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Sentinel lymph node mapping with computed tomography lymphangiography and intraoperative methylene blue peritumoral injection has a high detection rate with moderate agreement in dogs with oral neoplasms

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Objective
To investigate the feasibility of indirect CT lymphangiography (CTL) and intraoperative lymphangiography with methylene blue (IOL-MB) for sentinel lymph node (SLN) mapping in canine oral cancer and to report both agreement between the techniques and accuracy of identifying metastatic lymph nodes (LNs).

Methods
This prospective study included 38 client-owned dogs with gross macroscopic or incompletely excised microscopic oral neoplasms. All dogs underwent CTL, IOL-MB, and extirpation of bilateral mandibular and retropharyngeal LNs. The detection rate of SLNs using the combined techniques was evaluated, and agreement between CTL and IOL-MB was assessed.

Results
The combined techniques identified all metastatic cases (4 of 4 dogs, 6 of 6 LNs) and achieved an SLN detection rate of 97.4% (37 of 38 dogs), with moderate agreement between modalities (76.8%; $\kappa = 0.481$). Nine cases showed discrepancies between the techniques, including 1 involving a metastatic LN.

Conclusions
CTL and IOL-MB demonstrated moderate agreement and an excellent detection rate for SLNs. With moderate agreement between modalities, our results suggested that the relationship between mapped LNs and true SLNs is not always straightforward.

Clinical Relevance
Results of this study suggested that SLN mapping techniques are most effective when there is no evidence of overt clinical LN metastasis. Employing at least 2 modalities is advisable, as metastasis may impact SLN identification rates depending on the technique utilized.

Keywords: dog, oral neoplasm, computed tomography lymphangiography, sentinel lymph node, methylene blue lymphangiography

Sentinel lymph node (SLN) mapping, a rapidly advancing component of cancer staging for human patients, aims to identify the specific LN most likely to first harbor tumor metastasis and may also offer therapeutic benefits.[1–3] Sentinel lymph node biopsy enhances accuracy in cancer staging (ie, the presence of nodal metastasis), allowing for elective node dissection. In humans, clinical trials demonstrate its equivalence to conventional axillary LN dissection in early breast cancer patients with clinically negative LNs,[1,2] potentially reducing surgical complications compared to nonselective lymphadenectomy.[4–6] This targeted approach has recently gained popularity in veterinary medicine as clinicians have become aware of the difficulties in accurate detection of metastatic

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LNs with a traditional approach (ie, anatomical proximity or nodal enlargement).[7–12] Although the recent research has actively explored the feasibility of various SLN mapping techniques in veterinary medicine, including lymphoscintigraphy, radiographic lymphangiography, indirect CT lymphangiography (CTL), colorimetric lymphangiography (ie, methylene blue), and fluorescence lymphangiography (ie, indocyanine green),[10,12–19] standardized techniques for SLN evaluation are currently lacking.

Lymphoscintigraphy, using radioactive tracers, is considered the current gold standard for SLN mapping in human medicine.[20,21] While the feasibility of scintigraphy has also been studied in veterinary medicine,[10,15,18] its clinical application remains limited. This is largely due to the restricted availability of radioactive tracers and the specialized equipment required, as well as the stringent safety precautions associated with handling radioactive materials.[10,12,15,22] Computed tomography lymphangiography utilizing a water-soluble iodine-based contrast agent is an appealing modality in veterinary medicine.[12,14,16,19,23] Although this technique is not necessarily less expensive than indocyanine green (ICG) near-infrared fluorescence or lymphoscintigraphy in institutions equipped with near-infrared fluorescence systems and radioactive tracer facilities—particularly in cases where CT is not already part of routine surgical planning—it offers distinct advantages. Computed tomography is widely available and provides detailed information about the anatomical relationships of SLNs, as well as the size, architecture, and contrast enhancement patterns of LNs. Methylene blue (MB) is a commonly used intraoperative tracer as it is readily available, cost-effective, and simple to use[13,18,24]; however, when compared to radioactive tracers or ICG with near-infrared imaging, MB has an inferior detection rate of SLNs.[16,25,26] Recent studies[8,14,17,27] have provided evidence for successful applications of SLN mapping with MB in dogs with mammary cancers, mast cell tumors, and other superficial (palpable) tumors.

