A retrospective study on prophylactic regional lymphadenectomy versus nodal observation only in the management of dogs with stage I, completely resected, low-grade cutaneous mast cell tumors

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A retrospective study on prophylactic regional lymphadenectomy versus nodal observation only in the management of dogs with stage I, completely resected, low-grade cutaneous mast cell tumors

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Abstract

Background. While lymphadenectomy of metastatic lymph nodes (LNs) has been associated with improved outcome, the clinical utility of prophylactic lymphadenectomy in dogs with stage I cutaneous mast cell tumors (cMCTs) remains a controversial topic. To assess the therapeutic role of lymphadenectomy of uninvolved regional LNs, the long-term outcome of cMCT-bearing dogs with cytologically negative and surgically unresected regional LNs (observation only, OO) was compared with that of dogs with a surgically resected and histologically negative regional LNs (prophylactic regional lymphadenectomy, PRL).

Results. A retrospective analysis of 64 dogs with a low-grade, completely resected stage I cMCT was performed: 35 (54.7%) dogs were subjected to OO and 29 (45.3%) underwent PRL. Dogs were monitored for a median of 813 and 763 days in the OO group and PRL group, respectively. The number of dogs undergoing MCT progression was significantly higher in the OO group ($P = 0.028$) and curve comparison revealed a tendency to a better time to progression in the PRL group ($P = 0.058$). No significant difference in survival time ($P = 0.294$) was observed between dogs in the OO and PRL groups.

Conclusions. Our results showed that lack of immediate lymphadenectomy was associated with a higher risk for tumor progression. This preliminary judgement, reinforced by the findings that lymphadenectomy was well tolerated in all cases, and that histopathology provides the definitive assessment of the nodal pathological status, may suggest that prophylactic lymphadenectomy is indicated in the management of stage I MCTs. Larger prospective studies are warranted for generating clinical evidence of this latter hypothesis.

Keywords

Canine, lymphadenectomy, mast cell tumor, observation, stage I

Background

In canine cutaneous mast cell tumors (cMCTs), lymphatic drainage from the primary tumor has long been recognized as the most common initial route of metastatic spread, with the first site of metastasis identified as the draining nodal basin.[1–3]

While the therapeutic effect of regional lymphadenectomy has been documented in dogs with stage II MCTs, the role of prophylactic regional lymphadenectomy in animals with stage I disease remains a controversial topic.[4] In daily routine, dogs very often present with a well-differentiated cMCT and a cytologically negative lymph node (LN). It is difficult to advise owners about the need to surgically remove the LN alongside the primary tumor, as the amount and quality of information currently available does not offer a definitive answer to the question of the prognostic effect of prophylactic regional lymphadenectomy in early stage cMCTs.

Both an elective lymphadenectomy and a watchful-waiting policy have their proponents. The suspected high incidence rate of undetected or late LN metastasis in cMCTs is the main argument in favor of prophylactic lymphadenectomy, which is based on the rationale that further metastatic spread could be prevented at the level of the regional LN by eliminating the potential first neoplastic reservoir.[8]

Conversely, the main arguments against prophylactic lymphadenectomy include the morbidities from the procedure including risk of lymphedema, increased length of surgery and complications from wound healing with unclear benefit, and the interference with the protective immune response to metastatic disease by removal of unaffected regional LNs.[5,6] This being said, it must be stressed that such evidence derives from human medicine only, where patients undergo massive nodal dissection and immunity studies have been performed.

Additionally, the recent introduction of sentinel LN mapping in the diagnostic work-up of MCTs has introduced the question regarding the clinical usefulness of prophylactic regional lymphadenectomy. According to two recent studies, the sentinel LN was different to the regional LN in 42-60% of dogs with cMCT.[3,7]

Another problem of utmost importance concerning prophylactic lymphadenectomy indication is related to the diagnostic methods to classify a dog as node-positive or negative. The clinical examination, upon which the WHO classification is based, is far from being accurate, as palpation as well as imaging studies are unreliable predictors of nodal metastasis by themselves.

It has been recently shown that non-palpable or normal-sized LNs may harbor metastatic disease and approximately 50% of those nodes either had early metastatic (HN2 according to the Weishaar classification) or overtly metastatic (HN3) disease, whereas the other half of dogs had nodes with no evidence of metastatic disease (HN0) or minimal suspicion of metastasis (HN1).[8,9]

In this frame, cytologic evaluation of the regional LN is always advised for the assessment of metastatic involvement. Fine-needle aspiration (FNA) of the regional LN has been established as a cost-effective diagnostic tool to screen dogs for metastatic disease.

