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To map or not to map the cN0 neck: impact of sentinel lymph node biopsy in canine head and neck tumours

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Abstract

Tumor stage is a prognostic indicator for canine malignant head and neck tumors (MHNT). However, consensus is lacking on nodal staging in the absence of clinically apparent nodal disease (cN0 neck). This prospective observational study aims to determine the diagnostic accuracy of radiopharmaceutical and blue dye for sentinel lymph node biopsy (SLNB), to assess the correspondence between sentinel lymph node (SLN) and clinically expected regional lymph node (RLN) and the impact on staging of the procedure in dogs with MHNT and cN0 neck.

Twenty-three dogs with MHNT and cN0 neck underwent tumour excision and SLNB guided by preoperative lymphoscintigraphy and intraoperative gamma-probe and blue dye. Diagnostic performances and detection rate were calculated. Correspondence between SLN and RLN, number of nodes excised, histopathological status of the SLN and complications related to the procedure were recorded. The mapping technique identified at least one SLN in 19/23 dogs, with a detection rate of 83%. The SLN did not correspond to the RLN in 52% of dogs. Multiple nodes were removed in 61% of dogs. At histopathology, 8 (42%) dogs had SLN+, of which 4 differed from the RLN. Only minor self-limiting complications occurred in 5 (22%) dogs.

Radiopharmaceutical and blue dye guidance is accurate (sensitivity 88.9%; specificity 100%) for SLNB in dogs with MHNT and cN0 and allowed the extirpation of unpredictable and/or multiple

SLN with minimal morbidity. Incorporation of SLNB in the management of MHNT is desirable to correctly stage the cN0 neck, owing the unpredictability of the lymphatic drainage.

Keywords: sentinel lymph node, dog, oral tumours, maxillofacial surgery, staging

Introduction

Tumor staging is of outmost importance in the oncological management of canine malignant head and neck tumors (MHNT) and failure to identify metastatic spread to the lymphatic basin may result in patient understaging and incorrect treatment recommendations, potentially worsening the prognosis.[1-9] Despite a lack of standardization in lymph node assessment, especially in the absence of clinically evident nodal disease (cN0 neck), it is well established that detection of nodal metastases should rely on histopathological examination of the excised draining lymph nodes.[10-15] However, identification of the draining node(s) in the head and neck is not straightforward due to individual variation in the lymphatic pathways of the canine head and neck as well as a variable number of lymphocenters and/or lymph nodes per lymphocenter.[9,14,17-19] Furthermore, unpredictable patterns of nodal metastasis for MHNT have been documented, with contralateral dissemination in up to 24-62% of dogs, and involvement of mandibular nodes, which are most commonly sampled due to their superficial location, reported in only 54-87% of cases.[17,18,20,21]

Elective node dissection (END) with extirpation of multiple lymphocenters of the head has been advocated both in human and small animal oncology for accurate staging of the cN0 neck. Although the procedure seems to be well tolerated in the canine patient, with only minor complication being reported and a lower morbidity than in humans, it involves extensive dissection and potentially unnecessary extirpation of non-draining nodes.[16,21-23] Hence, lymphatic mapping techniques and

sentinel lymph node biopsy (SLNB) are gaining acceptance owing their potential to accurately detect the presence of lymphatic metastases by harvesting the first node that drains a tumor (the sentinel lymph node, SLN), while limiting extensive dissection.[23-25] Despite the development of different SLN mapping methods, preoperative lymphoscintigraphy with intraoperative gamma probing has cemented its role in human oncology and is regarded as the gold standard for SLN mapping of several malignancies, including MHNT.[26] This technique has been validated in dogs with naturally occurring tumors, including those of the head and neck.[9,19,27] Reported detection rates as high as 100% compare favorably with other mapping techniques and suggest that lymphoscintigraphy may be considered the gold standard for SLN mapping in canine patients as well.[19,27] Hence, we prospectively applied this mapping technique to determine its diagnostic accuracy for SLNB in canine MHNT, and to investigate the relationship between location of primary tumor and SLN identified with this technique. The impact of SLNB on tumor staging was also assessed in a cohort of dogs with naturally occurring MHNT. We hypothesized that lymphoscintigraphy and blue dye is an accurate technique for guiding SLNB in MHNT and that it would allow for extirpation of clinically unpredictable nodes that my harbor occult nodal metastases, thus causing an upstaging in some dogs.