The main lymphocenters of the head and neck are the parotid, mandibular, and medial retropharyngeal lymphocenters. Because of the complex anatomy and documented interindividual variability in the location of metastatic LNs[28–30] and SLNs,[12,16,17] further investigation on the SLN mapping with widely available techniques appears to be a timely topic. Therefore, the objective of this prospective study was to investigate the feasibility of CTL and intraoperative lymphangiography with MB (IOL-MB) for SLN mapping in canine oral cancer and to report both agreement between the techniques and accurate identification of subclinical metastatic LNs. We hypothesized that CTL and IOL-MB will have moderate agreement for identification of the SLN and that the combination of techniques will accurately identify the LN that harbors subclinical metastasis, when present.

Methods

Patient characteristics

Client-owned dogs with a primary tumor of the oral cavity or a scar from a previously incompletely excised cytologically or histopathologically diagnosed malignant tumor were prospectively enrolled from 4 institutions (University of Guelph Ontario Veterinary College [OVC], University of Minnesota, University of Missouri, and University of Florida) from January 2017 to May 2021. Institutions were informed of the study by email and provided access to a standardized data collection form and lymphangiography protocols. Preoperative staging was performed at the discretion of the veterinary oncologist or referring veterinarian prior to SLN mapping and included a subset or combination of CBC, biochemistry profile, 3-view thoracic radiographs, thoracic CT, abdominal ultrasound, regional LN cytology, and histopathology of the oral mass. Informed owner consent was obtained prior to study enrollment. Cases with clinically suspected metastatic LNs were excluded to focus solely on SLN mapping in individuals with clinically negative LNs. Exclusion criteria included palpable lymphadenomegaly relative to the contralateral side, cytological evidence of nodal metastasis, or significant nodal changes on imaging, where CT reports prioritized metastasis over reactive lymphadenopathy. Consequently, the identification of metastatic LNs in this study inherently aligns with the concept of subclinical metastatic LNs. Additionally, cases were excluded if complete removal of both mandibular and retropharyngeal LNs was not achieved. All dogs had a routine pre- and postcontrast CT scan of the head and neck prior to CTL.

Preoperative CT and indirect CT lymphangiography

The CT procedures were performed in 3 phases. Dogs were positioned in sternal recumbency and scanned under general anesthesia or sedation, ensuring no compression of head or face (eg, endotracheal tube tie, the dog's body weight on the tissues) to prevent lymphatic obstruction. Initially, a precontrast scan was performed according to the routine standard soft tissue protocol for head and neck CT using a helical multislice scanner **(Supplementary Table S1)**. Images were evaluated to ensure mandibular, medial retropharyngeal, and superficial cervical LNs were included. A routine intravenous contrast study was performed. Indirect CTL was performed by submucosal injection of a total of 2 mL of diluted iodinated contrast agent into 3 to 4 equal parts around the tumor at a 1:1 ratio with saline.[14,16] The total injection time for all sites was 2 minutes with each injection (approx 0.5 mL) performed over 30 seconds. The sites were massaged for 30 seconds following the final injection.[31] Indirect CTL images were captured at 1 and 3 minutes following completion of injection **(Figure 1)**. If no SLN was identified, repeat injection was performed as described above, followed by an additional scan at 12 minutes from the start of the first injection. If no SLN was identified after the repeated injection, it was considered a failure of the technique. Any changes in heart rate, blood pressure, temperature, and peripheral capillary oxygen saturation were monitored during the procedures in all dogs. Injection sites were examined for redness, swelling, and bleeding, and any changes were documented.