Until 2009, the reporting and interpretation of LN cytology had caused considerable confusion in comparing results from different settings. The introduction of the Krick criteria provided the opportunity to establish standard terminology and reporting guidelines for different diagnostic categories.[10] Based on cytology, five categories associated with escalating risk of malignancy have been proposed: “normal LNs”, “hyperplastic LNs”, “possible”, “probable” and “certain” metastasis based on the number of mast cells and the number and size of mast cell aggregates.[10] However, not unexpectedly, cytologic diagnosis of nodal metastasis may yield false-positive or false-negative results, leading to >25% of discrepant cases when cytology and histology are compared.[11,14]

Another classification has been proposed by Weishaar et al. to standardize the histological assessment of metastatic involvement in dogs with cMCTs. While the labeling of the categories HN1 and HN2 is misleading, and the system is not based on a standardized trimming approach of examined nodes that may result in similar high false-positive and false-negative results as the cytologic system, the proposed classification was found to correlate with clinical outcome in the original study.[9] Regardless, the question that arises is whether LNs with no to rare (0–3), scattered, individualized mast cells in sinuses and/or parenchyma (HN0) or greater than three individualized

mast cells in sinuses and/or parenchyma in a minimum of four high-power fields (HN1) represent no metastatic disease or whether dogs with such nodes will go on to develop macroscopic disease, stressing a different biology at play. If HN0/HN1 LNs are essentially clinically and prognostically insignificant, then this would limit lymphadenectomy to only those LNs harboring metastatic disease (HN2/HN3), thereby eliminating the need for consideration of routine lymphadenectomy. Such distinct difference would also further highlight the need of more accurately determining those nodes with true evidence of metastatic disease.

Thus, to investigate whether removal of potential clinically occult metastatic disease is associated with improved outcome, we first carried out an agreement study aimed at assessing the concordance between Krick's cytological classification and Weishaar's histological classification in diagnosing a LN as non-metastatic. Then, we retrospectively compared dogs with stage I, completely resected, low-grade cMCTs undergoing prophylactic regional lymphadenectomy (PRL group) with those where the regional LN was only monitored over time (observation only group, OO group).

We hypothesized that PRL provides a clinical benefit and is well tolerated.

Informed consent was obtained from animal owners for using data for the research purpose. Since this was a retrospective study, no approval from the Ethical Committee was required.

Results

Agreement study

Eighty-two cMCT-bearing dogs with cytologically negative regional LN undergoing subsequent lymphadenectomy and histological examination were reviewed: 48 (58.5%) LN aspirates were interpreted as normal and 34 (41.5%) as reactive. On the original histopathology reports, 48 (58.5%) LNs were interpreted as non-metastatic (HN0), 30 (36.6%) as pre-metastatic (HN1) and 4 (4.9%) as early metastatic (HN2). The negative predictive value of cytology in the identification of

cMCT nodal metastases was 95.1%. This was considered sufficient to confirm the reliability of cytology in the identification of dogs without LN metastasis and to perform the subsequent clinical study.

Clinical study

Overall, 64 dogs were included in the analysis: 35 (54.7%) were subjected to OO and 29 (45.3%) underwent PRL.

Patients and tumor characteristics

Among OO dogs, the most represented breeds were Labrador retrievers (n=10, 28.6%), Boxer (n=10, 28.6%) and American Staffordshire terriers (n=4, 11.4%). Of the remaining dogs, 3 were mixed-breed dogs, and 7 were breeds that were represented once or twice.

Median age was 6 years (range, 2-11) and median weight was 33 kg (range, 8.4-50.4). There were 18 females (15 spayed) and 17 males (12 neutered).

Tumors were located on limbs (n=16; 45.7%), trunk (n=9; 25.7%), head and neck (n=8; 22.9%), and inguinal region (n=2; 5.7%). Median maximum tumor diameter was 1.4 cm (range, 0.5-5.4); 33 (94.3%) cMCTs were not ulcerated, while 2 (5.7%) were. According to Krick's criteria, 27 (77.1%) LN aspirates were interpreted as normal and 8 (22.9%) as reactive. All dogs were asymptomatic at presentation.

Based on the Patnaik grading system, there were 5 (14.3%) grade 1 cMCTs, and 30 (85.7%) grade 2 cMCTs. All were Kiupel low-grade.

