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Adjuvant medical therapy provides no therapeutic benefit in the treatment of dogs with low-grade mast cell tumors and early nodal metastasis undergoing surgery

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Abstract

Lymph node (LN) metastasis is a negative prognostic factor in dogs with cutaneous mast cell tumors (cMCTs). While elective lymphadenectomy of metastatic LNs improves outcome, the benefit of adjuvant medical therapy in dogs with early metastatic (HN2) LNs is debated. The aim of this retrospective multicenter study was to evaluate the therapeutic benefit of adjuvant medical therapy following surgical removal of the primary low-grade cMCT (Patnaik grade 1-2 and Kiupel low-grade) and lymphadenectomy of HN2 LNs by analyzing survival rates and patterns of recurrence.

Seventy-three dogs were included: 42 received adjuvant medical treatment (chemotherapy and/or kinase inhibitors), and 31 did not. The median follow-up time for medically-treated dogs was 619 days: 2 experienced local recurrence, 3 nodal relapse and 4 distant relapse.

For dogs undergoing surgery only, the median follow-up time was 545 days. None of them experienced local recurrence, nodal or distant relapse. Time to progression was significantly shorter in dogs receiving adjuvant medical treatment ($P = 0.021$). A similar tendency was observed for overall survival ($P = 0.056$).

The current study shows that dogs with low-grade cMCTs, that undergo surgical excision of the primary tumor and elective lymphadenectomy of the HN2 regional LN harbor a good prognosis. The use of adjuvant medical treatment in these dogs does not seem to provide any benefit in terms of progression and survival.

Keywords: mastocytoma, canine, metastatic lymph node, HN2, lymphadenectomy, chemotherapy, prognosis

Introduction

Lymph node (LN) metastasis is an important predictor for disease recurrence and death in dogs with canine cutaneous mast cell tumors (cMCTs) and the removal of LNs with metastatic cMCTs has been associated with a better prognostic outcome.[1-5]

The WHO clinical staging system is based on the anatomic extent of the cMCT and is widely used for predicting survival outcome.[3,6,7] According to this system, stage II cMCTs are characterized by primary tumors being confined to the dermis and regional LN involvement. However, accurate detection of nodal involvement is hampered by several factors. The closest LN in the expected drainage area may not be the sentinel node and around 40% of LNs with metastatic disease would go unnoticed, if only the anatomically closest node was sampled rather than the sentinel node.[8] In addition, 50% of LNs with metastatic disease remain normal sized and cannot be diagnosed by palpation.[9] Lastly, accurate diagnosis of metastatic disease by discriminating neoplastic and non-neoplastic mast cells in a LN can be challenging.

While still suffering from numerous technical difficulties, standardized histological criteria have been proposed to more consistently characterize nodal involvement, and 4 histological patterns have been identified: HN0 (non-metastatic LN), HN1 (pre-metastatic LN), HN2 (early metastasis) and HN3 (overt metastasis).[1] Unfortunately, no standardization for trimming excised nodes has been proposed and even serial sectioning nodes at 0.2 cm

intervals would only arbitrarily detect individual mast cells or small clusters of such cells. Furthermore, the labeling of the categories HN1 and HN2 as "pre-metastastic" or "early metastatic disease", respectively, is misleading since these categories have not been shown to represent a progression of metastasis, but rather represent different morphologic categories that have been associated with prognostic outcome. Regardless, these nodal patterns are not included in the current WHO clinical staging system.

It has been recently shown that radical surgery of the primary cMCT together with regional lymphadenectomy of LNs with metastatic disease improves outcome.[4] However, in that study, HN2 and HN3 LNs were analyzed together, and all dogs received adjuvant medical treatment, consisting of chemotherapy, tyrosine kinase inhibitors (TKIs) or a combination of these. As a result, the benefit of adjuvant medical therapy in dogs with HN2 LNs in routine clinical practice remains to be investigated.

In this retrospective multicenter study, our objectives were to evaluate the therapeutic benefit of adjuvant medical therapy following surgical removal of primary low-grade (Kiupel-low and Patnaik grade 1-2) cMCT and their regional HN2 LNs by analyzing survival rates and patterns of recurrence. It was hypothesized that adjuvant medical treatment would not confer any survival benefit in these dogs.

