), and median weight was 28 kg (range, 4.5 to 53 kg). There were 36 female dogs (of which 27 were spayed) and 35 males (of which 15 were castrated).

The tumors were located on limbs (n=23; 32.5%), head and neck (n=15; 21.1%), inguinal region (n=14; 19.7%), trunk and tail (n=12; 16.9%), digits (n=4; 5.6%), and mammary region (n=3; 4.2%). Tumor diameter ranged from 1 to 7 cm (median, 3 cm); 44 (62.0%) cMCTs were not ulcerated, while 27 (38.0%) were. Sixty-two (87.3%) dogs were asymptomatic at presentation, whereas the remaining 9 (12.7%) dogs had signs of systemic effects of cMCT.

Based on the Patnaik grading system, there were 3 (4.2%) P-G1 MCTs, 47 (66.2%) P-G2 cMCTs and 21 (29.6%) P-G3 cMCTs. Based on the Kiupel grading system, there were 39 (54.9%) K-LG cMCTs and 30 (42.3%) K-HG cMCTs. The Kiupel grade was not available for 2 (2.8%) dogs.

The surgical margin status was available for 60 (84.5%) cMCTs. Histopathological evaluation revealed clean surgical margins in 22 (36.7%) cMCTs, clean but close margins in 6 (10%) cases, and incomplete margins in 32 (53.3%) cases.

Ki67 immunohistochemical labeling was available for 15 (21.1%) cases. Ki67 counts ranged from 1% to 99% with a median of 13%. Kit immunolabeling was available for 26 (36.6%) cases. Kit pattern 1 was observed in 3 cMCTs, Kit pattern 2 was present in 18; and Kit pattern 3 was found in 5. Mutational analysis was available for 30 (42.3%) cMCTs: 12 cMCTs were mutated (11 had an ITD on exon 11, and 1 had an ITD on exon 8), while the remaining 18 were wild type.

Based on Krick's criteria, all dogs had a cytological diagnosis of certain LN metastasis. Metastatic ipsilateral LNs included the inguinal (n=21; 29.6%), popliteal (n=16; 22.5%), superficial cervical (n=14; 19.7%), submandibular (n=10; 14.1%), axillary (n=7; 9.9%), retropharyngeal (n=2; 2.8%), and medial iliac (n=1; 1.4%) LN. Eight (11%) had normal size and consistency, while 63 (89%) were abnormal; 53 (75%) were mobile and 18 (25%) were fixed.

Treatment and outcome

Severe complications following lymphadenectomy were not reported for any of the 81 dogs undergoing LND. All dogs received adjuvant medical therapy, consisting of cytotoxic chemotherapy (vinblastine and prednisone: n=52; vinblastine, prednisone and lomustine: n=1; vinblastine, cyclophosphamide, prednisone: n=2; chlorambucil: n=1), TKI (n=17), or both concurrently (n=8).

Twelve (14.8%) dogs also received RT to the tumor and nodal bed.

The median follow-up time was 409 days (95% CI, 298-657).

Twelve (14.8%) dogs experienced local recurrence after a median of 199 days (range, 29-1499); incomplete surgical margins had been diagnosed in 8 (67%) of these cases. Fourteen (17.3%) dogs experienced nodal relapse after a median of 193 days (range, 28-592) and 9 (11.1%) developed distant relapse after a median of 218 days (range, 52-2152). Overall median TLR, TNR and TDR were not reached. Mean TTP was 1461 days.

At the end of the study, 50 (61.7%) dogs were alive, and 31 had died because of cancer-related (n=21; 25.9%) or unrelated causes (n=10; 12.3%). Median TSS was 2213 days (95% CI, 1410-3015) (Table 2).

There was no significant difference in TLR, TNR, TDR and TSS between dogs diagnosed with HN2 and HN3 lymph node status.

All dogs in the LNS group received adjuvant medical therapy, consisting of cytotoxic chemotherapy (vinblastine and prednisone: n=22; vinblastine, prednisone and lomustine: n=3; paclitaxel: n=1), TKI (n=20), or both (n=25). Twelve (16.9%) also received RT. Both the primary cMCT and the metastatic LN were included in the treatment field.

The median follow-up time was 620 days (95% CI, 59-1207).