Materials and methods

This prospective observational study was conducted in a single institution from May 2017 to July 2020. We included consecutive client-owned dogs with cytologically or histologically confirmed spontaneous MHNT at first presentation or T0, amenable to surgical excision, and absence of clinical/tomographic evidence of nodal metastases (cN0 neck). We excluded dogs with RLN lymphadenomegaly (subjectively defined based on shape, consistency, comparison with contralateral nodes and bodyweight) detected during clinical examination (palpable nodes) or CT (non-palpable nodes) and cytologically confirmed nodal metastases. Prior to enrollment, all dogs staged negative for distant metastases according to the current recommendations for each tumor type. Owners had to sign a written informed consent to diagnostic and surgical procedures as well as data recording. All the procedures were performed following Institutional guidelines for animal welfare under the control of the National Ministry of Public Health.

Signalment data, location of MHNT and of the clinically expected RLN were collected. The RLN was identified following previous anatomical studies.[28-29] For preoperative SLN mapping, dogs were induced under general anesthesia and regional lymphoscintigraphy was performed: Technetium-99 metastable labeled nano-sized human serum albumin (Nanoalbumon; Radiopharmacy Laboratory Ltd, Budaors, Hungary) was injected subcutaneously/submucosally in four quadrants at 1-2 mm from the margins of the tumor at a dose of 7.8 – 31 MBq/0.5 mL (median: 23.31MBq); a single-head

gamma-camera (Prism 2000 XP; Picker International, Highland Heights, OH, USA) was used to acquire static and dynamic planar images until the first draining lymphocenter(s) were visualized.[9] Sentinel lymph node biopsy was performed concurrently with MHNT excision. Depending on the estimated anesthetic risk of each dog and duration of the surgical procedure, preoperative SLN mapping and surgical excision of MHNT and SLN(s) were performed either on the same day or on two consecutive days to reduce the duration of anesthesia. Anesthetized dogs were aseptically prepared for surgery and sterile methylene blue 1% (SALF S.p.A.; Cenate Sotto, Bergamo, Italy) was injected peritumorally in four quadrants at a dose of 0.4 mL just before moving the patient to the operating theater, approximately 10-15 minutes before surgical incision. Lymphadenectomy was performed before tumor excision to minimize cross contamination of the surgical field with tumor cells. A hand-held gamma probe (Crystal probe SG04, Crystal Photonic GmbH, Berlin, Deutschland) was used to measure the radioactive counts (RC) through the skin in order to direct the surgical incision over the lymphocenter and to guide the extirpation of the radiolabeled hot node(s). A node was defined as "hot" if it had RC two times greater than background counts.[9,30] Surgeons excised every hot and/or blue node(s) belonging to the lymphocenter(s) identified by preoperative lymphoscintigraphy and measured RC again ex-vivo. The gamma-probe was then used to measure residual RC in the lymphocenter and additional lymph nodes having counts 10% or greater than the first SLN were also extirpated.[9,30] Furthermore, every visible node belonging to the draining lymphocenter(s) was excised, even if not hot and not blue, in order to reduce the risk of residual disease.[14] Primary MHNT was then excised according to current recommendations for tumor type and location.

The additional surgical time for SLNB, surgical complications, and any adverse reaction to methylene blue or radiopharmaceutical were recorded. Correspondence between SLN(s) identified by preoperative lymphoscintigraphy, SLN(s) identified intraoperatively with the gamma probe and blue dye, and clinically expected RLN was assessed. The number of excised lymphocenters and lymph nodes per lymphocenter was also recorded.

If the procedures were performed on consecutive days, dogs were hospitalized overnight and peritumoral injection of radiopharmaceutical was not repeated before the second procedure. Dogs were hospitalized postoperatively for at least 24 hours and discharged at the discretion of the attending clinician.

