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

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(Article begins on next page)

1 ORIGINAL ARTICLE

2

3 **Therapeutic impact of regional lymphadenectomy in canine stage II cutaneous mast cell**
4 **tumours**

5

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25 ABSTRACT

26 Lymph node (LN) metastasis in canine cutaneous mast cell tumours (cMCTs) is a well-known
27 negative prognostic factor. The role of lymphadenectomy in the treatment of stage II disease
28 remains controversial because of its uncertain therapeutic benefit. Aim of this retrospective
29 study was to investigate the impact of lymphadenectomy on tumour control and survival for dogs
30 with stage II cMCTs. Dogs with firstly occurring, histologically confirmed cMCT with LN
31 metastasis undergoing resection of the primary tumour and medical treatment thereafter were

32 retrospectively enrolled. Dogs were classified into two groups: LN sampling (LNS; diagnosis of
33 metastasis obtained by cytology) and regional LN dissection (LND; diagnosis obtained by histo-
34 pathology). To determine the therapeutic value of lymphadenectomy, the characteristics of
35 recurrence (local, nodal and distant) and survival were compared between groups. Evaluated
36 outcome variables included signalment, anatomic location, diameter, ulceration, substage,
37 surgical margins, Patnaik grading, Kiupel grading and medical treatment. Overall, 152 dogs were
38 included: 81 underwent LND as part of primary surgery and 71 LNS. The median follow-up
39 was 409 days for LND group and 620 days for LNS group. On univariable analysis, the risk of
40 developing local, nodal or distant relapse was significantly higher in the LNS group compared
41 with LND ($P < 0.001$) On multivariable analysis, the risk of tumour progression and tumour-
42 related death were 5.47 and 3.61 times higher in the LNS group, respectively ($P < 0.001$).
43 Regional lymphadenectomy may have therapeutic value and improve prognosis in dogs with
44 stage II cMCTs undergoing surgical removal of the primary tumour and medical treatment.

45

46 KEYWORDS

47

48 dog, lymphadenectomy, lymph node metastasis, mast cell tumour, prognosis, stage II

49

50 INTRODUCTION

51

52 The benefit of surgical extirpation of metastatic lymph nodes (LNs) in the surgical management
53 of dogs with solid cancer is unclear. Approximately, 20% of dogs with cutaneous mast cell
54 tumours

55 (cMCTs) have nodal metastasis (stage II) at initial diagnosis.¹ LN metastasis has been associated
56 with decreased survival time (ST) in several studies.^{2–3} Given the prognostic significance of
57 nodal metastases, assessment of the regional LNs by cytology and/or histology is a fundamental
58 diagnostic step in dogs with cMCT.^{3,5,6} pre-metastatic lesions.

59 Currently, the primary standard treatment for dogs with stage II cMCT comprises surgical
60 excision of the primary tumour with or with-out radiation therapy (RT) and adjuvant medical
61 treatment.^{7–9}

62 In this context, it has to be emphasized that the role of elective lymphadenectomy has historically

63 been related to surgical staging for recognizing the true disease extent by detecting overt
64 metastasis as well as pre-metastatic lesions.³ However, the benefits of lymphadenectomy may
65 extend beyond merely staging the burden of disease. If cancer morbidity is a function of the
66 burden of disease in the primary tumour site and the locoregional LNs, successful removal of the
67 primary tumour and metastatic LN would be expected to confer a significant survival advantage.
68 Yet, to the authors' knowledge, the therapeutic role of metastatic LN dissection has received
69 relatively little attention and only one retrospective study has suggested a favourable impact of
70 lymphadenectomy on tumour-specific survival (TSS) time in dogs with stage II cMCTs.¹⁰ In a
71 later study by Weishaar et al, dogs with extensive nodal involvement (HN2/HN3) had shorter
72 disease-free interval and ST when compared with dogs with a less advanced nodal involvement
73 (HN0/HN1) when evaluated with Gehan-Breslow-Wilcoxon test.³ However, in that study, the
74 population of included dogs was small and medical treatment was not administered to all
75 patients, thereby biasing the outcome results.
76 The aim of the current retrospective study was to explore the impact of lymphadenectomy on
77 tumour control and TSS for dogs with stage II cMCTs.