Thirty-one (43.7%) dogs experienced local recurrence; 19 (61%) of them had been removed with incomplete surgical margins; 51 (71.8%) dogs developed nodal relapse and 23 (32.4%) distant relapse. Overall median TLR, TNR, and TDR were 511, 170 and 1045 days, respectively. Median TTP was 170 days.

At the end of the study, 16 (22.5%) dogs were alive, and 55 had died because of cancer-related (n=45; 63.4%) or unrelated causes (n=10; 14.1%). Median TSS was 360 days (95% CI, 181-539) (Table 2).

The risk of developing local recurrence, nodal relapse or distant relapse was significantly higher in the LNS group compared with the LND group ($P < 0.001$). Overall, the risk of tumor progression was significantly higher in the LNS group (HR = 4.26 P<0.001, Table 2). The risk of tumor-related death was also significantly higher (HR=3.63, $P < 0.001$; Table 2; Figures 1 and 2).

Analysis of prognostic variables

On univariable analysis, variables significantly associated with an increased risk of tumor progression were age > 9 years, head and neck location, tumor diameter >3 cm, substage b, P-G3, K-HG, enlarged/firm LN, fixed LN, lack of lymphadenectomy and TKI administration (Table 3). Variables significantly associated with TSS were age > 9 years, lack of neutering, head and neck location, tumor diameter > 3 cm, substage b, P-G3, K-

HG, enlarged/firm LN, fixed LN, lack of lymphadenectomy and TKI administration (Table 4).

On multivariable analysis, age > 9 years, head and neck location, enlarged/firm LN and lack of lymphadenectomy were still significantly associated with tumor progression, whereas the variables associated with tumor-related death were head and neck location, K-HG and lack of lymphadenectomy. The lack of lymphadenectomy was the variable associated with the highest risk for tumor progression and the second after K-HG for tumor-related death (Tables 5 and 6).

Discussion

In the current study, a significant improvement in tumor control and TSS was observed in dogs that underwent regional LND during primary surgery for stage II cMCTs. Notably, the beneficial effects of LND were most pronounced among dogs younger than 9 years, with cMCTs arising in anatomic locations different than head and neck, smaller than 3 cm, of K-LG, and with no enlarged/firm regional LN. Most of these results are similar to previous reports.[1,12,16,17] Intuitively, it would appear that the explanation for these observations is that the patients who experienced greatest benefit were those a) with sufficient life ahead for a life-expectancy benefit to be measured and b) with a less aggressive manifestation of disease. Previously identified prognostic markers, Kiupel grade and gross enlargement and firmness of the regional LN, remained prognostically significant; the negative impact of these observations was not removed by the application of LND. Nevertheless, this is the first study including the extent of node involvement (sampled versus removed) as a death-related risk factor for cMCT.

Lymphadenectomy is increasingly employed in veterinary oncology for improved accuracy of clinical stage evaluation. It is accepted that LND is the superior technique for the diagnosis of LN metastases. The limits of cytology in over- or under-staging disease by obtaining false positive or false negative results, respectively, have been well documented.[18] Even though the sensitivity and specificity of cytological examination for the detection of LN metastasis in dogs with solid tumors (including cMCTs) have been reported to be as high as 100% and 96%, respectively,[6] in the specific case of cMCT, cytological diagnostic accuracy is hampered by an inability to accurately differentiate malignant from reactive mast cells in LN aspirates, possibly leading to false positive results.[19] In order to avoid this, in the current study strict criteria were applied to May-Grünwald-Giemsa-stained LN cytological smears to identify nodal metastatic disease. Criteria for the definition of LN metastasis included replacement of lymphoid cells 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 aggregates of more than three mast cells, according to Krick's criteria.[5] Additionally, cases were only included in the LNS group if the LN was interpreted as "certainly" metastatic according to Krick's criteria.[5]

Beside staging, our results have documented that LND is also important for survival. Nodal metastasis indicates aggressive tumor biology, but also may represent a source of subsequent metastasis, as hypothesized by the Halstedian theory.[20] In the LNS group, dogs had a significantly higher local recurrence rate (43.7% vs 14.8% in the LND group), a significant increase in nodal relapse (71.8% vs 17.3% in the LND group) and distant metastasis (32.4% vs 11.1% in the LND group). While it is difficult to clinically determine the tumor origin from which systemic metastasis derives, including the primary cancer versus the metastatic LN, the survival benefit observed in dogs undergoing LND cannot be

ignored, suggesting that tumor biology, including metastatic capability, differs between the primary site and the LNs.[21,22]It is certainly plausible that improved loco-regional control translates into a lower risk of distant spread, ultimately leading to a survival benefit.

