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Influence of locoregional lymph node aspiration cytology vs sentinel lymph node mapping and biopsy on disease stage assignment in dogs with integumentary mast cell tumors

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Abstract

Objective: To compare the effect of sentinel lymph node (SLN) histology vs locoregional lymph node (LRLN) fine needle aspiration (FNA) cytology on assigned disease stage and adjunctive treatment recommendations and describe the incidence of anatomic disparity between the LRLN and SLN.

Study design: A pre-post study refers to a study design type in which subjects are compared pre and post the intervention of interest.

Animals: Seventeen dogs undergoing primary excision of 20 cutaneous and subcutaneous mast cell tumors (MCT).

Methods: Client-owned dogs presenting to the Cornell University Hospital for Animals for surgical removal of a cytologically confirmed cutaneous or subcutaneous MCT >1 cm in diameter were enrolled. Cytological examination of FNA from the LRLN was compared with histology of the SLN. The SLN was identified by indirect computed tomographic lymphangiography (ICTL) after peritumoral injection of iopamidol and scanning at 1, 3, 5, 10, and 15 minutes. Histopathologic node score > 1 was considered metastatic. After case review by an oncologist, LRLN FNA cytology was compared with SLN histology for effect on changes in stage assignment and adjunctive treatment recommendations.

Results: Mast cell tumors were graded as 2 low (n = 11), 2 high (n = 2), and subcutaneous (n = 7). Optimal scan timing was 10 minutes after injection of iopamidol. Sentinel lymph node differed anatomically from LRLN in 5 of 18 scans. Metastases were detected by histology in 9 of 20 SLN compared with in 1 of 20 FNA of LRLN ($P = .001$), changing stage and adjunctive treatment recommendations 8 of 20 tumors. Only 6 of 19 LRLN FNA samples were diagnostic.

Conclusion: Sentinel lymph nodes were consistently identified with ICTL and differed from LRLN in one-quarter of tumors. Histopathological examination

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of SLN altered recommendations in half of the dogs compared with the previous standard of care.

Clinical significance: Indirect computed tomographic lymphangiography and SLN excision should be considered as a new standard for dogs with MCT.

1 | INTRODUCTION

Mast cell tumors (MCT) are the most common cutaneous neoplasm in dogs, with a reported prevalence of 0.27%, representing up to 21% of all canine cutaneous tumors.[1-3] Tumor related death despite treatment has been reported at 30.8%, with a median overall survival time of 1020 days.[4] Animal survival is associated with stage of disease. Disease progression can include metastasis to lymph nodes, solid organs, and bone marrow. Because metastasis via the lymphatic system in MCT disease is both common and prognostic, evaluation of lymph node status is an important part of animal staging. Researchers in one study identified histological change consistent with metastasis in almost 50% of normal-sized surgically excised lymph nodes with 15% premetastatic,[5] while researchers in another study found a survival difference of 0.8 vs 6.2 years in dogs with and without lymph node metastasis, respectively.[6] Surgical excision of locoregional lymph nodes (LRLN) has been associated with a survival advantage in dogs with stage II disease.[7] Thus, identification of lymphatic metastasis at the point of diagnosis of the primary tumor has major implications for both prognosis and adjunctive treatment recommendations, while excision of infiltrated nodes may confer a survival advantage.

Unfortunately, several challenges impede optimal lymphatic staging. First, while fine needle aspiration (FNA) cytology is easy to perform and minimally invasive, it is only 68% to 75% sensitive for nodal metastasis detection in MCT disease compared with lymph node histology.[8,9] Second, the LRLN predicted by anatomic proximity to the tumor may differ from the primary draining lymph node for the tumor lymphatic basin, known as the *sentinel lymph node* (SLN).[10] This discrepancy coupled with inability to readily identify the SLN may prevent nodal excision at the time of treatment of the primary tumor.

Various methods of SLN identification or mapping have been reported, including indirect computed tomographic lymphography (ICTL), radionuclide lymphoscintigraphy, indocyanine green fluorescence imaging, and methylene blue injection.[11] These modalities rely on injection of a marker substance at the site of the tumor followed by tracking the marker out to the recipient SLN. The tracking process may be performed preoperatively with imaging studies or intraoperatively with surgical dissection and direct visualization. Because the SLN may be any of several nodes at some distance to the primary tumor, preoperative minimally invasive methods of tracking such as ICTL or scintigraphy would appear advantageous. Additional advantages of ICTL include no handling of radioactive materials and relative availability of computed tomography (CT) compared with scintigraphic equipment in specialty practice. Researchers in previous veterinary studies have evaluated ICTL for lymphatic mapping in anal sac adenocarcinoma,[12] tumors of the head,[13] and mammary tumors.[14]

This report describes a single-center experience of the introduction of the ICTL technique for sentinel node mapping of cutaneous MCT. The preexisting facility standard of care for lymphatic staging consisted of blind or ultrasound guided FNA of the LRLN. The study objective was (1) to determine the proportion of canine MCT that exhibited anatomic disparity between the LRLN and the SLN, with the null hypothesis of no difference; (2) to determine the proportion of dogs for which ICTL identification of SLN followed by surgical excision and histological assessment changed adjunctive treatment recommendations compared with the preexisting standard of care of FNA cytology of LRLN, with the null hypothesis of no treatment changes; and (3) to identify the optimal imaging time postinjection for the ICTL.