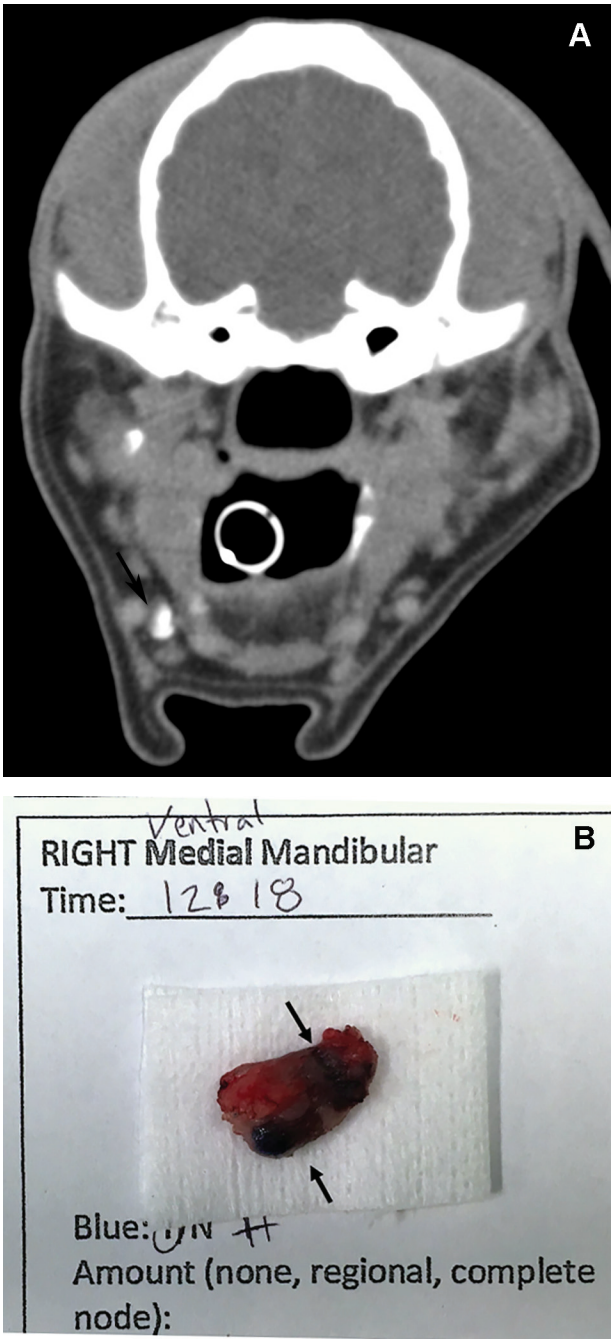

Figure 1—Sentinel lymph node (SLN) mapping techniques. A—Transverse indirect CT lymphangiography image obtained 3 minutes after completing the peritumoral injection. The LN was defined as an SLN when it was contrasted in this technique. A ventromedial mandibular LN was contrasted in this dog and was considered an SLN (black arrow). B—Intraoperative methylene blue lymphangiography. The LN that was stained ≥ 1 was considered to be the SLN. The right medioventral mandibular LN was defined as a SLN.

Lymph node size, short-long axis ratios (S:L ratios), and numbers in each location were recorded from CT images. Images were analyzed with open source image analysis software (OsiriX, version 11.0; Pixmeo). The S:L ratio was defined as the ratio between the maximum short axis and the maximum long axis of an LN, measured in both transverse and sagittal planes.[32]

Mandibular LNs were recorded as either dorsolateral (LMLN) or ventromedial LNs (MMLN). The LMLN was defined as any mandibular LN noted dorsolateral to the facial vein. The MMLN was defined as any mandibular LN noted ventromedial to the facial vein. Given that some MMLNs consist of a cluster of 2 or more LNs,[33,34] only the largest LN was included in the analysis to ensure standardization, reduce variability in cases with multiple nodes, and prioritize potentially clinically relevant node. Mandibular LNs and medial retropharyngeal LNs (MRLN) on each side were measured at the maximum length in transverse and sagittal planes by means of digital calipers, with the measurements conducted by 1 author (SB).[35] The measurement methods and representative initial cases were reviewed and verified by the corresponding author (MLO).

Intraoperative MB lymphangiography

Patients were positioned in dorsal recumbency and aseptically prepared for cervical lymphadenectomy and primary tumor excision within 28 days of the CT procedures. Methylene blue (Supplementary Table S1) was diluted with 0.9 mL saline for 1 mL total volume. If ICG (Seaford Pharmaceuticals Inc) was also used (OVC cases), the solution was prepared by mixing 0.1 mL (0.25 mg) of reconstituted ICG, 0.1 mL of MB (1.0 mg), and 0.8 mL of saline. Four-quadrant submucosal injection was performed over 30 seconds to 1 minute with a 23-gauge needle before or after draping of the patient (goal, < 5 to 10 minutes prior to the cervical skin incision). Following incision, LNs were inspected for blue staining. The time to LN identification and degree of blue dye visually observed were recorded. Semiquantitative scoring was used to assess the amount of blue staining both in situ and ex vivo that appeared on the LN surface (0 = no blue, 1 = regional, 2 = complete).[36] Lymph nodes stained ≥ 1 were considered SLNs (Figure 1). If all LNs were scored as 0, it was considered a failure of the technique. Cervical LN extirpation in all cases was performed through a ventral midline incision, as previously described.[37] The primary tumor and excised LNs were submitted for histopathology. Pathology reports were utilized for data analysis; pathology was not reviewed by a single pathologist.

Statistical analysis

Descriptive statistics were used to report age, body weight, type, and size of the primary tumor; number, size, and S:L ratios of LNs; time to detection or excision of SLNs by each modality; and patient SLN detection rate. Continuous data were evaluated for normality via the Kolmogorov-Smirnov test; normally distributed data are reported as mean ± SD, and nonnormal data are reported as median (range).