Among PRL dogs, the most represented breed was Labrador retriever (n=7, 24.1%). Of the remaining dogs, 7 (24.1%) were mixed-breed dogs, and 12 were breeds that were represented once or twice. Median age was 7 years (range, 1-13) and median weight was 27.4 kg (range, 5-55). There were 18 females (14 spayed) and 11 males (3 neutered).

The tumors were located on limbs (n=11; 37.9%), head and neck (n=8; 27.6%), trunk (n=4; 13.8%), mammary region (n=3; 10.3%), and inguinal region (n=3; 10.3%). Median maximum tumor

diameter was 1.3 cm (range, 0.3-9 cm); 27 (93.1%) cMCTs were not ulcerated, while 2 (6.9%) were.

All dogs were asymptomatic at presentation.

Based on the Patnaik grading system, there were 3 (10.3%) grade 1 cMCTs; and 26 (89.7%) grade 2 cMCTs. All were Kiupel low-grade. Nineteen (65.5%) LNs were interpreted as non-metastatic (HN0) and 10 (34.5%) as pre-metastatic (HN1).

The only difference among groups regarding demographic features and possible prognostic variables was a tendency towards a proportion of breeds predisposed to low-grade cMCTs in the OO group (Table 1).

Treatment and outcome

In the OO group, the median follow-up time was 813 days (range, 290-2900). Overall, 6 dogs (17.1%) experienced cMCT progression after a median of 822 days (range, 560-1380): 4 (11.4%) experienced local relapse, 2 (5.7%) experienced nodal metastases in the LNs that had been previously aspirated, and 1 (2.9%) developed visceral metastasis. Median TTP was not reached. Twelve (34.3%) dogs developed new cMCTs after a median of 734 days (range, 197-1409).

At the end of the study, 25 (71.4%) dogs were alive, 7 (20%) had died because of tumor-unrelated causes, and 3 (8.6%) had died because of cMCT-related causes after 1215, 1300 and 1471 days. Median ST was not reached.

In the PRL group, surgical complications related to lymphadenectomy did not occur, and no longer hospitalization was required compared with dogs undergoing surgical resection of the primary tumor only. The median follow-up time was 763 days (range, 181-2039). None experienced cMCT progression. Three dogs (10.3%) developed *de novo* cMCTs after 321, 417 and 1092 days.

At the end of the follow-up period, 28 (96.6%) dogs were alive, and 1 (3.4%) had died because of tumor-unrelated causes after 835 days.

The number of dogs undergoing cMCT progression was significantly higher in the OO group (P=0.028) and curve comparison revealed a tendency to a better TTP in the PRL group (P=0.058; Figure 1; Table 1). Similarly, the number of dogs developing new cMCTs was significantly higher in the OO group (P=0.037; Table 1).

No significant difference in ST (P=0.294) was observed between dogs in the OO and PRL groups (Figure 2).

On Cox proportional hazards regression analysis, no factor was significantly associated with an increased risk of cMCT progression or cMCT-related death.

cMCT progression and cMCT-related death were not affected by Krick cytological LN score (normal or reactive) in the OO group, or by Weishaar histological LN score (HN0 or HN1) in the PRL group.

Discussion

Over the past decade, meaningful treatments for canine cMCTs have been developed. Nevertheless, the significant uncertainty in staging work-up and the considerable variability in current practice, mainly due to the lack of prospective evidence, have led to the unstandardized management of localized disease. While lymphadenectomy is the current standard approach for clinically suspected or positive LNs, regardless of histological grade of the primary tumor,[4,21,22] whether clinically unaffected LNs should undergo prophylactic regional lymphadenectomy when the primary cMCT is resected or whether only the primary cMCT should be resected remains a dilemma. Thus, the goal of this retrospective study was to assess the therapeutic role of prophylactic lymphadenectomy of pathologically uninvolved regional LNs in canine cMCTs. Our results overall showed no significant differences in ST between operated dogs and those undergoing OO. However, a significantly higher

proportion of dogs developing tumor progression and new cMCTs was observed in the group of dogs not receiving an elective regional LN dissection as part of their primary therapy.

As a general rule, an accurate preoperative diagnosis and strict follow-up are required to provide minimally invasive treatment while ensuring the therapeutic effect by narrowing down the target based on the risk–benefit balance. In other words, when it comes to surgical management, based on the current evidence, the extent of LN dissection should be adapted to clinical stage, as this corresponds to metastatic spread. To do so, several critical aspects need to be taken into consideration.