Also, it is interesting to note that the histopathological LN status (HN2 versus HN3) did not show any significant difference in terms of outcome, suggesting that both classifications have the potential to behave aggressively, thereby requiring an additional medical intervention.

Patients with advanced mast cell neoplasia are known to suffer paraneoplastic, systemic consequences of their disease, even in the absence of detectable metastasis. In patients without detectable metastasis, morbidity and overall disease burden are correlated.[23] Therefore, a simple explanation for the observed outcome findings lies in the fact that LND removes an additional burden of cancer from the patient. Thus a potential driver for paraneoplastic morbidity consequences is also removed.

However, if the explanation for the observed findings was as simple as that given above, one would expect an improvement in overall survival and TNR following lymphadenectomy, but one would not intuitively expect an improvement in TLR and TDR. It is accepted that the observed differences in time to recurrence outcomes may have arisen due to an inherent bias or to chance. However, considering the possibility that the observed results are a true effect, this study provides evidence for a model of disease progression whereby metastatic foci in loco-regional LNs present a threat of bidirectional disease progression. In other words, the metastatic local LN can either act as a reservoir for neoplastic mast cells, which can then relocate to the primary tumor site or to other distant sites or it can exert a biological effect, which favors the development of neoplasia at those sites. Indeed, in humans with solid cancer, local reseeding from neoplastic cells located in the LNs is a well-known phenomenon, and is driven by chemoattractants released during

360 the post-surgery local wound-healing processes.[24] The same may hold true for dogs with
361 cMCTs.
362
363 The results of this study indicate prognostic benefits of regional LND of metastatic LNs for
364 dogs with surgically removed cMCT. However, the data should be interpreted with
365 caution. Every effort was made to minimize potential bias by accounting for all known
366 prognostic variables associated with both the tumors and patients; however, selection bias
367 regarding dogs' recruitment cannot be ruled out because of the retrospective nature of this
368 study. Decisions regarding whether to perform LND were made according to each
369 clinician's discretion, rather than random allocation or well-defined criteria. It is utterly
370 plausible that unknown owner and clinician perceptions or preferences may have impacted
371 the treatment decision. Also, while all dogs received some form of systemic treatment,
372 protocols were not standardized, rather the choice was left to the primary clinician. Any
373 confounding effect of adjuvant therapy choice could also have influenced outcome.
374 It must be noted that cytotoxic chemotherapy was more often offered to dogs in the LND
375 group and TKI therapy to dogs in the LNS group. This may reflect a clinical bias;
376 veterinarians managing dogs in the study generally perceived TKI therapy to offer a higher
377 probability of a durable response than cytotoxic chemotherapy to dogs with more
378 malignant disease or in which the goal of treatment was to stabilize the disease by
379 administering a cytostatic drug. By contrast, dogs with less malignant, down-staged disease
380 were considered better candidates for treatment comprising a finite course of vinblastine
381 and prednisolone. This latter treatment was regarded to confer a lower risk, lower cost,
382 shorter treatment duration and a good chance of a very good outcome for that patient
383 group.
384 Furthermore, although this study recruited cases regardless of the location of the
385 locoregional-draining node, inadvertently, it primarily evaluated dogs with readily

accessible LNs. This means that caution must be exercised in applying the conclusions of this study to dogs that were poorly represented. The morbidity associated with removal of an intra-cavitary LN would be expected to be greater than that for removal of a peripheral LN. This increase in morbidity might offset some of the survival advantage supposedly achieved and may create other problems not highlighted in this study.

Our study raises several important questions for the management of dogs with stage II cMCTs.

First, should LND of metastatic LNs become a standard component of surgical management of cMCTs? Given the outcome advantages and the lack of morbidity observed in this study, we believe the answer to this question is a qualified yes. In this study cohort, sufficient patients enjoyed a survival benefit that a statistically significant improvement was noted for the LND group as a whole. However, it should be noted that a proportion of individual patients did not enjoy a survival benefit. Further studies to define optimal application of LND recommendation would be useful.