78

79 MATERIALS AND METHOD

80 **Case selection**

81 Members of SIONCOV (Italian Society of Veterinary Oncology) were invited to review their
82 records for dogs with treatment-naïve, firstly occurring, histologically confirmed cMCT with
83 regional LN metastasis, confirmed either by cytology or histology. For the purpose of this study,
84 stage II refers to dogs with LN metastasis regardless of the dimension of the primary cMCT, to
85 avoid the confusions and ambiguities in the classification of World Health Organization stage III
86 disease. No time limits were defined for case enrolment and no minimum follow-up time was
87 established.

88 To be eligible for recruitment, dogs had to undergo wide surgical excision of the primary cMCT
89 and medical treatment (consisting of cytotoxic chemotherapy, tyrosine kinase inhibitors [TKIs]
90 or both) thereafter. Wide surgical excision was defined as a lateral margin of 2 to 3 cm and a
91 deep margin of one facial plane, depending on tumour size and location.

92 Information on clinical stage was obtained by means of the following: haematological and
93 biochemical analysis; cytological evaluation of the cutaneous nodule and regional LN; thoracic

94 radiographs; abdominal ultrasound, and fine-needle aspirates of liver and spleen.
95 The regional LN was defined as the closest LN in the expected lymphatic drainage, and was
96 identified either by palpation or by ultrasound.
97 Dogs were classified into two groups: LN sampling (LNS; diagnosis of regional LN metastasis
98 was made by cytology with no subsequent lymphadenectomy) and LN dissection (LND; dogs
99 undergoing both excision of the cMCT and regional lymphadenectomy and thus whose diagnosis
100 was obtained by histopathology). Decisions regarding whether to perform LNS or LND were
101 made according to each clinician's discretion.
102 Dogs were enrolled in the LNS group if LN cytology yielded a certain diagnosis of metastasis
103 according to Krick's criteria,⁵ whereas enrolment in the LND group was possible only following
104 histopathological confirmation of early (HN2) or overt (HN3) nodal metastasis according
105 to Weishaar et al.³ Dogs with concurrent multiple or subcutaneous MCTs, and those with stage
106 IV disease were excluded from the study. Dogs with nodal pre-metastatic disease on histology
107 (HN1 based on Weishaar et al)³ were also excluded. Background information recorded for each
108 dog included: signalment; primary tumour description (location, size, presence of ulceration);
109 clinical substage; site of nodal involvement; LN clinical characteristics (normal size and
110 consistency or abnormal [increased in size or with a firm consistency compared with the
111 contralateral]; mobile or fixed); histopathological evaluation of surgical margins (clean, clean but
112 close [presence of neoplastic cells within 1 mm from the surgical margin], incomplete);
113 histologic grade of the primary cMCT according to Patnaik and Kiupel classification
114 systems^{11,12};
115 Ki67-index (expressed in percentage by counting a total of 1000 cells in 10 high power field)¹³;
116 Kit-pattern¹⁴; c-Kit mutational status; date of surgery; medical treatment (cytotoxic
117 chemotherapy, TKIs or both); use of post-operative RT; local recurrence (defined as the
118 cytological evidence of a recurrent cMCT within 2 cm from previous scar); nodal relapse
119 (defined for the LNS group as nodal progressive disease with a more than 20% increase in size
120 or presence of new metastatic LNs, and for the LND group as presence of new metastatic LNs);
121 distant relapse (defined as the occurrence of visceral metastasis); date of death or last follow-up
122 examination, and cause of death. To determine the therapeutic value of lymphadenectomy, the
123 characteristics of relapse (local, nodal and distant) and the survival impact were compared
124 between the LNS and LND groups. While under medical treatment, dogs were monitored every 2

125 to 4 weeks. Afterwards, dogs were followed-up every 1 to 3 months, depending on clinicians'
126 discretion and owners' compliance.

127

128 |**Statistical analysis**

129 Descriptive statistics were used in the analysis of dogs and tumour characteristics. When
130 appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus
131 normality test. Values were expressed as mean \pm SD in case of normal distribution, or as median
132 with a range in case of non-normal distribution. The distribution of demographic features and
133 possible outcome variables between the LNS and LND groups were assessed with Student's
134 t test (numerical, parametric variables), the Mann-Whitney U test (numerical, non-parametric
135 variables) or the χ^2 test (categorical variables). The considered variables included breed
136 (predisposition to biologically aggressive MCTs, i.e., Shar pei, Labrador retriever and Golden
137 retriever),¹⁵ age, body weight, sex, anatomic location of the primary cMCT (head and neck,
138 trunk [including tail], limbs [excluding digital tumours], inguinal region [including perineal and
139 scrotal], mammary region and digits), macroscopic tumour diameter, ulceration, substage,
140 surgical margins, Patnaik grading (P-G1, P-G2 or P-G3), Kiupel grading (K-LG or K-HG),
141 Ki67-index, Kit staining pattern, c-Kit mutational status, medical treatment (cytotoxic
142 chemotherapy, TKI or both) and the use of post-operative RT. For age, weight and tumour
143 diameter, the median was used as cut-off value.