Cohen's κ test was used to evaluate the agreement between CTL and IOL-MB for SLN detection at the individual LN level. Higher κ values (0 to 1.0) indicate

stronger agreement, with thresholds defined as follows: 0.0 to 0.20 = slight agreement, 0.21 to 0.40 = fair agreement, 0.41 to 0.60 = moderate agreement, 0.61 to 0.80 = substantial agreement, and 0.81 to 1.0 = almost perfect or perfect agreement.[38] To compare S:L ratios, 2 independent-samples *t* tests were performed separately for the sagittal and transverse planes, assessing differences based on modality agreement (same vs different results). For nonnormally distributed data, a Mann-Whitney *U* test was used instead. The S:L ratio was the dependent variable, while independent variables included modality agreement (same vs different results) and LN metastatic status (metastatic vs nonmetastatic). Cohen d was calculated as a measure of effect size for *t* tests. Four outliers were removed prior to analysis to ensure accuracy and clarity of results. At the patient level, modality results were considered "same" when all LNs detected by one modality were also identified by the other, regardless of whether additional LNs were detected by either modality (inclusive agreement). All statistical tests were conducted separately for the sagittal and transverse planes.

Results

Patient characteristics

A total of 38 client-owned dogs with a primary tumor of the oral cavity or a scar from a previously incompletely excised tumor were included in this study from 4 academic institutions: 20 cases from OVC, 11 cases from the University of Minnesota, 6 cases from the University of Missouri, and 1 case from the University of Florida. Dogs included had a mean age of 9.22 ± 3.56 years and mean body weight of 28.59 ± 15.05 kg. Dogs consisted of neutered males (19 of 38 [50.0%]), spayed females (17 of 38 [44.7%]), and intact males (2 of 38 [5.2%]). The most frequent breeds were Labrador Retriever (6 of 38 [15.8%]) and 2 of 38 (5.3%) of each of the following: Shetland Sheepdog, Pug, German Shepherd Dog, and Golden Retriever. Mixed-breed dogs accounted for 11 of 38 (28.9%) of the study population.

Tumor characteristics

The anatomic location of the primary tumor or scar was reported as follows: rostral mandible in 15 of 38 cases (39.5%), caudal maxilla in 7 of 38 cases (18.4%), caudal mandible in 5 of 38 cases (13.1%), rostral maxilla in 4 of 38 cases (10.5%), frenulum in 2 of 38 cases (5.3%), and 1 of 38 (2.6%) of each of the following: lip, cheek, nasal cavity, soft palate, and tongue. The side of the primary tumor or scar was reported as follows: left in 20 of 38 cases (52.6%), right in 13 of 38 cases (34.2%), and both or midline in 5 of 38 cases (13.2%). In 7 cases, there was no visible mass due to the previous excisional biopsy. Histologic diagnosis of the primary tumor was malignant in 27 of 38 (71.1%) dogs and benign in 11 of 38 (28.9%) dogs. Malignant tumors included melanoma (8 of 38 [21.1%]), squamous cell carcinoma (6 of 38 [15.8%]), osteosarcoma (6 of 38 [15.8%]), fibrosarcoma (3 of 38 [7.9%]), sarcoma of unknown origin (2 of 38 [5.3%]), mast cell tumor (1 of 38 [2.6%]), and ameloblastic sarcoma (1 of 38 [2.6%]), and benign included ameloblastoma (5 of 38 [13.2%]) and 1 of 38 (2.6%) of each of the following: ameloblastic fibroma, fibromatous epulis, gingival fibrous hyperplasia, epithelial hyperplasia, synovial cyst, and amyloid-producing odontogenic tumor. In all 11 dogs, malignancy was clinically suspected based on preoperative cytology, biopsy, or biological behavior. The median maximum mass length was 30.5 mm (range, 0 to 100 mm).

Lymph node characteristics on CT

The median number of MMLN and MRLN were 1 (range, 1 to 3) and 1 (range, 1 to 2), respectively. All dogs had only a single LMLN. The median diameter in the sagittal plane for each node was as follows: MMLN maximum short axis, 7.10 mm (range, 2.90 to 17.50 mm); MMLN maximum long axis, 17.90 mm (range, 6.60 to 36.0 mm); LMLN maximum short axis, 7.55 mm (range, 3.50 to 13.80 mm); LMLN maximum long axis, 14.00 mm (range, 6.00 to 42.00 mm); MRLN maximum short axis, 10.15 mm (range, 2.60 to 18.00 mm); and MRLN maximum long axis, 31.4 mm (range, 9.30 to 64.40) mm. Median S:L ratios for MMLN, LMLN, and MRLN were 0.40 (range, 0.19 to 0.92), 0.53 (range, 0.22 to 0.88), and 0.33 (range, 0.13 to 0.81), respectively. Measurements of bilateral MRLNs in the sagittal plane were unable to be completed in 1 dog due to poor image quality.