Future studies might explore whether medical treatment is necessary for this whole population of dogs, as there is no clear consensus regarding systemic treatment for stage II cMCTs in terms of the need for, and choice of, adjuvant cytotoxic chemotherapy regimen, as highlighted in a recent Letter to the Editor in this journal.[25] In some patient groups, consider older patients and those with a lesser metastatic burden, it is conceivable that the survival advantage of LND is sufficient to achieve the full remainder of that patient's life expectancy, meaning that adjuvant medical therapy would no longer confer a survival advantage.

Second, should LND be performed systematically, regardless of the nodal disease status? Undoubtedly accurate surgical staging, including LND, recognizes the true extent of

disease by detection of occult node metastases (HN1). It remains to be explored whether lymphadenectomy of HN1 nodes further improves prognosis as compared to surgical excision of the primary cMCT only.

Last, the regional LN does not necessarily represent the sentinel LN, which is by definition the first node that receives direct lymphatic drainage from the tumor rather than the closest node to the primary tumor.[26,27] Different methods of identification of the sentinel LN have been used, including radioisotope injection, vital blue dye, or lymphangiography. For LNs not obviously metastatic, sentinel LNs techniques rather than anatomic sampling should be applied to accurately reflect the metastatic status. It could be suggested that if sentinel LN mapping had been used to drive LN extirpation, the difference between outcomes for the two patient groups might have been even greater.

In conclusion, the present study indicates a potential therapeutic value of metastatic regional lymphadenectomy in the context of surgical removal of cMCT and the administration of adjuvant systemic medical treatment. This finding was demonstrated by the evidence of a lower local recurrence, nodal relapse rate and distant metastatic rate with LND versus LNS. The authors propose that the need to secure loco-regional control of solid tumors will assume increasing importance as systemic therapies improve and the incidence of death from distant spread reduces.

References

1. Stefanello D, Buracco P, Sabattini S, Finotello R, Giudice C, Grieco V, Iussich S, Tursi M, Scase T, Di Palma S, Bettini G, Ferrari R, Martano M, Gattino F, Marrington M, Mazzola M, Elisabetta Vasconi M, Annoni M, Marconato L. Comparison of 2- and 3-

438 category histologic grading systems for predicting the presence of metastasis at the time of
439 initial evaluation in dogs with cutaneous mast cell tumors: 386 cases (2009-2014). J Am
440 Vet Med Assoc. 2015; 246: 765-769.
441 2. Pizzoni S, Sabattini S, Stefanello D, Dentini A, Ferrari R, Dacasto M, Giantin M,
442 Laganga P, Amati M, Tortorella G, Marconato L. Features and prognostic impact of distant
443 metastases in 45 dogs with de novo stage IV cutaneous mast cell tumours: A prospective
444 study. Vet Comp Oncol. 2017 Feb 23. doi: 10.1111/vco.12306.
445 3. Weishaar KM, Thamm DH, Worley DR, Kamstock DA. Correlation of nodal mast cells
446 with clinical outcome in dogs with mast cell tumour and a proposed classification system
447 for the evaluation of node metastasis. J Comp Pathol. 2014; 151: 329-338.
448 4. Murphy S, Sparkes AH, Blunden AS, Brearley MJ, Smith KC. Effects of stage and
449 number of tumours on prognosis of dogs with cutaneous mast cell tumours. Vet Rec 2006;
450 158: 287-291.
451 5. Krick EL, Billings AP, Shofer FS, Watanabe S, Sorenmo KU. Cytological lymph node
452 evaluation in dogs with mast cell tumours: association with grade and survival. Vet Comp
453 Oncol. 2009; 7: 130-138.
454 6. Langenbach A, McManus PM, Hendrick MJ, Shofer FS, Sorenmo KU. Sensitivity and
455 specificity of methods of assessing the regional lymph nodes for evidence of metastasis in
456 dogs and cats with solid tumors. J Am Vet Med Assoc. 2001; 218: 1424-1428.
457 7. Lejeune A, Skorupski K, Frazier S, Vanhaezebrouck I, Rebhun RB, Reilly CM,
458 Rodriguez CO Jr. Aggressive local therapy combined with systemic chemotherapy
459 provides long-term control in grade II stage 2 canine mast cell tumour: 21 cases (1999-
460 2012). Vet Comp Oncol. 2015; 13: 267-280.
461 8. Chaffin K, Thrall DE. Results of radiation therapy in 19 dogs with cutaneous mast cell
462 tumor and regional lymph node metastasis. Vet Radiol Ultrasound. 2002; 43: 392-5.