105 **Material and methods**

106

107 Medical records were reviewed to identify dogs with treatment-naive, firstly
108 occurring, histologically confirmed low-grade cMCT with regional LN
109 metastasis and no distant spread.
110 To be eligible for inclusion, dogs had to undergo complete staging work-up
111 and wide surgical excision of the primary cMCT and lymphadenectomy of
112 the regional or sentinel LN, regardless of size and mobility. For the cases seen
113 at one of the authors' institution (xxx), the sentinel LN was identified by means
114 of scintigraphy; in all other cases, the regional LN was removed. The regional
115 LN was defined as the closest LN in the expected lymphatic drainage,[22] and
116 was identified either by palpation, ultrasound or surgical exploration.
117 Information on clinical stage was obtained by means of the following:
118 hematological and biochemical analysis; cytological evaluation of the
119 cutaneous nodule and regional LN; thoracic radiographs; abdominal
120 ultrasound, and fine-needle aspirates of liver and spleen regardless of their
121 sonographic appearance. Viscera were considered metastatic, if mast cells
122 appeared in clusters or sheets, in very large numbers or atypical on
123 morphology, as previously documented.[23] Regarding histological grade of
124 the primary tumor, only Patnaik grade 1 or 2, and Kiupel low-grade cMCTS
125 were admitted. Additionally, only dogs with HN2 LNs according to Weishaar[1]
126 were included in the analysis. Slides were not reviewed, rather the reports
127 from the individual laboratories were used for the purpose of this study. Dogs
128 with distant metastasis were excluded.

Dogs were categorized into adjuvant medical therapy or no medical therapy groups. For inclusion, patients had to receive at least one treatment administration in the case of traditional chemotherapy, and at least two weeks of therapy in the case of a TKI.

The decision on whether or not to receive adjuvant medical therapy was made at the discretion of the clinician and owner.

Finally, to be included in the analysis, a follow-up of at least 6 months from surgery had to be available. Dogs who had recurrence or dead/euthanized for tumor related causes within 6 months from surgery were included in the analysis.

Regardless of treatment, dogs were followed-up by means of clinical rechecks every 3 months for the first year, and every 6 months thereafter. Imaging was repeated whenever indicated; specifically, at some institutions abdominal ultrasound was repeated every 6 months, regardless of the disease status, whereas at other institutions imaging was carried out in the case of symptoms occurrence or progressive disease.

Dogs with concurrent multiple or subcutaneous MCTs were excluded from the study.

Background information recorded for each dog included: signalment; primary tumor description (location, size, presence of ulceration); clinical substage; site of nodal involvement; histopathological evaluation of surgical margins (complete, or incomplete, if large portions of the MCT itself were present within the margins or if small groups of mast cells having the same

morphological features as those of the primary tumor were retrieved within histological margin sections); histologic grade of the primary cMCT according to Patnaik and Kiupel classification systems; Kit-pattern (if performed); c-kit mutational status (exons 8, 9, 10, 11) (if performed); date of surgery; medical treatment (chemotherapy, TKIs or both); use of post-operative radiation therapy (RT); local relapse (defined as the cytological evidence of a recurrent cMCT within 2 cm from previous scar); nodal relapse (defined as presence of new metastatic LNs confirmed by cytology)[3]; distant relapse (defined as the occurrence of cytologically-confirmed visceral metastasis); date of death or last follow-up examination, and cause of death.

To determine the therapeutic value of adjuvant medical treatment, the characteristics of relapse (local, nodal and distant) and the survival impact were compared between the two groups.