The primary tumor and all the excised nodes were processed for histopathology after 24 hours for radioactive decay. Sentinel lymph nodes were cut longitudinally at the level of the hilus and additionally sliced every 1.5 mm if thicker than 3 mm at their minor axis; all sections thus obtained were processed for histopathology. Histopathological reports included MHNT histological diagnosis (tumor type), the surgical margin status (infiltrated or tumor-free), and presence or absence of SLN metastasis (SLN+ versus SLN-)

Dogs were rechecked 7 and 14 days after surgery and further rechecks were scheduled if deemed necessary by the attending clinician or if required by the owner. Thereafter, in the absence of clinical signs related to suspected tumor progression, periodic rechecks (including clinical examination, thoracic radiographs, and abdominal ultrasound when deemed necessary) were scheduled every 3 months to monitor tumor progression. Descriptive statistics were used to summarize obtained data. Sensitivity and specificity evaluated the performance of SLNB with radiopharmaceutical and blue dye followed by histopathology. Positive and negative likelihood ratios (LR+; LR-) were calculated to assess the propensity of SLNB to rule in (LR+) or rule out (RL-) nodal metastases.[31] The strength of evidence of the likelihood ratios to rule in/out the presence of nodal metastases was classified according to Hayden and colleagues.[32] Likelihood ratios also indicate the magnitude of change from pretest assessment of the probability of SLN+ to the likelihood of SLN+ neck after knowing the result of the test. Positive and negative predictive values (PPV; NPV) were calculated considering a 35%-45% prevalence of nodal metastases in MHNT, estimated on the basis of the available literature.[17,18] The 95% confidence interval (C.I.) for sensitivity and specificity were calculated with Clopper and Pearson's method.[33] For likelihood ratios, 95% C.I. were calculated with Marill's method.[34]

Results

Twenty-three MHNT in 23 dogs were included in the study. Signalment data, location of the MHNT, clinically expected RLN and SLN(s) are detailed in Table 1.

The combined use of radiopharmaceutical and blue dye identified at least one SLN in 19/23 animals, leading to an estimated detection rate of 83%. A SLN was not detected in 4 dogs, all affected by thyroid tumors. Preoperative lymphoscintigraphy identified more than one draining lymphocenter in 4/23 (17%) dogs. Fifty-one lymph nodes were excised, with more than one lymph node removed in 14/23 animals (61%) and multiple nodes excised from the mandibular lymphocenter (from 1 to 4 nodes) and from the superficial cervical lymphocenter (from 1 to 3 nodes). Conversely, only 1 retropharyngeal node was identified in the study population. Fifteen non-hot, non-blue nodes were visualized during surgical dissection of the SLN and extirpated in 7 dogs; all these nodes belonged to the mandibular lymphocenter.

In 12 dogs (52%), lymphoscintigraphy identified at least 1 lymphocenter that did not correspond to the clinically expected RLN. Of these, in 5 cases the SLN was completely different from the RLN; in 5 cases lymphoscintigraphy identified multiple SLNs, one that corresponded to the RLN and one or more at unpredictable sites; in 3 cases identification of the RLN was not possible based on the location of the primary tumor, and lymphoscintigraphy permitted to excise at least one SLN (Table 1). In 2 dogs with MCT of the conjunctiva (case #16) and eyelid (case #20) the SLN was the parotid

node, which corresponded to the RLN based on the study by Suami et al (2013) but did not fully correspond based on previous anatomical studies that suggested an overlapping of lymphatic drainage of this area between the parotid and mandibular lymphocenters (Table 1).[28,29]

Based on ex-vivo RC count, 34 (67%) of the 51 excised nodes were hot, with at least one hot node being extirpated from the sentinel lymphocenter in every dog; 27/34 (79%) hot nodes were also blue stained, while 7 (21%) nodes in 6 dogs were hot but not blue stained. Seventeen nodes (33%) were excised despite having a RC of 0, of which 2 in 2 dogs with an oral melanoma (OMM) and squamous cell carcinoma (OSCC) were blue stained. At least one blue node was excised in all but 3 dogs with an adenocarcinoma of the parotid gland, an oral fibrosarcoma and an MCT (Table 1). Median dimensions of the excised nodes at the longest diameter was 15 mm, ranging from 2 to 30 mm.

Median surgical time for SLNB was 20 minutes, and no major complications related to this procedure were recorded in the included cases. Transitory self-limiting edema of the muzzle and seroma formation at the lymphadenectomy site occurred in 3 and 2 dogs, respectively, and remitted spontaneously within 7-15 days from surgery.

At histopathology, tumor types included: MCT (n=8), OMM (n=5), thyroid carcinoma (n=4), oral fibrosarcoma (FSA) (n=2), oral osteosarcoma (OSA) (n=1), soft-tissue sarcoma (n=1), parotid gland adenocarcinoma (n=1), OSCC (n=1). Nodal metastases were detected in 8 animals (42%), of which 4 (21%) had an unpredictable SLN and 1 had a parotid node excised and would have thus been under-staged without the mapping procedure (Table 1).