The median nodal diameters in the transverse plane were as follows: MMLN maximum short axis, 6.15 mm (range, 2.00 to 14.40 mm); MMLN maximum long axis, 12.55 mm (range, 5.00 to 30.40 mm); LMLN maximum short axis, 6.55 mm (range, 3.50 to 14.50 mm); LMLN maximum long axis, 12.00 mm (range, 6.00 to 48.10 mm); MRLN maximum short axis, 6.95 mm (range, 3.40 to 17.20 mm); and MRLN maximum long axis, 16.15 mm (range, 6.20 to 33.90 mm). Median S:L ratios for MMLN, LMLN, and MRLN were 0.51 (range, 0.24 to 0.83), 0.52 (range, 0.19 to 0.92), and 0.44 (range, 0.17 to 0.77), respectively.

Preoperative indirect CTL

Nodes were characterized as sentinel in 35 of 38 dogs (92.1%), with only MMLNs or MRLNs classified as SLNs in 11 of 38 dogs (28.9%). The median number of SLNs identified was 1 (range, 0 to 3) per dog. In 6 dogs, SLNs were not identified following the first injection. Contrast uptake was observed in 2 of these 6 cases; in one, SLN identification was successful after reinjection, while in the other, it remained unsuccessful. In all 6 cases where the initial injection was unsuccessful, the dogs had no history of prior surgical intervention. Reinjection was attempted in 5 of the 6 dogs, while the reason reinjection was not pursued in the remaining case was not specified. Following reinjection, contrast uptake within LNs was observed in 3 of the 5 dogs. No adverse events were reported from the CTL procedure. The location of the primary tumor and SLNs were reported in all dogs **(Table 1)**. Sentinel lymph nodes were observed bilaterally in 5 cases (13.2%).

Intraoperative MB lymphangiography

Bilateral cervical lymphadenectomy was performed in all 38 cases. In 3 dogs, 1 LN could not be

Table 1—Summary of sentinel lymph node (SLN) identification in indirect CT lymphangiography (CTL) and intraoperative lymphangiography with methylene blue (IOL-MB).

Case No.	Tumor location	CTL SLN No.	IOL-MB SLN No.	Agreement
1	Left rostral mandible	2*	1,2*	Y
2	Left rostral mandible	1*,2*,3*	1*,2*,3*,4,5	Y
3	Right rostral mandible	1,4*,6*†	4*,6*†	Y
4	Left rostral maxilla	2	1,4,5	N
5	Left caudal maxilla	2*	2*	Y
6	Left rostral mandible	1*†	1*†	Y
7	Left caudal maxilla	2*	1,2*	Y
8	Right rostral mandible	1*,4*,5*	1*,4*,5*	Y
9	Right caudal buccal cheek	4,5*	5*	Y
10	Right caudal maxilla	4*,5*	4*,5*	Y
11	Left caudal mandible	3*†	2,3*	Y
12	Left rostral nasal cavity	1,2*	2*	Y
13	Soft palate mass	2,5,6	3,4,5,6	N
14	Left rostral mandible	1*†	1*†	Y
15	Left rostral maxilla	No SLN identified	2,5	N
16	Right caudal mandible	No SLN identified	4†	N
17	Left rostral mandible	1*,2,3	1*†	Y
18	Bilateral rostral mandible	1,3,4	1,4,6†	N
19	Left rostral mandible	1,2	4†	N
20	Bilateral rostral mandible	1*†	1*,2,4,5	Y
21	Right rostral mandible	4*†	1,2,4*,5	Y
22	Left rostral maxilla	2*	1,2*,4,5	Y
23	Right rostral maxilla	2	1,4,5	N
24	Bilateral rostral mandible	No SLN identified	No SLN identified	Y
25	Left caudal maxilla	2*	2*	Y
26	Right rostral mandible	1*,5*	1*,4,5*	Y
27	Left caudal mandible	1*,2*,3	1*,2*	Y
28	Right frenulum	3,6*†	6*†	Y
29	Left caudal mandible	1*,2*	1*,2*	Y
30	Left caudal maxilla	2*	2*	Y
31	Bilateral rostral mandible	1*,4*†	1*,4†	Y
32	Left rostral mandible	1,4†	No SLN identified	N
33	Right mandibular lip	4*,5*	1,4*,5*	Y
34	Right rostral maxilla	4*,5*	4*,5*	Y
35	Left frenulum	1†	2	N
36	Right caudal mandible	4*†	1,2,4*,5	Y
37	Right caudal maxilla	5*	5*	Y
38	Left caudal maxilla	1*,2*	1*,2*	Y