Statistical analysis

Descriptive statistics were used in the analysis of dogs and tumor characteristics. When appropriate, data sets were tested for normality by use of the D'Agostino and Pearson omnibus normality test. 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 medical treatment and no medical treatment groups were assessed with Student's T-test (numerical, parametric variables), the Mann

177 Whitney U test (numerical, non-parametric variables) or the Chi-square test/
178 Fisher's exact test (categorical variables).
179 The considered variables included breed (predisposition to biologically
180 aggressive MCTs, i.e. Shar-pei, Labrador retriever and Golden retriever), age,
181 body weight, sex, anatomic location of the primary cMCT (head and neck,
182 trunk and limbs, inguinal/perineal/mammary region and digits),
183 macroscopic tumor longest diameter, ulceration, substage, and surgical
184 margins. For age, weight and tumor diameter, the median was used as the
185 cut-off value.
186
187 Time to local recurrence (TLR) was calculated from the date of surgery to
188 the date of local recurrence. Time to nodal relapse (TNR) was calculated
189 from the date of surgery to the date of new nodal involvement. Time to
190 distant relapse (TDR) was calculated from the date of surgery to the date of
191 diagnosis of visceral metastases. Time to progression (TTP) was calculated
192 from the date of surgery to the first occurrence of one or more of local
193 recurrence, nodal or distant relapse. Dogs with no recurrence or disease
194 progression at the date of the last visit or death were censored.
195 Overall survival (OS) was calculated from the date of surgery to the date of
196 death or to the date of the last visit if death did not occur. Only dogs
197 deceased for cMCT-related causes were considered as events.
198 Survival plots were generated according to the Kaplan-Meier product-limit
199 method. 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.

Cell Line Validation Statement

No cell lines were used in the current study.

Results

Patient and tumor characteristics

A total of 73 dogs fulfilled the inclusion criteria: 24 dogs were treated at xxx, 18 dogs at xxx, 12 dogs at xxx, Italy, 7 dogs at xxx, 7 dogs at xxx, 3 dogs at xxx, and 2 dogs at xxx.

Forty-two (57.5%) dogs received adjuvant medical treatment, and 31 (42.5%) did not. Patient and tumor characteristics are listed in Table 1.

There was good balance between groups regarding demographic features and possible outcome variables (Table 1).

Among the 42 dogs receiving adjuvant medical treatment, the tumors were located on limbs (n=25; 59.5%), head and neck (n=6; 14.3%), trunk (n=5;

11.9%), digits (n=2; 4.8%), inguinal region (n=2; 4.8%), mammary region (n=1; 2.4%) and axilla (n=1; 2.4%).

Forty-one (97.6%) dogs were asymptomatic at presentation (substage a), whereas one (2.4%) dog had signs of systemic effects of cMCT (vomiting, diarrhea, pruritus, and regional edema; substage b).

Based on the Patnaik grading system, there were 41 (97.6%) P-G2 cMCTs and 1 (2.4%) P-G1 cMCT. All (100%) cMCTs were Kiupel low grade.

Histopathological evaluation revealed clean surgical margins in 28 (66.7%) cMCTs, and incomplete margins in 14 (33.3%) cases.

Mutational analysis was available for 35 (83.3%) cMCTs: 5 cMCTs were mutated (4 had an ITD on exon 11, and 1 had an ITD on exon 8), while the remaining 30 were wild type.

The following metastatic ipsilateral LNs were removed: popliteal (n=15; 35.7%), superficial cervical (n=8; 19%), axillary (n=8, 19%), inguinal (n=6; 14.3%), and submandibular (n=5; 11.9%). Based on the Weishaar criteria, all (100%) LNs were classified as HN2. Four (9.5%) LNs were sentinel nodes identified by scintigraphy, whereas the other 38 were regional LNs. All sentinel LNs mirrored the regional LNs.

Among the 31 dogs not receiving adjuvant medical treatment, the tumors were located on limbs (n=20; 64.5%), inguinal region (n=4; 12.9%), head and neck (n=3; 9.7%), mammary region (n=1; 3.2%), trunk (n=1; 3.2%), digit (n=1; 3.2%), and axilla (n=1; 3.2%).