First, the identification of pathologically negative LNs contributes to the problem. Peripheral LNs are initially evaluated by means of physical examination and cytology, and a high degree of inaccuracy for these methods has been documented in the literature.[8,11,13]. While the ultimate goal of FNA is to obtain cytologic material sufficiently to render a diagnosis of metastatic or non-metastatic LNs confidently, based on the current literature, the proportion of clinically negative, histologically positive cases ranges, in a worrying way, from 10 to 50%.[8,11,14]

Krick et al. established standard cytologic reporting and terminology guidelines;[10] however, the reliability and accuracy of any reporting system is built on experience, not only with cytologic interpretations, but years of follow-up of cytologic specimens and their correlations with histopathology whenever available. In the current study, 82 cytologically negative nodes from the same institutions with the available corresponding histological reports on the surgical sample were retrospectively reviewed, yielding a false negative rate of approximately 5%.

Moreover, while FNA of the regional LN is quick and cost-effective, in routine clinical practice the acquisition of diagnostic and representative samples may be hampered by several issues, including sampling error, difficulties in approaching non-palpable LNs and lack of ultrasound guidance. In doubtful cases or non-diagnostic/poorly-representative samples, lymphadenectomy should be performed to obtain a histopathological diagnosis.

Second, the indications for PRL remain subjects of much debate, since there are widely divergent views concerning the efficacy of routine lymphadenectomy and no evidence-based guidelines. Resection of the primary cMCT and concurrent lymphadenectomy or resection of the primary cMCT only have both advantages and disadvantages.

The argument in favor of PRL is based on the possibility that clinically or even histologically normal nodes may contain isolated malignant cells which, if not removed, may worsen outcome.[4] It is hypothesized that neoplastic cells may lie dormant in the regional LN for a considerable amount of time, only to recur or spread at a later point. This phenomenon has been well documented in human patients with melanoma, and prophylactic lymphadenectomy of the sentinel LN is recommended for selected patients.[15,16]

Conversely, removing LNs that appear unaffected may be unnecessary and potentially harmful, and the following have been considered issues speaking against PRL, including higher morbidity associated with the procedure, increased duration of surgery and costs. Also, considering the host-tumor immunologic relationships, there may be concern raised for routinely removing unaffected LNs. Indeed, the extirpation of an immunologic defense organ may alter the host response to the tumor.[6]

In the current series, lymphadenectomy was well tolerated, with no reported surgical complications. Additionally, even if this study failed to demonstrate a survival benefit for dogs undergoing lymphadenectomy compared with the nodal observation group, among the operated cases there was a reduced incidence of MCT progression and new MCT development.

The first observation seems to be in line with the previously reported hypothesis that PRL might eliminate a potential neoplastic reservoir, representing at the same time a safe clinical procedure without evident complications. Indeed, in the current study HN1 LNs were considered as uninvolved, but still they contain an increased number of mast cells compared to normal nodes, which could represent a micro metastatic load rather than a reactive mast cell proliferation.[9,17]

It must be stressed that, according to our agreement study, 5% of dogs had a cytologically uninvolved LN, yet an early metastatic disease (HN2) based on histopathology. Also, the cytological slides were analyzed by board-certified clinical pathologists, which is not common clinical practice. As a consequence, the false-negative results may be higher if the LN cytology slides are not sent to experienced clinical pathologists. Additionally, if the LNs are not palpable, they may not undergo cytological evaluation, so the nodal status is often unknown at the time of surgery. Because the therapeutic role of HN2 LN extirpation has been previously demonstrated,[4] and due to the lack of complications related to lymphadenectomy (which has been documented here and in previous studies)[7,8,21], to recommend this additional surgical procedure may not be viewed as unnecessary or harmful. On the contrary, it may provide a clinical benefit, as shown by the reduced incidence of MCT progression found in the current series.

The finding that lymphadenectomy of clinically uninvolved LN also reduces the risk of *de novo* cMCT development is more difficult to explain. The most plausible explanation is that dogs in the OO group were more likely to develop new cMCTs, as predisposed breeds were more represented. More speculatively, it may be hypothesized that quiescent neoplastic cells residing in the regional LN may at some point regain the cell cycles and relocate at distant cutaneous sites, giving rise to overt disease.[18] In the current series, it was not investigated whether the new cMCTs were of the same histological grade and mutational status of the primary tumor, impeding any further comment. In both groups the median ST was not reached, therefore it cannot be excluded that a survival advantage in either group may emerge with longer follow-ups. A power analysis was not performed but, due to the overall favorable prognosis for dogs with stage I low-grade cMCTs, very few tumor related events are to be expected, thus a very large number of cases would be needed to find a difference.

Several limitations of this study should be noted.