144 Time to local recurrence (TLR) was calculated from the date of surgery to the date of local
145 recurrence. Time to nodal relapse (TNR) was calculated from the date of surgery to the date of
146 nodal recurrence for LND or nodal progression for LNS. Time to distant relapse (TDR) was
147 calculated from the date of surgery to the date of diagnosis of visceral metastases. Time to
148 progression (TTP) was calculated from the date of surgery to the first occurrence of one or more
149 of local recurrence, nodal or distant relapse. Dogs with no recurrence or disease progression at
150 the date of the last visit or death were censored. TSS was calculated from the date of surgery to
151 the date of death or to the date of the last visit if death did not occur. Only dogs deceased for
152 MCT-related causes were considered as events. Survival plots were generated according to the
153 Kaplan-Meier product-limit method. Survival estimates were presented as medians with the
154 corresponding 95% confidence intervals (95% CIs). The influence of potential prognostic

155 variables on tumour progression and TSS was investigated with univariable and multivariable
156 Cox's regression analyses. Data were analysed by use of commercial software programs
157 (SPSS Statistics v.19, IBM, Somers, New York, and Prism v.5.0, GraphPad, San Diego,
158 California). P-values ≤ 0.05 were considered significant.

159

160 RESULTS

161

162 **Patient and tumour characteristics**

163

164 A total of 152 dogs fulfilled the inclusion criteria. Among these dogs, 81 underwent LND as part
165 of primary surgery, and 71 underwent LNS. There was good balance between groups regarding
166 demographic features and possible outcome variables (Table 1). Only medical treatment differed
167 among groups: cytotoxic chemotherapy was more often administered to dogs in the LND group,
168 and TKI to dogs in the LNS group ($P < 0.001$). Among dogs undergoing LND, there were 22
169 (27.3%) mixed breed dogs, 11 (13.7%) Labrador retrievers, 6 (7.5%) Boxers, 6 (7.4%) French
170 bulldogs, 6 (7.4%) Golden retrievers, 3 (3.7%) Maltese terriers, 3 (3.7%) Shar peis, 3 (3.7%)
171 Bernese mountain dogs, 2 (2.5%) Brittany spaniels, 2 (2.5%) Pugs, 2 (2.5%) Pit bull terriers, 2
172 (2.5%) Dogo Argentino and one (1.2%) each of the following: Chihuahua, Alaskan malamute,
173 Pomeranian, English setter, Gordon setter, grand bleu de Gascogne, Bull mastiff, Jack Russell
174 terrier, Dachshund, Dalmatian, Poodle, Cane corso and Great Dane. Mean age was 8.3 ± 3.0
175 years (range, 3-16 years) and median weight was 24.3 kg (range, 2.5-58.7 kg). There were 48
176 female dogs (of which 39 were spayed) and 33 males (of which 6 were castrated).

177 The tumours were located on limbs ($n = 33$; 40.7%), head and neck ($n = 20$; 24.7%), digits ($n =$
178 11 ; 13.6%), inguinal region ($n = 7$; 8.6%), mammary region ($n = 6$; 7.5%), and trunk ($n = 4$;
179 4.9%). Tumour diameter ranged from 0.5 to 18 cm (median, 2.5 cm); 53 (65.4%) cMCTs were
180 not ulcerated, while 28 (34.6%) were ulcerated. Seventy-six (93.8%) dogs were asymptomatic at
181 presentation (substage a), whereas the remaining 5 (6.2%) dogs had signs of systemic effects of
182 cMCT (vomiting, diarrhoea, pruritus and regional edema; substage b). Based on the Patnaik
183 grading system, there were 2 (2.5%) P-G1 cMCTs; 58 (71.6%) P-G2 cMCTs and 21 (25.9%) P-
184 G3 cMCTs. Based on the Kiupel grading system, there were 53 (65.5%) K-LG cMCTs and