246 Thirty (96.8%) dogs were asymptomatic at presentation (substage a), whereas
247 one (3.2%) dog had signs of systemic effects of cMCT (substage b).
248 Based on the Patnaik grading system, there were 27 (87.1%) P-G2 cMCTs and
249 4 (12.9%) P-G1 cMCT. All (100%) cMCTs were Kiupel low grade.
250 Histopathological evaluation revealed clean surgical margins in 25 (80.6%)
251 cMCTs, and incomplete margins in 6 (19.3%) cases. Mutational analysis was
252 available for 12 (38.7%) cMCTs, and they were all wild type.
253 The following metastatic ipsilateral LNs were removed: inguinal (n=10; 32.3%),
254 popliteal (n=8; 25.8%), superficial cervical (n=8; 25.8%), axillary (n=4, 12.9%),
255 and submandibular (n=1; 3.2%). All (100%) LNs were classified as HN2
256 according to Weishaar et al., 2014. Fourteen (45.2%) LNs were sentinel nodes
257 identified by scintigraphy, whereas the other 17 were identified anatomically.
258 Eleven (78.6%) sentinel LNs mirrored the regional LNs. LNs identified as sentinel
259 nodes by scintigraphy were significantly more represented in the group of
260 dogs not receiving adjuvant medical treatment ($P < 0.001$).
261
262 *Treatment and outcome*
263 Among dogs receiving adjuvant medical treatment, 34 were treated with
264 vinblastine (administered at 2-3 mg/m^2 IV every 14 days for 8 cycles) and
265 prednisone, 7 with TKIs (toceranib administered at 2.4-2.8 mg/kg on a
266 Monday- Wednesday- Friday schedule for a median of 6 months), and 1 dog
267 received both.
268 Nine dogs in this group also received RT to the primary tumor bed.
269 The median follow-up time was 619 days (range, 182 to 1825 days).

Two (4.8%) dogs experienced local recurrence after 592 and 321 days, respectively. Both dogs had been diagnosed with complete surgical margins. Ki67 and c-kit mutational status were available for one dog only, and both suggested a non-aggressive biological behavior (Ki67 11%; wild-type status).

Interestingly, none of the 14 dogs that had incomplete surgical margins relapsed locally after a median follow-up of 637 days (range, 239 to 1709 days). Seven (50%) of these dogs also received adjuvant radiation therapy at the surgical scar. Ki67 was available for 7 dogs, ranging from 2 to 15%. c-kit mutational status was available for 11 dogs, and only 2 had an ITD (one on exon 8 and one on exon 11).

Three (7.1%) dogs experienced nodal relapse after a median of 321 days (range, 61-592) and 4 (9.5%) developed cytologically-proven distant relapse in the lungs (n=2) and liver and spleen (n=2) after a median of 230 days (range, 218-580).

At the end of the study, 37 (88.1%) dogs were alive, and 5 (11.9%) had died because of cancer-related causes after a median of 358 days (range, 218-673). Median OS was not reached.

By removing the 9 dogs receiving RT, 24 (82.7%) dogs were alive, and 4 (14.3%) had died because of cancer-related causes after a median of 497 days (range, 218-673). Median OS was not reached.

Among dogs treated with surgery only, none received adjuvant prednisone and none was irradiated.

The median follow-up time was 545 days (range, 185 to 2603 days).

None of the dogs experienced local recurrence, despite 6 of them having histological evidence of incomplete margins. None of the dogs had Ki67 or c-kit mutational status analyzed. The median follow-up for these dogs was 688 days (range, 316 to 2241 days).

Additionally, none of the dogs in the surgery only group experienced nodal relapse or distant relapse.

At the end of the study, 19 dogs in the group were still alive. Twelve dogs were dead for unrelated causes after a median of 693 days. Median OS was not reached.

When considered individually, there was no significant difference in TLR, TNR, TDR and OS between dogs treated with adjuvant medical treatment or surgery only. However, overall TTP was significantly worse in dogs receiving adjuvant medical treatment (P = 0.021). Regarding OS, there was a tendency to a worse outcome in dogs receiving medical treatment (P = 0.056; Figure 1). By excluding the 9 dogs receiving RT, P values for TTP and OS were 0.015 and 0.051, respectively. No additional prognostic factors were shown to be significant on survival analysis.