First, dogs did not undergo sentinel LN removal and, as a result, this study may have misdirected their lymphadenectomy in up to 60% of cases.[7] As a consequence, it cannot be excluded that the extirpation of the sentinel rather than the regional LN would have improved outcome. Additionally, lymphocenters sometimes consist of more than a single LN. Therefore, it is possible that the entire lymphocenter was not removed during the lymphadenectomy, leaving additional regional LNs behind.

Second, the histological classification of HN0/HN1 nodes may have been impacted by the number of sections. Unlike human cancer pathology, there are currently no guidelines in veterinary medicine on how to section a LN and on how many sections need to be examined. In the current study, all LNs were sectioned along the major axis at the level of the hilus; thus, cell aggregates qualifying for HN2 nodal stage may have been missed.

Third, this study suffers the bias which are inherent to retrospective analysis. Lymphadenectomy is a procedure with a considerable treatment burden, and the surgeon's decision as to whether to perform such a procedure may depend not only on disease characteristics such as stage or histology, but also on the anatomic location, tumor size, or owner's willingness. Consequently, dogs requiring a difficult surgical procedure (including, among others, axillary lymphadenectomy) would find themselves in a no-lymphadenectomy group, whereas those with an easily accessible MCT and/or regional LN may undergo lymphadenectomy more commonly. Also, even if surgical complications related to lymphadenectomy were not reported, it may be possible that minor sequalae were not documented; also, no quality-of life assessment was carried out, potentially hiding disadvantages of the additional treatment burden. Surely, no longer hospitalization after surgery was necessary for dogs undergoing lymphadenectomy.

Additionally, only dogs with completely resected, low-grade MCTs were included in the study, and this information is often retrieved only after surgery. Nevertheless, provided that cytologic grading may help predicting the histological grade, due to the high rate of locoregional relapse, high-grade MCTs will require lymphadenectomy in any case.[19-22]

Last, even though the median follow-up time was not significantly different among groups, dogs undergoing OO were monitored for a median of 813 days versus a median of 763 days for dogs undergoing nodal dissection. It cannot be excluded that, with a longer follow-up in operated dogs, the outcome differences may cancel out.

In conclusion, whether regional dissection of clinically negative LNs should be part of the primary resection for MCTs remains a problem of legitimate concern.

Our results showed that lack of immediate lymphadenectomy was associated with a higher risk for tumor progression. This preliminary judgement, reinforced by the findings that lymphadenectomy was well tolerated in all cases, and that histopathology provides the definitive assessment of the nodal pathological status, may suggest that prophylactic lymphadenectomy is indicated in the management of stage I MCTs. Larger prospective studies are warranted for generating clinical evidence of this latter hypothesis.

Material and methods

The aim of this retrospective multi-institutional study was to assess the therapeutic role of PRL of grossly and cytologically unremarkable regional LNs in canine low-grade, completely resected cMCT. To do so, the long-term outcomes of MCT-bearing dogs with cytologically unremarkable and surgically unresected regional LN were compared with those of dogs with a surgically resected and histologically normal or minimal risk regional LN.

The regional LN was defined as the closest LN in the expected lymphatic drainage, and was identified either by palpation or by ultrasound.[12]

Regional LNs were considered cytologically unremarkable if classified as normal or with reactive lymphoid hyperplasia according to Krick.[10] They were considered histologically unremarkable if classified as HN0 or HN1 according to Weishaar.[9]

To assess the consistency of cytology in the correct identification of uninvolved LNs, the clinical study was preceded by an agreement study aimed at assessing the concordance between Krick's cytological classification and Weishaar's histological classification in diagnosing a LN as non-metastatic.

Agreement study

For the agreement study, a subset of dogs with cytologically unremarkable LNs undergoing subsequent lymphadenectomy and histological examination were identified from the same oncology centers participating in the clinical study. Two of the four centers had the cytologic and histologic preparations read out by internal board-certified veterinary anatomic pathologists. The remaining two centers had both cytological and histologic samples submitted to the same private laboratory and were read out by two board-certified clinical pathologists and two anatomic pathologists. All cytological preparations had been obtained by FNA, with or without an ultrasound-guide using 27G or 25G needles. Smears were generated from obtained sample material and air-dried, and then stained with May Grünwald-Giemsa. All histological samples were fixed in 10% neutral-buffered formalin and paraffin-embedded following routine processing. Serial sections were cut and routinely stained with hematoxylin and eosin for histologic evaluation. Replicate sections were stained with toluidine blue or Giemsa to highlight mast cell granules. For each dog, the histological findings were correlated with the cytological findings, in order to evaluate the negative predictive value of cytology in the identification of MCT nodal metastases; i.e., the probability that dogs with a cytological result of an unremarkable LN will have a histologically unremarkable LN as well.