185 27 (33.3%) K-HG cMCTs. The Kiupel grade was not available for one (1.2%) dog.
186 Histopathological evaluation revealed clean surgical margins in 47 (58.1%) cMCTs, clean but
187 close margins in 1 (1.2%) case, and incomplete margins in 33 (40.7%) cases. Ki67
188 immunohistochemical labeling was available for 30 (37.0%) cases. Ki67 counts ranged from 1%
189 to 65% with a median of 7%. Kit immunolabelling was available for 28 (34.6%) cases.
190 Perimembranous kit labeling (Pattern 1) was observed in 8 cMCTs, focal/stippled kit
191 labeling (Pattern 2) was present in 9; and diffuse cytoplasmic kit labeling (Pattern 3) was found
192 in 11. Mutational analysis was available for 43 (53.1%) cMCTs: 12 cMCTs were mutated (10
193 had an ITD on exon 11 and 2 had an ITD on exon 8), while the remaining 31 were wild
194 types. The following metastatic ipsilateral LNs were removed: popliteal (n = 31; 38.4%),
195 submandibular (n = 20; 24.7%), superficial cervical (n = 13; 16.0%), inguinal (n = 13; 16.0%)
196 and axillary (n = 4, 4.9%). Sixteen (20%) had normal size and consistency, while 65 (80%) were
197 abnormal; 69 (85%) were mobile and 12 (15%) were fixed. Based on the Weishaar study, 28
198 (34.6%) LNs were classified as HN2 and 53 (65.4%) as HN3. Among dogs undergoing LNS,
199 there were 18 (25.4%) mixed breed dogs, 15 (21.1%) Labrador retrievers, 6 (8.5%) Boxers, 6
200 (8.5%) Golden retrievers, 4 (5.6%) American Staffordshire terriers, 2 (2.8%) Dobermanns,
201 2 (2.8%) Shih tzus, 2 (2.8%) Pinschers, 2 (2.8%) Pit bull terriers and one each of the following:
202 Irish setter, German shepherd dog, Australian terrier, Beagle, West Highland white terrier,
203 Dogue de Bordeaux, Cane corso, Bernese Mountain dog, Yorkshire terrier, Rottweiler,
204 Griffon, Shar pei, Fila San Miguel, and Cavalier King Charles spaniel. Mean age was 8.9 ± 3.0
205 years (range, 1-14 years), and median weight was 28 kg (range, 4.5-53 kg). There were 36
206 female dogs (of which 27 were spayed) and 35 males (of which 15 were castrated). The tumours
207 were located on limbs (n = 23; 32.5%), head and neck (n = 15; 21.1%), inguinal region (n = 14;
208 19.7%), trunk and tail (n = 12; 16.9%), digits (n = 4; 5.6%) and mammary region (n = 3;
209 4.2%). Tumour diameter ranged from 1 to 7 cm (median, 3 cm); 44 (62.0%) cMCTs were not
210 ulcerated, while 27 (38.0%) were ulcerated. Sixty-two (87.3%) dogs were asymptomatic at
211 presentation, whereas the remaining 9 (12.7%) dogs had signs of systemic effects
212 of cMCT. Based on the Patnaik grading system, there were 3 (4.2%) P-G1 MCTs, 47 (66.2%) P-
213 G2 cMCTs and 21 (29.6%) P-G3 cMCTs. Based on the Kiupel grading system, there were 39
214 (54.9%) K-LG cMCTs and 30 (42.3%) K-HG cMCTs. The Kiupel grade was not available for
215 two (2.8%) dogs. The surgical margin status was available for 60 (84.5%) cMCTs.