Discussion

The role of regional lymphadenectomy in dogs with cMCT remains a subject of debate.[1-5,10] Lymphadenectomy for overt metastatic LNs (HN3) is known to

confer a survival advantage. However, the benefit of elective regional lymphadenectomy in dogs with LNs harboring micrometastatic disease (HN2) is less clear. In addition to wide surgical excision of the primary cMCT, a previous study suggested that LN dissection improved outcome when compared to wide surgical excision alone.[4] However, in that study, patients with early (HN2) and overt (HN3) LN metastases were considered as a single group.

Furthermore, the role of adjuvant medical treatment following elective lymphadenectomy in dogs with a micrometastatic nodal load remains unclear. Some of these uncertainties must stem, in part, at least, from the grouping of cases that are prognostically distinct, as alluded to above with HN2 and HN3 LNs. One of the objectives of this study was to investigate a clearly-defined subset of the MCT-bearing population, with the aim of extrapolating conclusions that could confidently be applied to other patients with those defining characteristics.

The current study shows that dogs with low-grade cMCTs that underwent surgical excision of the primary tumor and elective lymphadenectomy of the HN2 regional LN harbored a good prognosis. Median OS was not reached in either group, with a minimum follow-up of 6 months after surgery.

The current study also shows that dogs undergoing adjuvant medical treatment had a shorter TTP compared with those treated with surgery only. Unexpectedly, not only did dogs not seem to benefit from adjuvant medical

342 treatment, but also the risk of tumor progression was significantly higher in
343 dogs receiving adjuvant chemotherapy or TKIs.
344 A direct detrimental effect of the adjuvant medical therapy has not been
345 clearly demonstrated. However, that possibility must surely be examined. It
346 may be hypothesized that chemotherapy may have depressed the host
347 defense immune system, thereby preventing it from identifying and killing
348 remaining tumor cells.[11-13]
349 It must also be considered possible that there were clinical differences
350 between the patients that received adjuvant medical therapy and those that
351 did not. Although testing for known prognostic factors failed to demonstrate a
352 statistically relevant difference between the two groups in this study, this does
353 not constitute a proof that there were no clinical nuances which led to a
354 different decision making process with some cases. This unconscious bias may
355 have led to the adjuvant medical therapy group being loaded with cases
356 which subjectively were thought to have a poorer prognosis.
357 The overall rationale for adjuvant chemotherapy for node-positive cMCTs is
358 the delivery of a systemic modality of anti-cancer treatment that may reduce
359 risk of death from micrometastatic disease. Since the risk of life-shortening
360 consequences of disease progression is sufficiently low in the case cohort
361 described in this study, the case for adjuvant chemotherapy does not stand
362 up to scrutiny.
363
364 In the current study, the majority of recurrences after lymphadenectomy
365 developed in distant sites such as the lung, liver and spleen. Interestingly, all 4

366 dogs that developed distant metastasis received adjuvant medical
367 treatment. When evaluating the known prognostic factors in these dogs, only
368 tumor longest diameter in one of them (>3 cm), anatomic location in a
369 second dog (head), and surgical margin status (incomplete) in a third one,
370 may have foreshadowed an aggressive biological course. These distant
371 relapses may be caused by lymphatic and/or hematogenous spread that
372 may already have occurred at the time of surgery or could not be prevented
373 by dissection of the LNs or medical treatment. Conversely, the sentinel LNs
374 may have not been recognized and removed in these dogs, leaving behind
375 unrecognized micrometastatic disease, favoring distant spread. Alternatively,
376 sectioning of the affected node may have simply missed more overt disease
377 that would have resulted in an HN3 classification. These same reasons may
378 explain the higher nodal relapse rate observed in dogs receiving medical
379 treatment.
380
381 Local recurrence occurred in 2 (4.8%) dogs in the group receiving medical
382 therapy, and in none of the dogs receiving surgery only. While complete
383 tumor resection appears capable of decreasing the risk of local recurrence,
384 both dogs experiencing relapse were reported to have clean surgical
385 margins.
386
387 A recurrence rate of 11% has already been documented for cMCTs resected
388 with clean margins.[14] Possible reasons behind this local regrowth of
389 completely excised tumors may lie in the width of the clean margins, and on