During the follow-up period (median 250 days, range 25 – 1185 days), 1 dog with OMM experienced locoregional recurrence (histologically confirmed metastases to another node located medially to the second third of the left jaw at 180 days), 2 dogs with oral FSA and OMM had local and distant tumor progression (120 days and 180 days respectively), and 1 dog with an adenocarcinoma of the salivary gland had distant progression (800 days) (Table 1). The 4 dogs that developed progressive disease had SLN-. (Table 1).

Sensitivity and specificity were 88.9% (95% C.I. 51.8 – 99.7%) and 100% (95% C.I. 69.2 – 100%), respectively. As specificity was 100%, LR+ tended to infinity and only the lower limit of the 95% was calculated (3.34); LR- was 0.11 (95% CI 0 – 0.424) indicating a moderate increase in the likelihood of having pN- in case of a negative test (Hayden 1999). PPV and NPV were 100% (95% C.I. 57.9% - 100%) and 94.4% (95% C.I. 63.7% - 99.8%), respectively, when considering a prevalence of nodal metastases of 35%; they were 100% (95% C.I. 57.9% - 100%) and 91.7% (95% C.I. 63.7% - 99.8%) with an estimated prevalence of nodal metastases of 45%.

Discussion

In this study, preoperative lymphoscintigraphy and intraoperative gamma probing and blue dye allowed for extirpation of at least one SLN in 19/23 dogs with MHNT, leading to a detection rate of 83%, with minimal morbidity. Arguably, identification of the draining lymphocenter based on the anatomical location of the primary tumor may be misleading, due to individual variations of drainage pathways and tumor-induced lymphangiogenesis.[17,18,20,14] Although Suami and colleagues (2013) described three lymphatic basins in the canine head and neck (mandibular, parotid, dorsal superficial cervical), an overlapping of the lymphatic vessels in this area was observed; furthermore, the inclusion of only 2 live dogs and the mapping of the superficial areas only are two main limitations of that study that may have hampered the accuracy and repeatability of their results, owing the wide range of anatomical variations between dog breeds and of possible different locations of MHNT.[28] Moreover, consistency is often lacking among anatomical studies and lymphosomes do not always correspond.[28,29] Hence, some authors recommend the extirpation of multiple lymphocenters of the head for nodal staging in dogs with MHNT and cN0 neck.[16-19,22] With this technique, contralateral dissemination was identified in 24-62% of dogs with lateralized tumors,[14,18] and in 21-45% of cases metastases were detected in lymph nodes other than the mandibular.[14,17,18,20] Similarly, in the sample population intraoperative radiopharmaceutical plus blue dye guided the extirpation of at least one

SLN other than the clinically expected RLN in 52% of dogs and corresponding to the ipsilateral mandibular lymphocenter in only 22% of dogs, underscoring the unpredictability of the lymphatic drainage of the canine head and neck.

In human medicine END is suggested in patients with OSCC and OMM that have an estimated risk of occult nodal metastases > 20%, which means that 70-80% of patients potentially undergo unnecessary extensive dissection with increased morbidity and costs.[23] Lately, SLNB has been proposed as an alternative to END in humans with OSCC and OMM to reduce the surgical extent and morbidity associated with END while still allowing to detect occult nodal metastases.[23,35] Reported results are encouraging, with sensitivity as high as 87% and NPV of 94% for early stage OSCC.[35] Likewise, in canine surgical oncology interest has been drawn on SLNB, with several mapping techniques being described for various tumor types including MHNT, and detection rates as high as 100% reported for lymphoscintigraphy.[19,21,27] In the sample population, we report a detection rate of 83%, which is lower than in previous studies, due to the inclusion of 4 dogs with thyroid tumors in which a SLN was not identified.[19,27] Both hematogenous and lymphatic spread have been reported for thyroid tumors in dogs, and the impossibility to detect a SLN in these 4 dogs may suggest a lack of tumor-associated lymphatic drainage in early-stage thyroid carcinomas rather than a failure of SLNB. Reported rates of nodal metastases for thyroid carcinomas varies significantly among studies (7-38%), probably due to the lack of standardization in staging modalities and inclusion of early to advance stage tumors.[36-38] Recently, metastases to surgically excised deep cervical lymph nodes have been described in 45% of dogs with thyroid carcinoma, several of which had negative prognostic factors such has bilateral tumors, lymphatic or vascular invasion and capsular invasion.[39] Conversely, the 4 thyroid tumors included in the present study were early-stage and no negative prognostic factors were recognized, except for the ectopic location of 1 thyroid carcinoma.[36] Although the impact of recognized prognostic variables and tumor stage on the likelihood of lymphatic spread has not been assessed yet for canine thyroid carcinoma, it is reasonable to assume that early stage tumors without negative prognostic factors may have a lower risk of lymphatic dissemination. The lack of tumor