Clinical study

Once it was established that cytology was reliable in identifying dogs without regional LN metastasis, the clinical study could take place.

For this part, the medical records of 4 oncology centers were reviewed to identify dogs with treatment-naive, firstly occurring, completely resected, low-grade cMCT, with either cytologically and/or histologically unremarkable regional LNs.

To be eligible for recruitment, dogs had to undergo complete staging and wide surgical excision of the primary cMCT and have a minimum follow-up of 180 days. Information on clinical stage was obtained by means of hematological and biochemical analysis, cytological evaluation of the cMCT and regional LN, thoracic radiographs, abdominal ultrasound, and FNA of liver and spleen.

Dogs were classified into two groups: OO and PRL. Decisions regarding whether to perform OO or PRL were made according to each clinician's discretion.

Dogs with recurrent, concurrent multiple, subcutaneous or high-grade MCTs or those with stage II-IV disease were excluded from the study. Also, dogs were excluded if they had received neoadjuvant or adjuvant antitumoral treatment, and if the cMCTs had been removed with incomplete margins.

Background information recorded for each dog included: signalment; cMCT description (location, size, presence of ulceration); clinical substage; date of surgery; local relapse (defined as the cytological evidence of a recurrent cMCT within 2 cm from previous scar); nodal relapse (defined as the development or cytologically or histologically-confirmed LN metastases); distant relapse (defined as the occurrence of visceral metastasis); development of *de novo* cMCTs (defined as the occurrence of a new cMCT at a distant cutaneous site having a different regional LN), date of death or last follow-up examination, and cause of death.

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

Regardless of the group, dogs were monitored post-operatively by means of clinical examination, blood testing, and abdominal ultrasound, performed every 3 months during the first year, and every 6 months thereafter. In case of progression of any type, dogs underwent a complete re-staging.

Statistical analysis

Descriptive statistics were used in the analysis of dogs and tumor characteristics. When appropriate, data sets were tested for normality with the Shapiro-Wilk test. Values were expressed as mean ± SD in case of normal distribution, or as median with a range in case of non-normal distribution.

The distribution of demographic features and possible prognostic variables between the OO and PRL groups were assessed with Student's T-test (quantitative, parametric variables), the Mann-Whitney *U* test (quantitative, non-parametric variables) or the Chi-square test/Fisher's exact test (categorical variables).

The considered variables included breed (purebred and predisposition to biologically aggressive cMCTs, that is, Shar-pei, Labrador retriever and Golden retriever; and predisposition to low-grade cMCTs, that is, Boxer, French Bouledogue, Weimaraner, Pug, American Staffordshire terrier), age, body weight, sex, neutering status, substage, anatomic site of the primary cMCT (head and neck, trunk and limbs, inguinal/perineal/mammary region and digits), substage, macroscopic tumor largest diameter, ulceration, and development of new cMCTs.

The influence of the above variables and of lymphadenectomy on cMCT progression and cMCT-specific survival was investigated with Cox proportional hazards regression analysis. Survival plots were assessed by means of Kaplan-Meier survival plots, generated according to the Kaplan-Meier product-limit method.

For age, weight and tumor diameter, the median was used as the cut-off value.

Time to cMCT progression (TTP) was calculated from the date of surgery to the first occurrence of one or more of local, nodal or distant relapse. Dogs with no recurrence or disease progression at the date of the last visit or death were censored.

cMCT-specific survival time (ST) 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 cMCT-related causes were considered as events.

Data were analyzed by use of commercial software programs (SPSS Statistics v. 19, IBM, Somers, New York, and Prism v. 5.0, GraphPad, San Diego, California). P values $\leq .05$ were considered significant.

Declarations

Authors' contributions

LM conceived the study design, included cases, and was responsible for writing the majority of the manuscript. SS was responsible for data analysis and data interpretation, and prepared tables and figures. MK was responsible for data interpretation and supervised final edits. LM, SS, RF, DS, EF, WB, UB, AR, SDM, AF, MG, LA and MC all participated in study recruitment and management of patients. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The retrospective study described here involved review of medical records from privately-owned dogs, all receiving care as prescribed by licensed veterinarians. Under such circumstances, no formal review by an Institutional Animal Care and Use Committee is required.

Consent for publication

Not applicable.