390 the sample sectioning technique (radial versus tangential sectioning).
391 Although no technique of surgical margin trimming is currently considered
392 unconditionally the best, tangential sectioning allows the histological
393 examination of a considerably greater percentage of the total margin
394 surface area, and has been recently shown to detect significantly more
395 incomplete surgical margins as compared with radial sectioning.[15] In the
396 present study, both cases experiencing local relapse despite clean surgical
397 margins were diagnosed using the radial sectioning technique.
398 Alternatively in some cMCTs neoplastic cells may have acquired a more
399 invasive phenotype.
400
401 Interestingly, 14 (33.3%) and 6 (19.4%) dogs in the medically treated group
402 and in the surgically treated group, respectively, had incomplete margins
403 based on histopathology. None of these tumors relapsed after median follow-
404 up of 637 and 688 days, respectively. It must be noted that in the medically
405 treated group, 7 of 14 (50%) dogs with incomplete margins were irradiated at
406 the surgical bed.
407 Failure to achieve histopathological clean margins in cMCT excision
408 frequently does not lead to local relapse.[16,17] There are several explanations
409 for the observed findings. It can be challenging to determine whether mast
410 cells at the surgical margins are inflammatory cells that have been attracted
411 by tumor-derived chemokines, or if they are neoplastic in nature.[18] Secondly,
412 and similar to the definition of "clean", there is no consensus in the literature
413 regarding the definition of "incomplete" surgical margins.[15,19]

Stromberg and Meuten warned that "the true margin between tumor and non-neoplastic tissues is in the patient". The pathologist examines a margin that is artificially created by surgery and histopathological processes. Therefore, what pathologists observe within their histological slides may occasionally misrepresent what is still present within the patient.[20]

The observation has been made that failure to remove metastatic LNs from patients with cMCTs increased the likelihood of local tumor recurrence. It has thus been postulated that lymphadenectomy may remove a reservoir for neoplastic mast cells, thereby impeding a process of *bidirectional* disease progression (local and distant).[4]

Fourth, the immune system may have been triggered by the surgical procedure, thereby sterilizing the margins.[13]

Finally, it has been previously documented that incompletely resected cMCTS with low proliferation activity may simply not recur.[21] Unfortunately in the current study, Ki67 was only performed in a minority of cMCTS, and in those a low proliferation activity was documented. However, the small number, and therefore the poor power, makes it impossible to draw definitive conclusions.

There are weaknesses to this study that limit the extrapolability of the conclusions. First, its retrospective nature only allowed capture of information that was reported in the medical records. Conformity between groups was tested by analysis of known prognostic factors. In a prospective study, clinical justifications could have been given for decisions to use or not to use

adjuvant medical therapy and the possibility that clinical nuance skewed the patient populations could be explored.

Second, although a minimum follow-up of 6 months was mandated for inclusion, the average follow-up remained less than 2 years. Local recurrence or metastatic disease that would have occurred later in the disease process remained undetected. Also, even though dogs were followed-up regularly, regardless of the treatment group, it may be possible that patients undergoing medical treatment were monitored more often, possibly catching up failures earlier.

Third, with relatively low patient numbers experiencing local recurrence or metastasis, the effect of medical treatment on outcome may have been ascribable to a type II error.

Fourth, the HN2 LN classification requires a subjective judgment. Histological review by a panel of pathologists might have led to reclassification of some cases, potentially altering the composition of either or both patient groups. Also, c-kit mutational status and Ki67 proliferation activity were not routinely performed.

Finally, the LN selected for removal was identified on anatomic grounds in the majority of cases; the sentinel LN was removed in only a minority. A significant reservoir of neoplastic cells may have been left behind in some animals. However, this would have lead to a higher local and distant failure in both groups.