progression that we report without adjuvant therapies after a median follow-up of 572 days further corroborates the hypothesis of the absence of lymphatic network in these early-stage thyroid carcinomas. This consideration is consistent with the prolonged survival times previously reported for surgically resected early-stage thyroid tumors and with metastases being more frequently detected during necroscopy (60-80%) than at the time of treatment (7-38%).[37,38]

In the study population, at least one "hot" node was extirpated in each dog, and only 7/34 hot nodes were not blue stained, confirming the correspondence between RC and blue dye.[9,30] In human medicine adverse reactions to methylene blue injection are not rare, and some authors suggest the omission of blue dye owing the high detection rates reported with use of the radiopharmaceutical alone.[40] In dogs, however, side effects to vital dyes have not been observed neither in previous studies nor in the sample population. [9,30] Hence, it is the authors' opinion that blue dye should be used in association with radiopharmaceutical to improve intraoperative visualization of the target lymph nodes and avoid inadvertent damage to surrounding neurovascular structures. Conversely, two excised nodes belonging to the sentinel lymphocenter detected preoperatively were blue but not "hot", and more interestingly one of these nodes was metastatic at histopathological examination. In the latter case, the dog had a OSCC of the tongue and the histological architecture of the lymph node was completely effaced by metastatic cells; thus, it may be hypothesized that this lymph node did not drain enough radiopharmaceutical to be detected intraoperatively due to neoplastic occlusion of the lymphatic network, as previously reported.[42,43] Additional nodes without RC and not blue-stained were extirpated from the mandibular lymphocenter in 7 dogs in order to reduce the risk of residual nodal disease.[14] This decision was mainly dictated by the explorative nature of this study, although it is worth mentioning that intraoperative identification of the SLN in the mandibular lymphocenter may not always be straightforward, given the close proximity of multiple nodes in this location, and non-hot nodes may thus be accidentally removed during dissection.[9] However, it should be noted that none of these nodes had occult metastases at histopathology, underscoring the accuracy of the combined technique and confirming that non-hot/non-blue nodes may not need to be removed.

Intraoperative gamma probing guided the extirpation of multiple SLNs in 10 dogs, owing the ability of this technology to accurately detect residual RC in the surgical field.[15,19] Furthermore, in 2 dogs with MCT of the conjunctiva and eyelid, the technique guided the extirpation of the parotid lymph, which is not often excised in clinical practice given the difficulty to identify lymphatic structures in that area.[14,30] The latter dog (case #20) had an overt metastatic parotid node (HN3[41]) and received adjuvant chemotherapy based on the SLNB findings. The possibility to detect multiple nodes and to evaluate the parotid lymphocenter represent some advantages of intraoperative guided SLNB compared to preoperative mapping alone or END. Indeed, although Smith and colleauges (1995) originally described a technique for END that allowed for extirpation of mandibular, retropharyngeal, and parotid lymphocenters,[16] more recently published studies reported the excision of bilateral mandibular and retropharyngeal nodes only, while the parotid nodes were not assessed (Skinner 2016, Grimes 2019).[14,18] Hence, with the latter form of END there is potential to miss nodal metastases to the parotid lymphocenter.

According to our results, another potential advantage of SLNB is the reduced surgical extent compared to END. In the sample population, only 4 animals underwent the excision of multiple lymphocenters, while for the majority of the dogs, lymphoscintigraphy guided the dissection towards a single lymphocenter, thus reducing the surgical extent. Although END seems to be well tolerated in the canine patient, with only minor complications reported, studies assessing the actual complication rate and morbidity of the procedure are still lacking.[15,18] With SLNB we recorded only self-limiting complications occurring in 26% of dogs, underscoring the tolerability of this procedure.