463 9. Thamm DH, Turek MM, Vail DM. Outcome and prognostic factors following adjuvant
464 prednisone/vinblastine chemotherapy for high-risk canine mast cell tumour: 61 cases. J Vet
465 Med Sci. 2006; 68: 581-587.
466 10. Baginski H, Davis G, Bastian RP. The prognostic value of lymph node metastasis with
467 grade 2 MCTs in dogs: 55 cases (2001-2010). J Am Anim Hosp Assoc. 2014; 50: 89-95.
468 11. Patnaik AK, Ehler WJ, MacEwen EG. Canine cutaneous mast cell tumor: morphologic
469 grading and survival time in 83 dogs. Vet Pathol 1984; 21: 469-474.
470 12. Kiupel M, Webster JD, Bailey KL, Best S, DeLay J, Detrisac CJ, Fitzgerald SD,
471 Gamble D, Ginn PE, Goldschmidt MH, Hendrick MJ, Howerth EW, Janovitz EB, Langohr
472 I, Lenz SD, Lipscomb TP, Miller MA, Misdorp W, Moroff S, Mullaney TP, Neyens I,
473 O'Toole D, Ramos-Vara J, Scase TJ, Schulman FY, Sledge D, Smedley RC, Smith K, W
474 Snyder P, Southorn E, Stedman NL, Steficek BA, Stromberg PC, Valli VE, Weisbrode SE,
475 Yager J, Heller J, Miller R. Proposal of a 2-tier histologic grading system for canine
476 cutaneous mast cell tumors to more accurately predict biological behavior. Vet Pathol
477 2011; 48: 147-155.
478 13. Vascellari M, Giantin M, Capello K, Carminato A, Morello EM, Vercelli A, Granato
479 A, Buracco P, Dacasto M, Mutinelli F. Expression of Ki67, BCL-2, and COX-2 in canine
480 cutaneous mast cell tumors: association with grading and prognosis. Vet Pathol. 2013; 50:
481 110-121.
482
483 14. Kiupel M, Webster JD, Kaneene JB, Miller R, Yuzbasiyan-Gurkan V. The use of KIT
484 and tryptase expression patterns as prognostic tools for canine cutaneous mast cell tumors.
485 Vet Pathol. 2004; 4: 371-377.
486 15. Moirano SJ, Lima SF, Hume KR, Brodsky EM. Association of prognostic features and
487 treatment on survival time of dogs with systemic mastocytosis: A retrospective analysis of
488 40 dogs. Vet Comp Oncol. 2018; 16: E194-E201.

16. Kiupel M, Webster JD, Miller RA, Kaneene JB. Impact of tumour depth, tumour location and multiple synchronous masses on the prognosis of canine cutaneous mast cell tumours. J Vet Med A Physiol Pathol Clin Med. 2005; 52: 280-286.

17. Gieger TL, Théon AP, Werner JA, McEntee MC, Rassnick KM, DeCock HE. Biologic behavior and prognostic factors for mast cell tumors of the canine muzzle: 24 cases (1990-2001). J Vet Intern Med. 2003; 17: 687-692.

18. Ku CK, Kass PH, Christopher MM. Cytologic-histologic concordance in the diagnosis of neoplasia in canine and feline lymph nodes: a retrospective study of 367 cases. Vet Comp Oncol. 2017; 15: 1206-1217.

19. Mutz ML, Boudreaux BB, Royal A, Merchant S, Pucheu-Haston C, Griffith EH, Gieger TL. Cytologic comparison of the percentage of mast cells in lymph node aspirate samples from clinically normal dogs versus dogs with allergic dermatologic disease and dogs with cutaneous mast cell tumors. J Am Vet Med Assoc. 2017; 251: 421-428.

20. Halsted WS. The results of radical operations for the cure of carcinoma of the breast. Ann Surg 1907; 46: 1–19.

21. Akita H, Doki Y, Yano M, Miyata H, Miyashiro I, Ohigashi H, Ishikawa O, Nishiyama A, Imaoka S. Effects of neoadjuvant chemotherapy on primary tumor and lymph node metastasis in esophageal squamous cell carcinoma: additive association with prognosis. Dis Esophagus. 2009; 22: 291-297.