216 Histopathological evaluation revealed clean surgical margins in 22 (36.7%) cMCTs, clean but
217 close margins in 6 (10%) cases, and incomplete margins in 32 (53.3%) cases.
218 Ki67 immunohistochemical labeling was available for 15 (21.1%) cases. Ki67 counts ranged
219 from 1% to 99% with a median of 13%. Kit immunolabelling was available for 26 (36.6%)
220 cases. Kit Pattern 1 was observed in 3 cMCTs, Kit Pattern 2 was present in 18 and Kit Pattern
221 3 was found in 5. Mutational analysis was available for 30 (42.3%) cMCTs: 12 cMCTs were
222 mutated (11 had an ITD on exon 11 and 1 had an ITD on exon 8), while the remaining 18 were
223 wild types. Based on Krick's criteria, all dogs had a cytological diagnosis of certain LN
224 metastasis. Metastatic ipsilateral LNs included the inguinal (n = 21; 29.6%), popliteal (n = 16;
225 22.5%), superficial cervical (n = 14; 19.7%), submandibular (n = 10; 14.1%), axillary (n = 7;
226 9.9%), retropharyngeal (n = 2; 2.8%), and medial iliac (n = 1; 1.4%) LN. Eight (11%) had
227 normal size and consistency, while 63 (89%) were abnormal; 53 (75%) were mobile and 18
228 (25%) were fixed.

230 **Treatment and outcome**

231
232 Severe complications following lymphadenectomy were not reported for any of the 81 dogs
233 undergoing LND. All dogs received adjuvant medical therapy, consisting of cytotoxic
234 chemotherapy (vinblastine and prednisone: n = 52; vinblastine, prednisone and lomustine: n = 1;
235 vinblastine, cyclophosphamide, prednisone: n = 2; chlorambucil: n = 1), TKI (n = 17) or both
236 concurrently (n = 8). Twelve (14.8%) dogs also received RT to the tumour and nodal bed. The
237 median follow-up time was 409 days (95% CI, 298-657). Twelve (14.8%) dogs experienced
238 local recurrence after a median of 199 days (range, 29-1499); incomplete surgical margins had
239 been diagnosed in 8 (67%) of these cases. Fourteen (17.3%) dogs experienced nodal relapse after
240 a median of 193 days (range, 28-592) and 9 (11.1%) developed distant relapse after a median of
241 218 days (range, 52-2152). Overall median TLR, TNR and TDR were not reached. Mean TTP
242 was 1461 days. At the end of the study, 50 (61.7%) dogs were alive, and 31 had died because of
243 cancer-related (n = 21; 25.9%) or unrelated causes (n = 10; 12.3%). Median TSS was 2213 days
244 (95% CI, 1410-3015, Table 2). There was no significant difference in TLR, TNR, TDR and TSS
245 between dogs diagnosed with HN2 and HN3 LN status. All dogs in the LNS group received
246 adjuvant medical therapy, consisting of cytotoxic chemotherapy (vinblastine and prednisone:

247 n = 22; vinblastine, prednisone and lomustine: n = 3; paclitaxel: n = 1), TKI (n = 20) or both (n =
248 25). Twelve (16.9%) also received RT. Both the primary cMCT and the metastatic LN were
249 included in the treatment field. The median follow-up time was 620 days (95% CI, 59-1207).
250 Thirty-one (43.7%) dogs experienced local recurrence; 19 (61%) of them had been removed with
251 incomplete surgical margins; 51 (71.8%) dogs developed nodal relapse and 23 (32.4%) distant
252 relapse. Overall median TLR, TNR and TDR were 511, 170 and 1045 days, respectively. Median
253 TTP was 170 days. At the end of the study, 16 (22.5%) dogs were alive, and 55 had died because
254 of cancer-related (n = 45; 63.4%) or unrelated causes (n = 10; 14.1%). Median TSS was 360
255 days (95% CI, 181-539, Table 2). The risk of developing local recurrence, nodal relapse or
256 distant relapse was significantly higher in the LNS group compared with the LND group (P <
257 0.001). Overall, the risk of tumour progression was significantly higher in the LNS group (HR =
258 4.26, P < 0.001, Table 2). The risk of tumour-related death was also significantly higher (HR =
259 3.63, P < 0.001; Table 2, Figures 1 and 2).

260

261 **Analysis of prognostic variables**

262

263 On univariable analysis, variables significantly associated with an increased risk of tumour
264 progression were: age >9 years, head and neck location, tumour diameter >3 cm, substage b, P-
265 G3, K-HG, enlarged/firm LN, fixed LN, lack of lymphadenectomy and TKI administration
266 (Table 3). Variables significantly associated with TSS were: age >9 years, lack of neutering,
267 head and neck location, tumour diameter >3 cm, substage b, P-G3, K-HG, enlarged/firm LN,
268 fixed LN, lack of lymphadenectomy and TKI administration (Table 4). On multivariable
269 analysis, age >9 years, head and neck location, enlarged/firm LN and lack of lymphadenectomy
270 were still significantly associated with tumour progression, whereas the variables associated
271 with tumour-related death were head and neck location, K-HG and lack of lymphadenectomy.
272 The lack of lymphadenectomy was the variable associated with the highest risk for tumour
273 progression and the second after K-HG for tumour-related death (Tables 5 and 6).