Numeric coding for SLNs: 1, left ventromedial mandibular; 2, left dorsolateral mandibular; 3, left medial retropharyngeal; 4, right ventromedial mandibular; 5, right dorsolateral mandibular; 6, right medial retropharyngeal.

*Sentinel LN identified by both modalities. †The modality characterized only the ventromedial LNs or medial retropharyngeal LNs as the SLNs.

N = Different. Y = Same.

identified, resulting in a total of 225 nodes removed. The median time from injection to removal of the first LN was 13 minutes (range, 2 to 98 minutes), and the median time to complete nodal extirpation was 45 minutes (range, 18 to 115 minutes). Excluding the case that required 98 minutes for the first SLN removal, the time ranges were 2 to 35 minutes for the first node and 18 to 74 minutes for total nodal extirpation. The reason for the prolonged time in the outlier case was not reported. Lymph nodes were characterized as SLN in 36 of 38 dogs (94.7%), with only MMLNs or MRLNs classified as SLNs in 9 of 38 dogs (23.7%). The median number of SLNs identified was 2 (range, 0 to 5) per dog. No adverse events were reported from IOL-MB. The location of the primary tumor and SLNs were reported in all dogs (Table 1). A contralateral SLN to the primary tumor was the only SLN in 1 dog (eg, a left dorsolateral LN was the only SLN in a dog with the right mandibular tumor). Additionally, SLNs were observed bilaterally in 10 cases (26.3%).

Agreement CTL and IOL-MB

In 37 dogs, SLNs were identified by at least 1 method, yielding an overall 97.4% (range, 37 of 38) SLN detection rate when the 2 modalities were combined (Table 1). In 29 of 38 dogs (76.3%), the same SLN was identified in both modalities. At the LN level, SLN results agreed between CTL and IOL-MB modalities in 175 of 225 LNs (76.8%; $\kappa = 0.485$; $P < .001$).

In the transverse plane, the mean S:L ratio was 0.487 (SD = 0.130) in cases where the 2 modalities agreed and 0.523 (SD = 0.121) in cases where they disagreed. Although there was a trend toward a difference between the 2 groups, the difference was not statistically significant ($t[104.309] = 1.788$; $P = .077$), with a small effect size (Cohen d = 0.121). In the sagittal plane, the S:L ratio data failed the normality test in cases where the 2 modalities agreed (Kolmogorov-Smirnov $P = .005$). A Mann-Whitney *U* test revealed a significant difference in mean ranks between cases where the modalities agreed (mean

Table 2—Characteristics of dogs with metastatic LNs.

	Tumor			LN			SLN	
Case No.	Type	Location	Size (mm)	Location	S:L ratios (sagittal)	S:L ratios (transverse)	CTL	IOL-MB
13	Melanoma	Soft palate	6	Left LM	0.67	0.67	Y	N
27	Melanoma	Left mandible	8	Left MM	0.37	0.49	Y	Y
				Left LM	0.5	0.4	Y	Y
28	Mast cell tumor	Right frenulum	60	Right RP	0.23	0.46	Y	Y
38	Melanoma	Left maxilla	31	Left MM	0.38	0.68	Y	Y
				Left LM	0.54	0.48	Y	Y

LM = Dorsolateral mandibular. MM = Ventromedial mandibular. N = Not identified. RP = Medial retropharyngeal. Y = Identified.

rank = 107.42) and disagreed (mean rank = 128.27; z = −1.998; *P* = .046). The median S:L ratio was 0.417 (range, 0.131 to 0.924) in cases where the 2 modalities agreed and 0.450 (range, 0.236 to 0.833) in cases where they disagreed.

Metastatic LN characteristics

Four 10.5% [4 of 38] dogs had at least 1 metastatic LN, with a total of 6 metastatic LNs identified (6 of 225 [2.7%]; **Table 2**). In 1 of 4 dogs, metastasis was exclusively identified in MRLN. All 6 metastatic LNs were identified as SLN by CTL (100%), whereas 5 of 6 metastatic LNs (83%) were detected by IOL-MB. In the combination of both modalities, at least 1 metastatic LN was identified in all 4 dogs, encompassing all 6 metastatic LNs. Due to the small number of cases with nodal metastasis, statistical analysis was not feasible. The S:L ratio of the metastatic LN not detected by IOL-MB appeared relatively high, exceeding the median values in both planes.