2 | MATERIALS AND METHODS

2.1 | Animals and study overview

This prospective interventional pre-post study was conducted between September 2017 and December 2019 and approved by the Cornell University IACUC (approval No. 2017-0061), and informed client consent was obtained for all included dogs. Client-owned dogs presenting to the Cornell University Hospital for Animals for surgical removal of a cytologically confirmed cutaneous or subcutaneous MCT >1 cm in diameter were recruited. Dogs were subsequently excluded when the tumor represented recurrence of a previously excised tumor, the dog had renal disease representing a contraindication to iodinated contrast use, or the owners elected to forego

surgery after receipt of staging results. Dogs with more than one primary tumor were not excluded. Hospital protocols for routine preoperative management, staging, and surgical excision of the primary tumor or tumors were unaltered for study purposes. Lymphatic staging was conducted in all dogs both according to the preexisting standard of care (FNA cytology of the LRLN) and the intervention under study (ICTL followed by surgical excision of the SLN identified by the ICTL and histopathology of the nodal tissue), and the results of each modality and the effect on subsequent adjunctive treatment recommendations were compared for each dog.

Routine preoperative blood work consisting of a complete blood count and serum chemistry profile was performed for all dogs. Fine needle aspiration of the primary MCT was evaluated by a board-certified clinical pathologist (A.W.N.) and assigned a grade of low or high according to previously published criteria.[15] Preoperative imaging for staging purposes consisting of thoracic radiographs or thoracic CT and abdominal ultrasound ± FNA cytology of the liver/spleen was recommended for all dogs and performed according to the owners wishes and financial resources. Contrast CT of the primary tumor or tumors was performed preoperatively while dogs were under sedation as part of the ICTL, and IV fluid support was maintained for all dogs for at least 12 hours after the procedure. Curative intent surgical excision was performed while dogs were under general anesthesia by board-certified surgeons for all MCT. En bloc excision was performed with proportional lateral margins for MCT >1 cm but ≤2 cm in diameter and with 3-cm lateral margins for MCT >2 cm in diameter.[16] A deep margin of one fascial plane was taken for all tumors. All dogs received appropriate analgesic protocols for their procedures as determined by a board-certified anesthesiologist and/or surgeon, consisting of opioid analgesia and epidural blockade/transversus abdominis plane blockade/splash blocks as appropriate.

2.2 | Lymph node cytology and imaging

The LRLN was designated by the attending board-certified surgeon after visual review of published lymphatic drainage patterns[17,18] and was based on anatomic location of the primary tumor or tumors. Fine needle aspiration of the anatomic LRLN was performed by a surgical resident or board-certified surgeon and obtained via blind palpation when the node could be readily palpated. When the node could not be readily palpated or the blindly obtained sample was considered nondiagnostic, the FNA was performed by an imaging resident or board-certified imaging specialist with ultrasound guidance. Three samples and smears were performed, and cellularity was confirmed on each sampling occasion. The exact technique used (needle and syringe size, milliliters of aspiration) was at the clinicians' discretion. Locoregional lymph node FNA cytology was evaluated by a single board-certified clinical pathologist (A.W.N.) for presence of lymphatic tissue and evidence of MCT metastasis by using a modification of previously published criteria.[6] For the purposes of this study, the previously described categories of "normal," "reactive lymphoid hyperplasia," and "possible metastasis" were combined and used to indicate "no metastasis," while the categories of "probable metastasis" and "certain metastasis" were combined and used to indicate "metastasis present." The categories of "possible" and "probable" metastasis differed according to the incidence of mast cell aggregates identified per slide, and this category grouping was used to mirror the clinical application of both this and the histopathologic histologic node (HN) score[19] in making chemotherapy recommendations.

After identification and aspiration of the LRLN, ICTL was performed. Dogs were sedated and positioned in the CT unit in as neutral a position as possible to minimize compression of the lymphatic pathways between the primary tumor or tumors and potential draining nodes. One milliliter of iopamidol (iodine 370 mg/mL, Isovue; Bracco, Milan, Italy) was diluted 50:50 with sterile saline, and 0.5 mL of this mixture was injected into the tissues immediately adjacent to the tumor in a simultaneous four-quadrant peritumoral approach for a total injection volume of 2 mL, according to previously established protocols.[12] The peritumoral area was gently massaged for 30 seconds to facilitate mobilization of the contrast material. Computed tomography of the local anatomic region was performed with a 16-slice Toshiba Aquilion CT scanner (Canon, Glen Mills, Pennsylvania), with slice thickness of 0.5 to 2 mm and a scan timing of 1, 3, 5, 10, and 15 minutes postinjection. Scanner settings were kV = 120, with mA determined by SureExpose (Canon Medical Systems, NJ, USA). Acquired data were reconstructed in Carestream PACS (Carestream, Fort Myers, Florida), and images were reviewed by a board-certified radiologist. Lymphatic pathways were identified by contrast uptake emanating from the primary tumor and followed. The sentinel node was defined as the first lymph node in the lymphatic pathway to uptake contrast material (Figure 1). When separate lymphatic pathways in different directions identified more than one node simultaneously, the first contrast enhancing node in each pathway was designated a sentinel node. After study completion, all ICTL were reviewed by a blinded board-certified radiologist (P.J.). The scan time postinjection that provided a diagnostic mapping study for the greatest proportion of tumors was identified and designated as the optimal scan time.