Competing interests

There are no competing interests.

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Figure legends

Figure 1. Time to progression for 64 dogs with surgically-removed stage I low grade mast cell tumor undergoing prophylactic regional lymphadenectomy (PRL, solid line) or regional lymph node observation only (OO, dashed line). There is a tendency to a better TTP in the PRL group (P = 0.058).

Figure 2. Survival time for 64 dogs with surgically-removed stage I low grade mast cell tumor undergoing prophylactic regional lymphadenectomy (PRL, solid line) or regional lymph node observation only (OO, dashed line). Difference not statistically significant (P = 0.294).

Figures

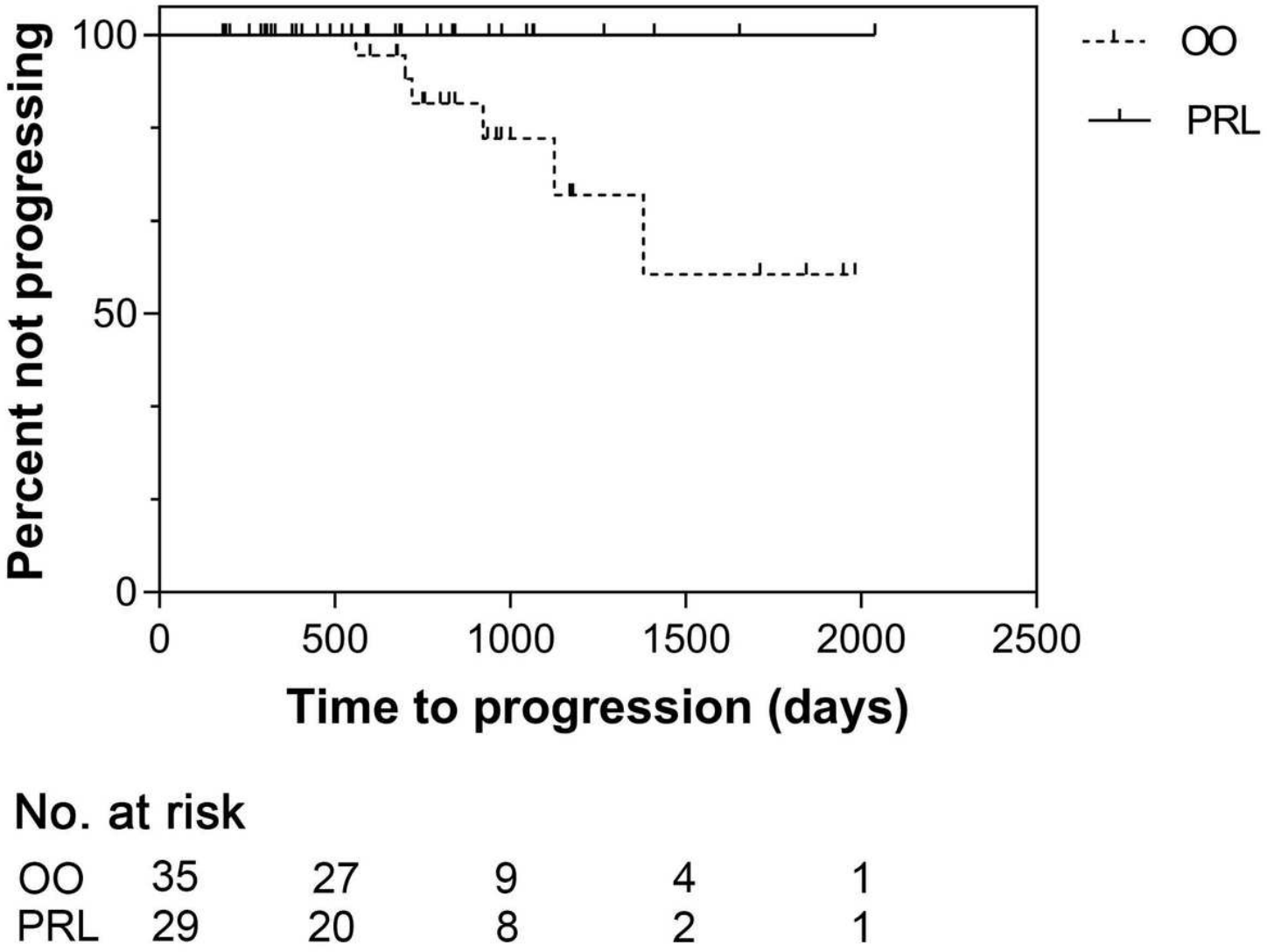

Figure 1

Time to progression for 64 dogs with surgically-removed stage I low grade mast cell tumor undergoing prophylactic regional lymphadenectomy (PRL, solid line) or regional lymph node observation only (OO, dashed line). There is a tendency to a better TTP in the PRL group (P = 0.058).