Discussion

This prospective study yielded a high detection rate of the SLN (97.4%) with moderate agreement (Cohen κ = 0.485) between CTL and IOL-MB, supporting the primary hypothesis. Notably, the combined techniques successfully identified all metastatic LNs as SLNs. This finding supports the secondary hypothesis; however, the results should be interpreted with caution due to the limited number of metastatic cases in the study population. Overall, these results emphasize the value of employing multiple modalities for SLN mapping, consistent with previous studies[13,16,19] that reported moderate discrepancies in agreement between different techniques.

Interestingly, several cases had metastasis exclusively to the MMLNs or MRLNs. Since the LMLN is generally the most easily accessible for palpation and cytology, these findings emphasize the importance of including the MMLNs and MRLNs in the staging evaluation. Previous studies[34,39] have highlighted the difficulty of evaluating MMLNs based on palpation and cytology alone, as well as the low diagnostic yield of ultrasound-guided aspiration of MRLNs in dogs. In addition, the presence of metastasis to the contralateral lymphocentrums is consistent with previous studies[12,16,28] that have reported unpredictable metastatic or drainage patterns to cervical LNs in dogs with head and neck neoplasms. In this study population, no parotid LN was characterized as SLN.

Nonselective lymphadenectomy is not a benign procedure and may lead to complications such as increased morbidity, tissue trauma, and prolonged anesthesia time.[4–6] These outcomes were not assessed in this study, as they were beyond its scope. In human medicine, there has been a gradual reduction in the number of nonselective lymphadenectomy procedures and the average number of LNs removed, with no significant change in the annual detection of cancer patients with LN metastases.[40] This trend highlights the diagnostic advantages inherent in SLN mapping techniques, which allow for the targeted identification of LNs most likely to harbor tumor metastases. Furthermore, recent studies[17,18] in veterinary medicine indicate that complications associated with lymphadenectomy using SLN mapping techniques are predominantly minor and generally do not require intervention.

Despite the encouraging detection rate in this study, discrepancies between the 2 modalities were observed in 9 cases, including 1 involving a metastatic LN, emphasizing the biological complexity of lymphatic mapping, particularly in the presence of metastasis. Previous studies[41,42] have shown that partially enhanced or heterogeneously opacified SLNs often reflect structural changes, such as follicular or sinus proliferation, or varying degrees of metastatic infiltration that disrupt lymphatic flow, whereas completely enhanced SLNs typically represent normal lymphatic tissue. These alterations in lymphatic pressure are a major reason that SLN mapping should not be performed in cases with overt nodal metastasis.[42] A recent study[43] in dogs with melanoma and mast cell tumors, however, found no association between SLN enhancement patterns and metastatic status. Variations in contrast agents, tumor types, and lymphocentra of interest may partly explain these inconsistencies across studies. Both previous studies and findings from this study suggest that increased tumor burden, extranodal invasion in the SLN, and/or biokinetics of the contrast agent may alter lymphatic flow, potentially resulting in a false negative and cancer understaging when relying only on SLN is sampled.[13,14,44] In a recent study by Beer et al,[45] all mapped SLN and clinically suspicious non-SLNs were removed in dogs with mast cell tumors. Given that 49 of 223 LNs (22.0%) in our study had different results between 2 mapping modalities (CTL and IOL-MB), it seems reasonable to

remove all LNs identified by either modality in case of disagreement. Also, the importance of evaluating the second-tier LN, when identified, has been highlighted in a recent study.[46]

We included the 7 patients that had incompletely excised tumors (ie, excisional biopsy). A recent study[47] reported that SLN detection rates are generally not affected by prior local excision (curative intent or marginal excision), whereas another study[48] has shown that surgical intervention can alter lymphatic drainage, with complete agreement between pre- and postoperative mapping observed in fewer than half of cases. In our study, we enrolled cases with either a primary tumor of the oral cavity or a scar from a previously incompletely excised tumor that was cytologically or histopathologically confirmed as malignant. While prior local excision may still represent a potential source of error, given that all 7 dogs got an excisional biopsy, the available evidence suggests it is unlikely to significantly impact SLN mapping outcomes.