274

275 **DISCUSSION**

276 In the current study, a significant improvement in tumour control and TSS was observed in dogs
277 that underwent regional LND during primary surgery for stage II cMCTs. Notably, the beneficial

278 effects of LND were most pronounced among dogs younger than 9 years, with cMCTs arising in
279 anatomic locations different than head and neck, smaller than 3 cm, of K-LG, and with no
280 enlarged/firm regional LN. Most of these results are similar to previous reports.^{1,12,16,17}
281 Intuitively, it would appear that the explanation for these observations is that the patients who
282 experienced greatest benefit were those (1) with sufficient life ahead for a life-expectancy benefit
283 to be measured and (2) with a less aggressive manifestation of disease. Previously identified
284 prognostic markers, Kiupel grade and gross enlargement and firmness of the regional LN,
285 remained prognostically significant; the negative impact of these observations was not removed
286 by the application of LND. Nevertheless, this is the first study including the extent of node
287 involvement (sampled vs removed) as a death-related risk factor for cMCT. Lymphadenectomy
288 is increasingly employed in veterinary oncology for improved accuracy of clinical stage
289 evaluation. It is accepted that LND is the superior technique for the diagnosis of LN metastases.
290 The limits of cytology in over- or under-staging disease by obtaining false positive or false
291 negative results, respectively, have been well documented.¹⁸ Even though the sensitivity and
292 specificity of cytological examination for the detection of LN metastasis in dogs with solid
293 tumours (including cMCTs) have been reported to be as high as 100% and 96%, respectively,⁶ in
294 the specific case of cMCT, cytological diagnostic accuracy is hampered by an inability to
295 accurately differentiate malignant from reactive mast cells in LN aspirates, possibly leading to
296 false positive results.¹⁹ In order to avoid this, in the current study strict criteria were applied to
297 May-Grünwald-Giemsa-stained LN cytological smears to identify nodal metastatic disease.
298 Criteria for the definition of LN metastasis included replacement of lymphoid cells by mast cells,
299 and/or the presence of aggregated, poorly differentiated mast cells with pleomorphism,
300 anisocytosis, anisokaryosis, and/or decreased or variable granulation, and/or greater than five
301 aggregates of more than three mast cells, according to Krick's criteria.⁵ Additionally, cases were
302 only included in the LNS group if the LN was interpreted as "certainly" metastatic according to
303 Krick's criteria.⁵ Besides staging, our results have documented that LND is also important for
304 survival. Nodal metastasis indicates aggressive tumour biology, but also may represent a source
305 of subsequent metastasis, as hypothesized by the Halstedian theory.²⁰ In the LNS group, dogs
306 had a significantly higher local recurrence rate (43.7% vs 14.8% in the LND group), a significant
307 increase in nodal relapse (71.8% vs 17.3% in the LND group) and distant metastasis (32.4% vs
308 11.1% in the LND group). While it is difficult to clinically determine the tumour origin from

309 which systemic metastasis derives, including the primary cancer vs the metastatic LN, the
310 survival benefit observed in dogs undergoing LND cannot be ignored, suggesting that tumour
311 biology, including metastatic capability, differs between the primary site and the LNs.^{21,22} It is
312 certainly plausible that improved loco-regional control translates into a lower risk of distant
313 spread, ultimately leading to a survival benefit. Also, it is interesting to note that the
314 histopathological LN status (HN2 vs HN3) did not show any significant difference in terms of
315 outcome, suggesting that both classifications have the potential to behave aggressively, thereby
316 requiring an additional medical intervention. Patients with advanced mast cell neoplasia are
317 known to suffer paraneoplastic, systemic consequences of their disease, even in the absence of
318 detectable metastasis. In patients without detectable metastasis, morbidity and overall disease
319 burden are correlated.²³ Therefore, a simple explanation for the observed outcome findings lies
320 in the fact that LND removes an additional burden of cancer from the patient. Thus a potential
321 driver for paraneoplastic morbidity consequences is also removed.