To validate our results, we statistically evaluated the diagnostic accuracy of SLNB with radiopharmaceutical and blue dye. The fact that specificity was higher than sensitivity suggests that the procedure is more useful to confirm than to rule out nodal metastases. However, the expected rate of false negatives based on these results is low (approximately 10%) and the high NPV (91.7 – 94.4 %) indicate that in the sample population the technique was useful in ruling out nodal metastases as well. These results are encouraging and underscore the accuracy of the technique, although they

313 should be confirmed on a larger sample and considering the prevalence of nodal metastases for each
314 tumor type.
315 In one dog with an OMM of the rostral mandible, SLNs (left mandibular nodes) were negative for
316 metastases but this dog developed locoregional relapse to a lymph node located rostral to the
317 mandibular nodes at 180 days (Table 1). This case was considered a possible false negative based on
318 previous experience in human medicine.[23] In humans, the ability to identify the draining lymph node
319 is site-specific, and SLNB is reportedly less sensitive for floor of the mouth tumors due to the "shine-
320 through effect".[23] In these cases, the high radioactivity of the area around the tumor may obscure the
321 visualization of the SLN. Similarly, it may be hypothesized that in our case the actual SLN was not
322 identified because it was in the flare of the OMM, and an upper echelon lymph node, that was not
323 metastatic already, was excised instead. It should be noted that the gamma probe used in the present
324 study has an integrated tungsten 40° collimator, which enables detection of radiation from a specific
325 direction and is considered optimal for breast cancer in humans. However, interchangeable
326 collimators between 20-90° are commercially available to improve the gamma sensitivity, and the
327 potential benefits of the use of different collimators to discriminate between tumor and SLN signal
328 in specific anatomical location, such as the floor of the mouth, warrant further investigations.
329 Three dogs with an oral FSA, an OMM and a parotid gland adenocarcinoma developed distant
330 metastases despite having SLN-, thus suggesting that the metastatic process occurred through an
331 hematogenous rather than lymphatic way. While nodal metastases from oral FSA and salivary gland
332 tumors reportedly occur in 3-10% and 11% of dogs, respectively, locoregional spread is commonly
333 observed in OMM, with reported rates of nodal metastases as high as 59-74% .[1,3,44-46] However, both
334 in dogs and humans OMM locoregional metastases are not always correlated with distant spread; this
335 observation has led to the "marker hypothesis" which states that removal of a metastatic node has
336 merely staging significance and loco-regional control but does not improve outcome, although
337 extirpation of positive SLNs in canine OMM has not been previously assessed for improved overall

338 survival.[46-48] In the future, the significance of SLN+ versus SLN- should be evaluated taking into
339 account the biological behavior of each tumor type.
340 In our study, occult nodal metastases were detected in 42% of dogs with cN0 neck, and of those, 5
341 (22%) had a SLN that would have not been excised without the mapping procedure due to a lack of
342 correspondence between the SLN and the RLN (n=4) or to its location in the parotid lymphocenter
343 (n=1), thus underscoring the importance of the correct identification of the draining lymph node for
344 tumor staging in the cN0 neck. Histopathological identification of occult nodal metastasis is a crucial
345 step of SLNB, and in human medicine it is well established that serial sectioning and
346 immunohistochemistry on excised nodes increase the ability to identify occult nodal metastases.[23,48]
347 Likewise, at our institution SLNs are routinely processed with serial sectioning potentially increasing
348 the sensitivity of the technique, although in canine oncology studies assessing the potential
349 advantages of serial sectioning for the detection of occult nodal metastases are still lacking.[14,15]
350 Another question that is yet to be answered, is how to treat dogs with SLN+ following SLNB. In
351 human medicine, patients that have occult nodal metastasis to the SLN undergo adjunctive surgery
352 to remove the remainder of the lymphatic basin.[23,50] In canine oncology there is still a gap of
353 knowledge on the potential benefits of additional lymph node dissection in dogs with metastatic SLN.
354 Excision of early metastatic lymph nodes (HN2[41]) in dogs with low-grade MCT resulted in a good
355 long-term survival even without adjuvant treatment, suggesting a potential therapeutic effect of
356 metastatic nodal removal.[51] However, there is still no evidence of the therapeutic effects of
357 lymphadenectomy in dogs with MHNT and SLN+/SLN-, and SLNB or END are currently performed
358 for staging purposes only. These issues should be addressed in future studies comparing the tumor-
359 specific outcome of dogs with a positive SLN that undergo additional lymphadenectomies versus
360 medical treatment or watchful waiting after SLNB. Despite its high diagnostic accuracy,
361 lymphoscintigraphy is not readily available for most veterinary facilities due to the costs of the
362 technology required and the need for specific permissions to store the radiotracer; thus, more
363 accessible intraoperative SLN mapping techniques should be validated by assessing their diagnostic

accuracy in comparison with lymphoscintigraphy to allow for a greater dissemination of SLNB in veterinary oncology.