22. Fruhwirth GO, Diocou S, Blower PJ, Ng T, Mullen GE. A whole-body dual-modality radionuclide optical strategy for preclinical imaging of metastasis and heterogeneous treatment response in different microenvironments. J Nucl Med. 2014; 55: 686–694.

23. Blackwood L, Murphy S, Buracco P, De Vos JP, De Fornel-Thibaud P, Hirschberger J, Kessler M, Pastor J, Ponce F, Savary-Bataille K, Argyle DJ. European consensus document on mast cell tumours in dogs and cats. Vet Comp Oncol. 2012; 10: e1-e29.

514 24. Karnoub AE, Weinberg RA. Chemokine networks and breast cancer metastasis. Breast
515 Dis. 2006-2007; 26: 75-85.
516 25. Schulman FY. Is lymph node metastasis of canine grade 2 MCTs justification for
517 adjuvant therapy? Vet Comp Oncol. 2015; 13: 151.
518 26. Brissot HN, Edery EG. Use of indirect lymphography to identify sentinel lymph node
519 in dogs: a pilot study in 30 tumours. Vet Comp Oncol. 2017; 15: 740-753.
520 27. Worley DR. Incorporation of sentinel lymph node mapping in dogs with mast cell
521 tumours: 20 consecutive procedures. Vet Comp Oncol. 2014; 12: 215-226.
522
523

524 **Table 1.** Distributions of variables potentially associated with prognosis in 152 dogs with stage II
525 cutaneous mast cell tumor treated by surgical excision of the primary tumor and systemic medical
526 therapy with or without concurrent lymphadenectomy.
527

Variable	**Lymph node dissection** (*n* = 81)	**Lymph node sampling** (*n* = 71)	***P***
Breed			0.492
Shar Pei, Labrador retriever, Golden retriever	20	22	
other	61	49	
Age			0.200
mean ± SD	8.3 ± 3.0 years	8.9 ± 3.0 years	
Weight			0.126
median (range)	24.3 (2.5-58.7) kg	28.0 (4.5-53.0) kg	
Sex			0.290
male	33	35	
female	48	36	
Neutering status			0.655
no	36	29	
yes	45	42	
Anatomic location			0.874
head and neck	20	15	
trunk and limbs	37	34	
inguinal, mammary, digital	24	22	

Tumor diameter *median (range)*	2.5 (0.5-18.0) cm	3.0 (1.0-70) cm	0.786
Ulceration *no* *yes*	53 28	44 27	0.655
Substage *a* *b*	76 5	62 9	0.167
Lymph node size and consistency *normal* *abnormal*	16 65	8 63	0.152
Lymph node mobility *mobile* *fixed*	69 12	53 18	0.103
Surgical margins *clean and clean but close* *incomplete*	48 33	28 32	0.138
Patnaik grading *grades I and II* *grade III*	60 21	50 21	0.617
Kiupel grading *low grade* *high grade*	53 27	39 30	0.224
Ki67-index			0.571

median (range)	7% (1-65%)	13% (1-99%)	
Kit-staining pattern			0.179
I	8	3	
II/III	20	23	
c-Kit mutational status			0.406
non mutated	31	18	
mutated	12	12	
Medical treatment			<0.001*
cytotoxic chemotherapy	56	26	
TKI	17	20	
cytotoxic chemotherapy and TKI	8	25	
Radiation therapy			0.8246
no	69	59	
yes	12	12	

528 * = significant; SD = standard deviation; TKI = tyrosine kinase inhibitor.

529

530

Table 2. Time to progression, survival time and evaluation of the risk of developing tumor progression and tumor-related death in 152 dogs with stage II cutaneous mast cell tumor treated by surgical excision of the primary tumor and systemic medical therapy with or without concurrent lymphadenectomy.