322 However, if the explanation for the observed findings was as simple as that given above, one
323 would expect an improvement in overall survival and TNR following lymphadenectomy, but one
324 would not intuitively expect an improvement in TLR and TDR. It is accepted that the observed
325 differences in time to recurrence outcomes may have arisen due to an inherent bias or to chance.
326 However, considering the possibility that the observed results are a true effect, this study
327 provides evidence for a model of disease progression whereby metastatic foci in loco-regional
328 LNs present a threat of bidirectional disease progression. In other words, the metastatic local LN
329 can either act as a reservoir for neoplastic mast cells, which can then relocate to the primary
330 tumour site or to other distant site or it can exert a biological effect, which favours the
331 development of neoplasia at those sites. Indeed, in humans with solid cancer, local reseeding
332 from neoplastic cells located in the LNs is a well-known phenomenon, and is driven by
333 chemoattractants released during the post-surgery local wound healing processes.²⁴ The same
334 may hold true for dogs with cMCTs. The results of this study indicate prognostic benefits of
335 regional

336 LND of metastatic LNs for dogs with surgically removed cMCT. However, the data should be
337 interpreted with caution. Every effort was made to minimize potential bias by accounting for all
338 known prognostic variables associated with both the tumours and patients; however, selection
339 bias regarding dogs' recruitment cannot be ruled out because of the retrospective nature of this

340 study. Decisions regarding whether to perform LND were made according to each clinician's
341 discretion, rather than random allocation or well-defined criteria. It is utterly plausible that
342 unknown owner and clinician perceptions or preferences may have impacted the treatment
343 decision. Also, while all dogs received some form of systemic treatment, protocols were not
344 standardized, rather the choice was left to the primary clinician. Any confounding effect of
345 adjuvant therapy choice could also have influenced outcome. It must be noted that cytotoxic
346 chemotherapy was more often offered to dogs in the LND group and TKI therapy to dogs in the
347 LNS group. This may reflect a clinical bias; veterinarians managing dogs in the study generally
348 perceived TKI therapy to offer a higher probability of a durable response than cytotoxic
349 chemotherapy to dogs with more malignant disease or in which the goal of treatment was to
350 stabilize the disease by administering a cytostatic drug. By contrast, dogs with less malignant,
351 down-staged disease were considered better candidates for treatment comprising a finite course
352 of vinblastine and prednisolone. This latter treatment was regarded to confer a lower risk, lower
353 cost, shorter treatment duration and a good chance of a very good outcome for that patient group.
354 Furthermore, although this study recruited cases regardless of the location of the loco-regional
355 draining node, inadvertently, it primarily evaluated dogs with readily accessible LNs. This means
356 that caution must be exercised in applying the conclusions of this study to dogs that were poorly
357 represented. The morbidity associated with removal of an intra-cavitary LN would be expected
358 to be greater than that for removal of a peripheral LN. This increase in morbidity might offset
359 some of the survival advantage supposedly achieved and may create other problems not
360 highlighted in this study. Our study raises several important questions for the management
361 of dogs with stage II cMCTs. First, should LND of metastatic LNs become a standard
362 component of surgical management of cMCTs? Given the outcome advantages and the lack of
363 morbidity observed in this study, we believe the answer to this question is a qualified yes. In this
364 study cohort, sufficient patients enjoyed a survival benefit that a statistically significant
365 improvement was noted for the LND group as a whole. However, it should be noted that a
366 proportion of individual patients did not enjoy a survival benefit. Further studies to define
367 optimal application of LND recommendation would be useful. Future studies might explore
368 whether medical treatment is necessary for this whole population of dogs, as there is no clear
369 consensus regarding systemic treatment for stage II cMCTs in terms of the need for, and choice

370 of, adjuvant cytotoxic chemotherapy regimen, as highlighted in a recent Letter to the Editor in
371 this journal.²⁵ In some patient groups, consider older patients and those with a lesser metastatic
372 burden, it is conceivable that the survival advantage of LND is sufficient to achieve the full
373 remainder of that patient's life expectancy, meaning that adjuvant medical therapy would no
374 longer confer a survival advantage. Second, should LND be performed systematically, regardless
375 of the nodal disease status? Undoubtedly accurate surgical staging, including LND, recognizes
376 the true extent of disease by detection of occult node metastases (HN1). It remains to be explored
377 whether lymphadenectomy of HN1 nodes further improves prognosis as compared with surgical
378 excision of the primary cMCT only. Last, the regional LN does not necessarily represent the
379 sentinel LN, which is by definition the first node that receives direct lymphatic drainage from the
380 tumour rather than the closest node to the primary tumour.^{26,27} Different methods of
381 identification of the sentinel LN have been used, including radioisotope injection, vital blue dye,
382 or lymphangiography. For LNs not obviously metastatic, sentinel LNs techniques rather than
383 anatomic sampling should be applied to accurately reflect the metastatic status. It could be
384 suggested that if sentinel LN mapping had been used to drive LN extirpation, the difference
385 between outcomes for the two patient groups might have been even greater.