The decision to include various tumor types was dictated by the explorative nature of our study and did not preclude the evaluation of the diagnostic performances of radiopharmaceutical and blue dye for SLNB and of its impact on tumor staging. However, to better understand the role of SLNB in the oncological management of MHNT the impact of this procedure on prognostication should be assessed. Furthermore, the accuracy and morbidity of SLNB and END should be compared in order to confirm the potential benefits of a targeted lymph node approach.

Given the unpredictability of the lymphatic drainage of the canine head and neck, with SLN not corresponding to the clinically expected RLN in 63% of cases in the present study, lymph node staging of dogs with MHNT and cN0 neck should rely on SLNB. The use of a mapping technique with intraoperative guidance has the potential to raise the diagnostic accuracy of the procedure by allowing for detection of multiple SLNs and extirpation of target lymph nodes that may be missed with END, thus reducing the risk of false negatives. Despite the impact of a targeted lymph node approach on correct staging of MHNT, the actual prognostic implications of the detection of occult nodal metastases in highly aggressive tumors such as OMM as well as the possible therapeutic role of the excision of cN0 and SLN+ in less aggressive MHNT are yet to be clarified.

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Case	Signalment	Tumor location	RLN (Suami et al 2013; Bezuidenhout 2013)	SLN	SLN hot	SLN blue	SLN Status	Tumor type	Progressive Disease	Outcome
#1	Pug, M, 3.5y, 9.7kg	Left parotid region - cutaneous	Left mandibular vs left parotid	Left superficial cervical	yes	yes	HN2	Cutaneous MCT, grade 2 Patnaik, Low Kiupel	no	Alive at 1185 days
#2	Labrador R., M, 1y, 32kg	Nose	Left mandibular	Right mandibular	no	no	HN0	Cutaneous MCT, grade 1 Patnaik, Low Kiupel	no	Alive at 760 days
				Right mandibular	yes	yes	HN0			
				Right mandibular	yes	yes	HN0			
				Left mandibular	no	no	HN0			
				Left mandibular	yes	yes	HN0			
				Left. mandibular	yes	yes	HN0			
#3	Dachshund, Fn, 11y, 5kg	Sublingual thyroid	ND	ND	ND	ND	ND	Follicular carcinoma	no	Alive at 685 days
#4	Golden R., M, 12y, 37kg	Rostral mandibula	ND	Left mandibular	no	no	-	Melanoma	LRR at 180 days DM at 525 days	Euthanasia at 525 days
				Left mandibular	no	yes	-			
				Left mandibular	yes	yes	-			
#5	Mixed-breed, M, 12y, 47kg	Left parotid gland	Left medial retropharyngeal, left parotid	Left mandibular	no	no	-	Parotid adenocarcinoma	DM at 800 days	Death at 854 days
				Left mandibular	no	no	-			
				Left mandibular	no	no	-			
				Left mandibular	no	no	-			
				Left parotid	yes	no	-			
#6	Mixed-breed, Fs, 10y, 23kg	Right thyroid	Right medial retropharyngeal	ND	ND	ND	ND	Papillary carcinoma solid variant	no	Alive at 630 days
#7	Pinscher, M, 15y, 6.4kg	Tongue	Right medial retropharyngeal	Left mandibular	no	yes	+	Squamous cell carcinoma	no	Death due to uncontrolled epilepsy at 25 days
				Left mandibular	no	no	-			
				Left mandibular	no	no	-			
				Left medial retropharyngeal	yes	yes	-			
				Right mandibular	yes	no	-			
				Right mandibular	no	no	-			
				Right medial retropharyngeal	yes	yes	-			
#8	Mixed-breed, Mn, 12y, 22.3kg	Left temporal region - cutaneous	Left mandibular vs left parotid	Left superficial cervical	yes	yes	HN2	Subcutaneous MCT	no	Death at 150 days due to LR of another MCT (hindlimb)
				Left superficial cervical	yes	yes	HN0			
				Left superficial cervical	yes	yes	HN0			
#9	Italian Hund, M, 9y, 19.4kg	Right thyroid	Right medial retropharyngeal	ND	ND	ND	ND	Follicular carcinoma	no	Alive at 515 days