	Overall	**Lymph node dissection** (*n* = 81)	**Lymph node sampling†** (*n* = 71)	**Hazard ratio** (95% CI)	***P*‡**
Median time to progression (95% CI)	462 days (276-648)	NR	170 days (110-230)	4.26 (2.63-6.92)	<0.001*
Median time to local recurrence (95% CI)	NR	NR	511 days (103-919)	4.02 (2.06-7.87)	<0.001*
Median time to nodal relapse (95% CI)	600 days (263-915)	NR	170 days (86-254)	6.05 (3.34-10.96)	<0.001*
Median time to distant relapse (95% CI)	2152 days (1749-2684)	NR	1045 days (556-1534)	4.22 (1.94-9.18)	<0.001*
Median tumor specific survival (95% CI)	811 days (287-1335)	2213 days (1410-3015)	360 days (181-539)	3.63 (2.15-6.13)	<0.001*

* = significant; † = reference category; ‡ = derived by univariate analysis; CI = confidence interval; NR = not reached.

539 **Table 3.** Univariable Cox regression analysis of variables potentially associated with increased risk

540 of tumor progression in 152 dogs with stage II cutaneous mast cell tumors.

Variable	**No. of cases**	**Hazard ratio** (95% CI)	***P***
Breed		1.37	0.230
Shar Pei, Labrador retriever, Golden retriever	42	(0.82-2.30)	
other‡	110		
Age†		2.48	<0.001*
≤ 9 years	88	(1.57-3.91)	
> 9 years‡	64		
Weight†		1.16	0.507
≤ 26 kg	75	(0.74-1.82)	
> 26 kg‡	76		
Sex		1.27	0.288
male‡	68	(0.82-1.98)	
female	84		
Neutering status		1.38	0.153
no‡	65	(0.89-2.15)	
yes	87		
Anatomic location		1.87	0.008*

head and neck‡	35	(1.17-2.98)	
other	117		
Tumor diameter†		1.87	0.012*
≤ 3 cm	93	(1.15-3.03)	
> 3 cm‡	45		
Ulceration		1.16	0.521
no	97	(0.74-1.83)	
yes‡	55		
Substage		2.38	0.008*
a	138	(1.25-4.52)	
b‡	14		
Surgical margins		1.59	0.053
clean and clean but close	76	(0.99-2.53)	
incomplete‡	65		
Patnaik grading		2.18	0.001*
grades I and II	110	(1.38-3.45)	
grade III‡	42		
Kiupel grading		2.32	<0.001*
low grade	92	(1.47-3.66)	

high grade‡	57		
Lymph node size and consistency *normal* *abnormal‡*	24 128	4.67 (1.70-12.79)	0.003*
Lymph node mobility *mobile* *fixed‡*	122 30	2.12 (1.28-3.50)	0.003*
Lymphadenectomy *no‡* *yes*	71 81	4.26 (2.63-6.92)	<0.001*
Medical treatment *Cytotoxic chemotherapy* *TKI‡*	82 70	1.67 (1.32-2.12)	<0.001*
Radiation therapy *no* *yes‡*	128 24	1.28 (0.74-2.22)	0.375

* = significant; † = median used as cut-off value; ‡ = reference category; CI = confidence interval; TKI = tyrosine kinase inhibitor (either alone or combined with cytotoxic chemotherapy); NR = not reached.

545 **Table 4.** Univariable Cox regression analysis of variables potentially associated with increased risk
546 of tumor-related death in 152 dogs with stage II cutaneous mast cell tumors.

Variable	**No. cases**		**Hazard ratio** (95% CI)	***P***
Breed			1.50	0.181
Shar Pei, Labrador retriever, Golden retriever	42		(0.83-2.70)	
other‡	110			
Age†			3.28	<0.001*
≤ 9 years	88		(1.98-5.46)	
> 9 years‡	64			
Weight†			1.01	0.972
≤ 26 kg	75		(0.62-1.65)	
> 26 kg‡	76			
Sex			1.50	0.103
male‡	68		(0.92-2.43)	
female	84			
Neutering status			1.65	0.044*
no‡	65		(0.01-2.67)	
yes	87			
Anatomic location			1.86	0.015*

head and neck‡	35		(1.13-3.08)	
other	117			
Tumor diameter†			2.54	0.001*
≤ 3 cm	93		(1.50-4.32)	
> 3 cm‡	45			
Ulceration			1.60	0.057
no	97		(0.99-2.61)	
yes‡	55			
Substage			2.90	0.005*
a	138		(1.37-6.14)	
b‡	14			
Surgical margins			1.49	0.132
clean and clean but close	76		(0.89-2.49)	
incomplete‡	65			
Patnaik grading		)	2.63	0.001*
grades I and II	110		(1.59-4.35)	
grade III‡	42			
Kiupel grading			3.05	<0.001*
low grade	92		(1.85-5.04)	
high grade‡	57			