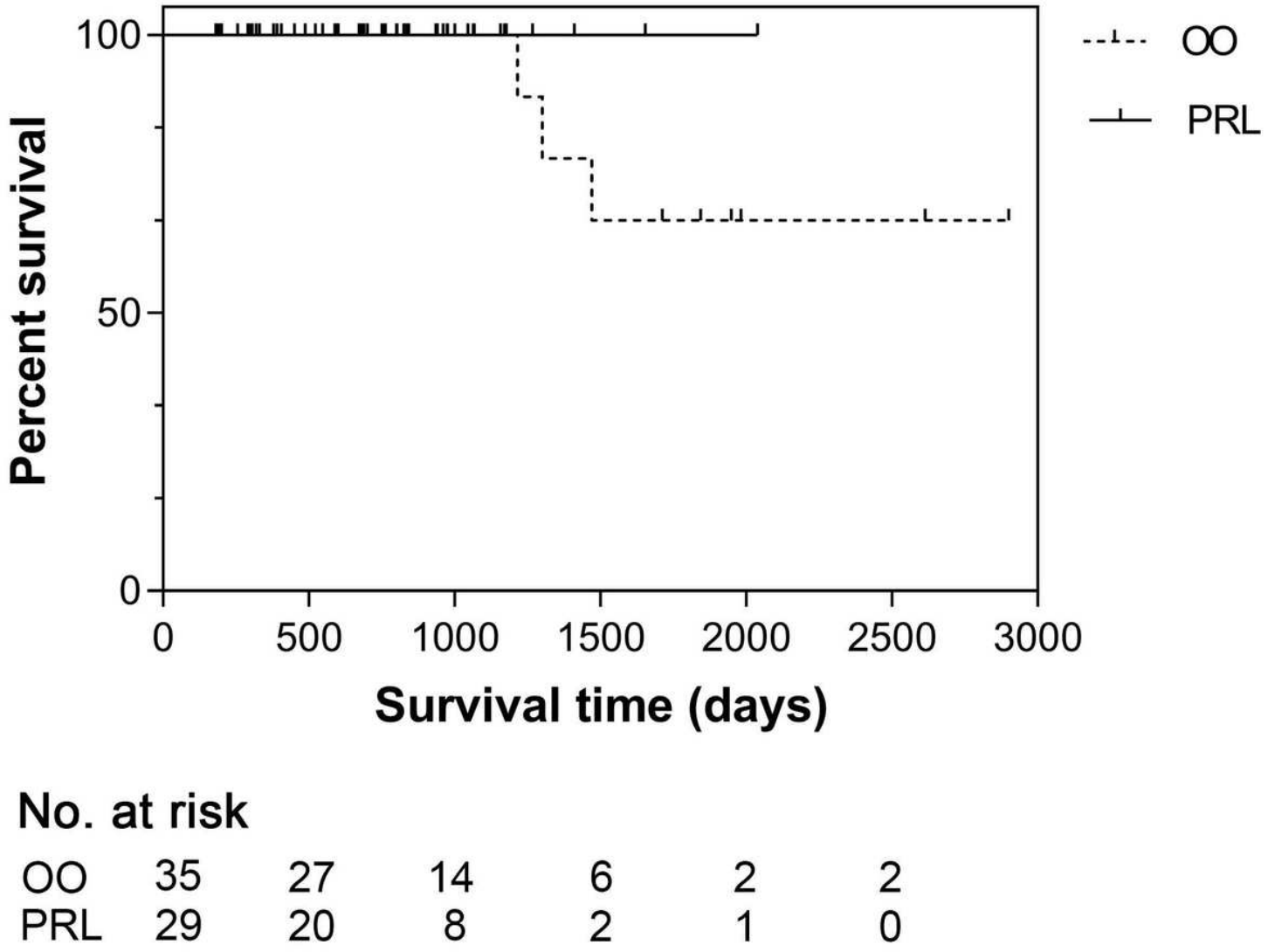

Figure 2

Survival time for 64 dogs with surgically-removed stage I low grade mast cell tumor undergoing prophylactic regional lymphadenectomy (PRL, solid line) or regional lymph node observation only (OO, dashed line). Difference not statistically significant (P = 0.294).

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