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

393

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397

398 **Conflict of interest**

399 The authors have no conflict of interest to declare.

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401
402 TABLE 1 Distributions of variables potentially associated with prognosis in 152 dogs with stage II
403 cutaneous mast cell tumour treated by surgical
404 excision of the primary tumour and systemic medical therapy with or without concurrent
405 lymphadenectomy

406
407 TABLE 2 Time to progression, survival time and evaluation of the risk of developing tumour progression
408 and tumour-related death in 152 dogs with stage II cutaneous mast cell tumour treated by surgical
409 excision of the primary tumour and systemic medical therapy with or without
410 concurrent lymphadenectomy

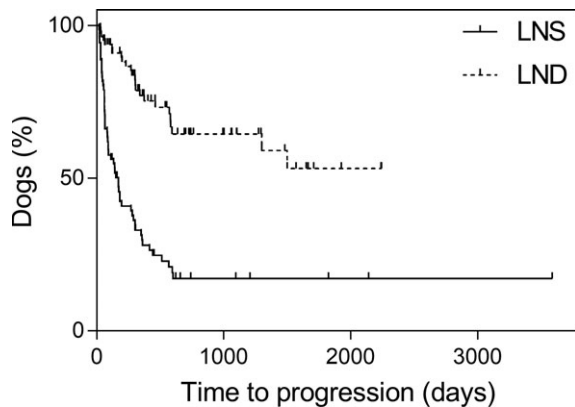
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412 TABLE 3 Univariable Cox regression analysis of variables potentially associated with increased risk of
413 tumour progression in 152 dogs with stage II cutaneous mast cell tumours

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415 TABLE 4 Univariable Cox regression analysis of variables potentially associated with increased risk of
416 tumour-related death in 152 dogs with stage II cutaneous mast cell tumours

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418 TABLE 5 Multivariable Cox regression analysis of variables potentially associated with increased risk of
419 tumour progression in 152 dogs with stage II cutaneous mast cell tumours

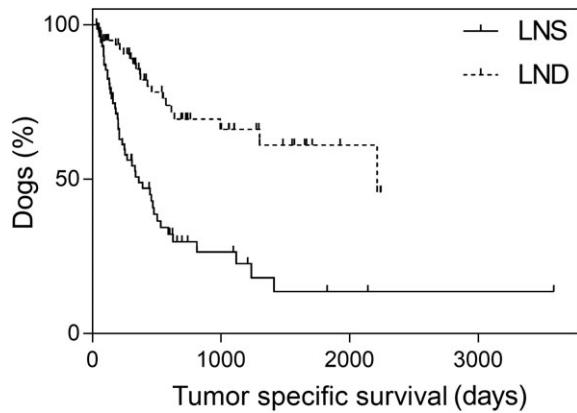
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421 TABLE 6 Multivariable Cox regression analysis of variables potentially associated with increased risk of
422 tumour-related death in 152 dogs with stage II cutaneous mast cell tumours

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426 FIGURE 1 Time to progression for dogs with stage II cutaneous mast cell tumour treated by surgical excision of the
427 primary tumour, systemic medical treatment and metastatic lymph node sampling (LNS) or dissection (LND). In the
428 LND group, dogs had a significantly longer time to progression (median, not reached vs 170 days, respectively; $P <$
429 0.001)

430



431
 432 FIGURE 2 Tumour-specific survival (TSS) for dogs with stage II cutaneous mast cell tumour treated by surgical
 433 excision of the primary tumour, systemic medical treatment and metastatic lymph node sampling (LNS) or
 434 dissection (LND). In the LND group, dogs had a significantly longer survival time (median, 2213 days vs 360 days,
 435 respectively; $P < 0.001$)

436

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