#10	Boxer, M, 9y, 42kg	Left neck - cutaneous	Left superficial cervical	Left superficial cervical	yes	no	HN0	Subcutaneous MCT	no	Alive at 485 days
				Left superficial cervical	yes	no	HN0			
#11	Rhodesian, Mn, 8y, 45.8kg	Caudal right mandibula	Right mandibular	Right mandibular	yes	yes	-	Amelanotic melanoma	no	Death at 90 days due to appendicular OSA
				Right mandibular	yes	yes	+			
#12	Chow-chow, Fn, 1y, 20.7kg	Caudal left mandibula	Left mandibular	Left mandibular	no	no	-	Fibrosarcoma grade 1	no	Alive at 485 days
				Left mandibular	yes	yes	-			
				Left mandibular	no	no	-			
#13	Am-staff, Mn, 3y, 25kg	Left auricular region - cutaneous	Left mandibular vs left parotid	Left prescapular	yes	yes	HN1	Cutaneous MCT, grade 2 Patnaik, Low Kiupel	no	Alive at 395 days
				Left prescapular	yes	yes	HN1			
#14	Labrador R., Fn, 11y, 29.4kg	Middle right mandibula	Right mandibular	Right mandibular	no	no	-	Pleomorphic sarcoma grade 3	LR and DM at 120 days	Euthanasia at 120 days
				Right medial retropharyngeal	yes	no	-			
#15	Fox terrier, Fn, 9y, 8kg	Left temporal region - cutaneous	Right mandibular vs right parotid	Right superficial cervical	yes	yes	HN2	Cutaneous MCT, grade 2 Patnaik, Low Kiupel	no	Alive at 260 days
#16	Tibetan terrier, M, 2y, 10kg	Upper left conjunctiva (T0)	Left parotid vs left mandibular	Left parotid	yes	no	HN0	MCT	no	Alive at 250 days
#17	Flat-coated R., Fn, 12y, 28kg	Left thyroid	Left medial retropharyngeal	ND	ND	ND	ND	Medullary carcinoma	no	Alive at 240 days
#18	Labrador R., M,	Right labial vestibulum	Right mandibular	Right mandibular	yes	yes	-	Melanoma	DM at 180 days LR at 210	Euthanasia at 210 days
				Right mandibular	yes	yes	-			
#19	Mixed-breed, Fn, 11y, 10.25kg	Rostral-right mandibula	Left mandibular	Right mandibular	yes	yes	-	Osteosarcoma	no	Death at 192 days due to uncontrolled diabetes mellitus
				Left mandibular	yes	yes	-			
				Right medial retropharyngeal	yes	yes	-			
#20	English setter, F, 6m, 15kg	Right third eyelid	Right parotid vs right mandibular	Right parotid	yes	yes	HN3	MCT	no	Alive at 160 days
#21	Mixed-breed, M, 8y, 35kg	Left upper lip	Left mandibular	Left mandibular	yes	yes	-	Melanoma	no	Alive at 90 days
				Left mandibular	no	no	-			
				Left mandibular	yes	yes	+			
				Left mandibular	no	no	-			
#22	Bernese, Fn, 4.5y, 38.5kg	Right neck - subcutaneous	Right superficial cervical	Right superficial cervical	yes	yes	-	STS	no	Alive at 90 days
#23	Bernese, M, 6y, 42.5kg	Left upper lip	Left mandibular	Left mandibular	yes	no	-	Melanoma	no	Alive at 60 days
				Left mandibular	yes	yes	+			

Table 1. Signalment, location of mHNT, location of RLN, location of SLN identified by preoperative lymphoscintigraphy, gamma probe and blue guidance, tumour type and outcome of the 23 dogs included in the study. M: male; Mn: neutered male; F: female; Fn: neutered female; ND: not determined; MCT: mast cell tumour; LRR: loco-regional recurrence; LR: local recurrence; DM: distant metastases.

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