Lymph node size and consistency *normal* *abnormal‡*	 24 128		5.01 (1.57-15.99)	0.006*
Lymph node mobility *mobile* *fixed‡*	 122 30		3.07 (1.79-5.25)	<0.001*
Lymphadenectomy *no‡* *yes*	71 81		3.63 (2.15-6.13)	<0.001*
Medical treatment *cytotoxic chemotherapy* *TKI*	82 70		1.58 (1.22-2.06)	0.001*
Radiation therapy *no* *yes‡*	128 24		1.17 (0.61-2.24)	0.637

* = significant; † = median used as cut-off value; ‡ = reference category; CI = confidence interval; TKI = tyrosine kinase inhibitor (either alone or combined with cytotoxic chemotherapy).

Table 5. Multivariable Cox regression analysis of variables potentially associated with increased risk of tumor progression in 152 dogs with stage II cutaneous mast cell tumors.

Variable	**Hazard ratio**	**95% CI**	***P***
Age > 9 years†	1.84	1.06-3.18	0.029*
Head and neck location	2.09	1.18-3.71	0.012*
Tumor diameter > 3 cm†	1.43	0.80-2.55	0.223
Substage b	1.75	0.70-4.38	0.228
Patnaik grade 3	1.16	0.50-2.70	0.724
Kiupel high grade	1.75	0.80-3.79	0.158
Enlarged/firm lymph node	3.38	1.14-9.98	0.028*
Fixed lymph node	1.13	0.60-2.14	0.704
Lack of lymphadenectomy	5.47	3.16-9.46	<0.001*
TKI administration‡	1.10	0.82-1.48	0.516

* = significant, † = median value, ‡ = tyrosine kinase inhibitor (either alone or combined with cytotoxic chemotherapy).

Table 6. Multivariable Cox regression analysis of variables potentially associated with increased risk of tumor-related death in 152 dogs with stage II cutaneous mast cell tumors.

Variable	**Hazard ratio**	**95% CI**	***P***
Age > 9 years§	1.75	0.94-3.25	0.075
Lack of neutering	1.37	0.76-2.44	0.294
Head and neck location	2.10	1.08-4.11	0.029*
Tumor diameter > 3 cm§	1.56	0.80-3.04	0.189
Substage b	2.03	0.67-6.25	0.217
Patnaik grade 3	1.66	0.68-4.00	0.262
Kiupel high grade	3.89	1.70-8.91	0.001*
Enlarged/firm lymph node	3.21	0.93-11.07	0.064
Fixed lymph node	1.96	0.98-3.91	0.058
Lack of lymphadenectomy	3.61	1.96-6.65	<0.001*
TKI administration‡	1.08	0.74-1.56	0.686

* = significant; † = median value, ‡ = tyrosine kinase inhibitor (either alone or combined with cytotoxic chemotherapy).

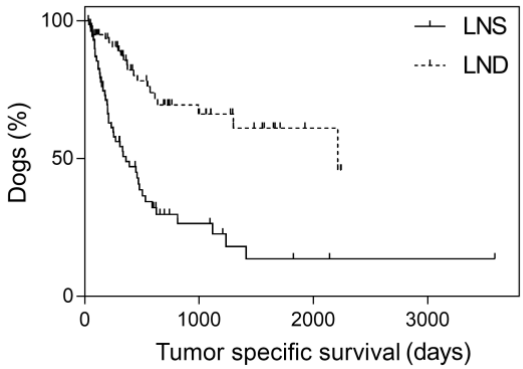
.

562 **Figure legends**

563 **Figure 1.** Time to progression for dogs with stage II cutaneous MCT treated by surgical
564 excision of the primary tumor, systemic medical treatment and metastatic lymph node
565 sampling (LNS) or dissection (LND). In the LND group, dogs had a significantly longer
566 time to progression (median, not reached versus 170 days, respectively; $P < 0.001$).

567

568 **Figure 2.** Tumor-specific survival for dogs with stage II cutaneous MCT treated by
569 surgical excision of the primary tumor, systemic medical treatment and metastatic lymph
570 node sampling (LNS) or dissection (LND). In the LND group, dogs had a significantly
571 longer survival time (median, 2213 days versus 360 days, respectively; $P < 0.001$).



572