In conclusion, by applying strict inclusion criteria, we have demonstrated that dogs with low grade (P-1/2, K-low) cMCTS and HN2 regional LNs that undergo surgical excision of the primary tumor and lymphadenectomy, have an excellent prognosis and do not seem to benefit from adjuvant medical treatment. Lymphadenectomy may afford a survival benefit via the debulking of microscopic LN metastases, as previously shown.[4]

Data availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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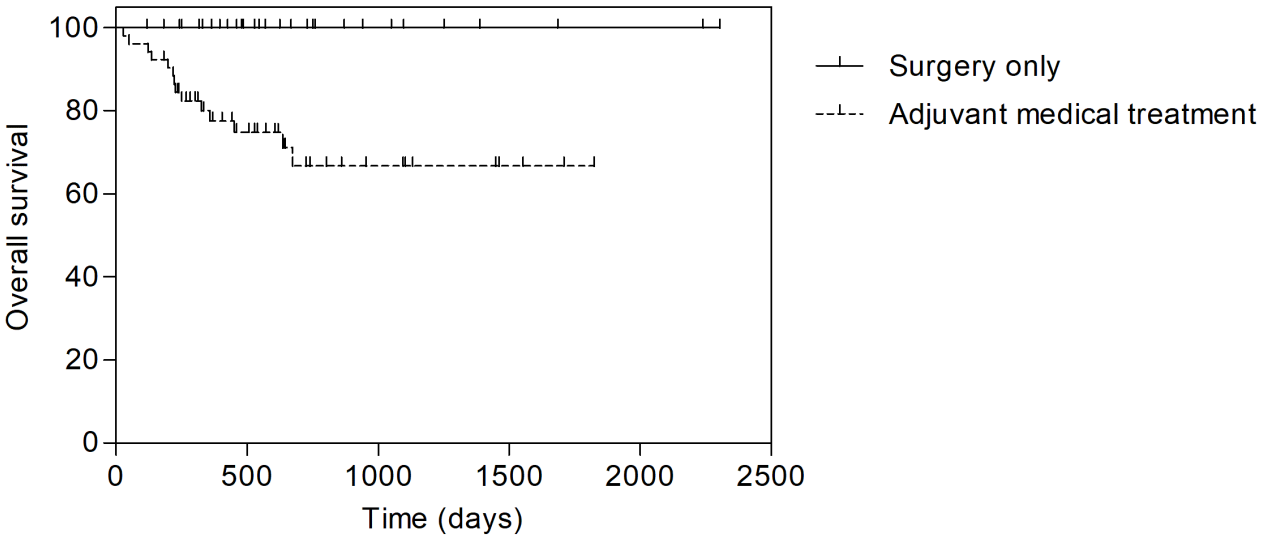
Figure legend

Figure 1. Kaplan–Meier survival plots for 73 dogs with low grade cutaneous MCT and HN2 regional lymph node according to Weishaar et al., 2014. There was a tendency to a worse outcome in dogs receiving adjuvant medical treatment following surgical removal and lymphadenectomy (P = 0.056).


Overall survival
100
80
60
40
20
0
0
500
1000
1500
2000
2500
Time (days)
Surgery only
Adjuvant medical treatment

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573

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575 **Table 1.** Distributions of variables potentially associated with prognosis in 73 dogs
576 with low grade cutaneous MCT and HN2 regional lymph node according to
577 Weishaar *et al.*, 2014, treated by surgery with or without adjuvant medical
578 treatment.
579

Variable	**Adjuvant medical treatment** (*n* = 42)	**Surgery only** (*n* = 31)	***P***
Breed			0.777
Shar-pei, Labrador retriever and Golden retriever	10	6	
other	32	25	
Age			0.323
median (range)	8 (3-11) years	7 (4-16) years	
Weight			0.514
median (range)	23.4 (5.8-49.0) kg	26.0 (6.0-65.3) kg	
Sex			>0.999
male	18	14	
female	24	17	
Neutering status			0.237
no	19	19	
yes	23	12	
Anatomic location			0.597

head and neck	6	3	
trunk and limbs	30	21	
inguinal, mammary, digital	6	7	
Tumor diameter			0.329
median (range)	2.0 (0.4-6.0) cm	2.2 (0.3-8.5) cm	
Ulceration			>0.999
no	37	28	
yes	5	3	
Substage			>0.999
a	41	30	
b	1	1	
Surgical margins			0.288
complete	28	25	
incomplete	14	6	
Patnaik grading			0.156
grade I	1	4	
grade II	41	27	

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