Mechanical influences from patient positioning and external compression can also affect lymphatic flow and potentially contribute to unsuccessful SLN detection. Previous studies[11,14] have utilized various positioning methods to minimize compression and optimize lymphatic drainage, and massage has been shown to facilitate contrast movement through lymphatic channels. In our study, precautions were taken to avoid external compression, including careful placement of the endotracheal tube tie and supporting the body to prevent pressure on lymphatic regions. However, it is noteworthy that the patient in which SLN mapping failed was the heaviest dog in the cohort (81 kg, compared to the second heaviest at 57 kg). Although the impact of body weight on lymphatic flow has not been previously evaluated, the substantial body mass may have contributed to lymphatic obstruction.

Unlike previous studies, this study excluded cases with clinically apparent nodal metastasis, determined by palpation, radiologic findings, and cytology, which likely contributed to the low prevalence of nodal metastasis in this population (2.7%). In 1 metastatic LN that was negative on IOL-MB, the S:L ratio was relatively high, exceeding the median value in both planes. Additionally, the S:L ratio was lower in the sagittal plane, with a trend toward lower values in the transverse plane, in cases where the 2 mapping modalities agreed, indicating a more elongated or oval nodal shape in these cases. These observations raise the possibility that micrometastasis may have been missed with routine histopathology.[49] While some studies[35,43] have reported no significant differences in nodal shape between metastatic and non-metastatic LNs, others[50] have shown significant differences between normal and malignant nodes. In that study,[50] the mean S:L ratios for normal, reactive, lymphoma, and metastatic nodes were 0.44, 0.59, 0.75, and 0.71, respectively. Additionally, metastatic LNs have been described as having variable shapes, with smaller nodes tending to be more oval and larger nodes becoming more rounded.[32] In this study, the decision to measure the S:L ratio in both sagittal and transverse planes was based on prior methodologies aimed at improving accuracy. Although no significant differences in S:L ratios were identified between metastatic and nonmetastatic nodes in this study, consistent with previous reports, [35,43] this finding may be attributable to early-stage disease or a potential type II error.

This study had limitations, including a small sample size, a low LN metastatic rate (2.7%), the multiinstitutional nature of the study involving multiple clinicians and pathologists, measurement of LN size by 1 author, and the inclusion of diverse tumor types. While these limitations are difficult to control for in clinical research, to further evaluate the accuracy of SLN mapping techniques, future studies should include a larger sample size of malignant tumors with a higher potential for nodal metastasis (eg, melanoma, squamous cell carcinoma). The low metastatic rate may also be due in part to the proportion of dogs (11 of 38 [28.9%]), ultimately diagnosed with a benign process because preoperative diagnosis was suspicious for malignancy in all cases. Additionally, different types of iodinated contrast agents were used across institutions (Supplementary Table S1). Although all contrast agents used were water soluble, variations in their chemical properties may have influenced imaging outcomes. Prior studies have demonstrated that CTL with aqueous contrast provides the advantage of more rapid SLN identification compared to lipid-based contrast agents.[13,14] Further research is warranted to clarify the potential impact of contrast agent selection on SLN mapping accuracy.

Another limitation of this study is that that timing of injection to SLN identification and removal was not standardized. With IOL-MB, injection was performed prior to skin incision to not interrupt lymphatic flow. However, the time from injection to removal of the first LN was variable, with a range of 2 to 98 minutes, which could affect the results of SLN mapping. The median number of SLNs identified using IOL-MB was 2, which was higher than the median of 1 identified with CTL. This may be due to the inclusion of second- or third-tier SLNs in cases where a longer time to SLN identification location and removal occurred. There are multiple factors that could affect timing, including but not limited to final aseptic patient preparation, equipment setup, and the experience of the surgeon making this timing difficult to synchronize in a clinical setting. The combination of patient preparation prior to injection and preoperative CTL may help to yield better SLN results and less inclusion of second- or third tier LNs. Nonetheless, the importance of evaluating second-tier LNs has been recognized in recent studies.[48]

In conclusion, combining CTL and IOL-MB techniques resulted in a high SLN detection rate with moderate agreement between modalities (76.8%). The observed discrepancies between modalities highlight how alterations in lymphatic flow and tumor-associated factors may affect SLN identification. These findings challenge the assumption that all mapped nodes represent definitive SLNs, as multiple

factors may contribute to false negatives. Sentinel lymph node mapping should be considered primarily in cases without overt nodal metastasis, and using multiple techniques may enhance detection accuracy. Further studies are needed to better understand the impact of metastatic burden and lymphatic flow dynamics on SLN mapping outcomes.

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Supplementary Materials

Supplementary materials are posted online at the journal website: avmajournals.avma.org.