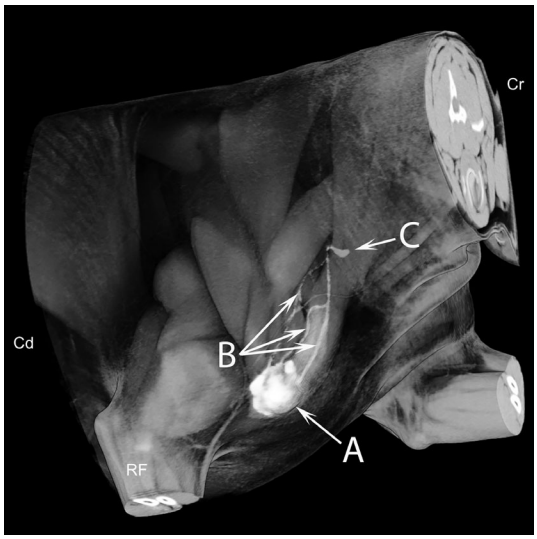

FIGURE 1 Three-dimensional maximal intensity projection reconstruction of computed tomographic data: the primary tumor, the lymphatic pathway draining the tumor, and uptake into the sentinel lymph node in a dog with a cutaneous mast cell tumor. A, mass; B, lymphatics; C, superficial cervical lymph node; Cr, cranial; Cd, caudal; RF, right forelimb

2.3 | Lymph node extirpation and histopathology

Indirect computed tomographic lymphangiography findings were reviewed with each client, and consent to proceed with SLN extirpation was obtained, and ICTL findings were reviewed by the surgeon to assess SLN size and surgical landmarks to guide extirpation. The SLN were excised by a board-certified surgeon while dogs were under general anesthesia during the same surgical episode and after the excision of the primary tumor or tumors. Gloves and instruments were changed between the procedures. The additional surgical time required for SLN excision was recorded, together with the surgeon's impression of the ease of lymph node identification (visual analog scale where 0 = most easy and 100 = most difficult). Histopathological assessment after fixation in formalin and routine preparation was performed for all excised primary tumors and SLN. All primary tumors were stained with hematoxylin–eosin, and grades were assigned by a board-certified pathologist (J.P.K.) according to the research findings of Kiupel et al[20] and Patnaik et al.[21] Concordance between the cytological Camus low/high grade and histological Kiupel low/high grade was assessed. Surgical margins were assessed, with an incomplete margin defined as the presence of tumor cells at the cut edge of tissue. The SLN was additionally stained with toluidine blue or Giemsa, and HN score was assigned by a single board-certified pathologist (J.P.K.) by following the proposed HN0-3 system.[19] The SLN was considered metastatic if the HN score was >1, corresponding to early or overt metastasis. After lymph node extirpation, all dogs were assessed by physical examination or owner phone interview at 48 hours and 2 weeks postoperatively, and any changes consistent with lymphedema or lymph node extirpation site incisional complications were recorded.

2.4 | Case review and adjunctive treatment recommendations

A board-certified medical oncologist (C.B.) blinded to case identification reviewed a clinical summary for each case that included the results of bloodwork, thoracic and abdominal imaging tests, FNA cytology of the LRLN, and primary tumor information including size, location, histologic grade, mitotic index, and surgical margin status. Additional treatment recommendations including surgical recut on the primary tumor and/or adjunctive chemotherapy or radiation therapy were recorded. The oncologist was then provided with the additional SLN information including histology report and HN score assignment. Any change in treatment recommendations resulting from this additional information was recorded.

2.5 | Sample size analysis and statistics

Previous studies[10] in which scintigraphy was employed to identify the SLN have provided evidence that the SLN may differ from the LRLN in 40% of tumors. A sample size of 20 tumors was calculated to provide a power of 0.95 and an actual α of .0169 to detect a difference of 40% vs the null hypothesis of 0% difference.

Continuous descriptive data were represented as mean ± SD when they were normally distributed. Normality was assessed by using the Shapiro–Wilk test. Differences in category proportions were tested by using a χ^2 test (where $n > 5$) or Fisher's exact test (for $n \leq 5$), as appropriate. An estimate of the proportion of tumors for which the SLN and the LRLN differed anatomically was calculated and compared with the null hypothesis of no difference. An estimate of the proportion of dogs for which cytological LRLN assessment and histopathological SLN assessment resulted in differing conclusions regarding nodal metastatic status and changes in adjunctive treatment recommendations was calculated and compared with the null hypothesis of no changes. The associated 95% CI for estimates of proportions was calculated by using logit transformation.

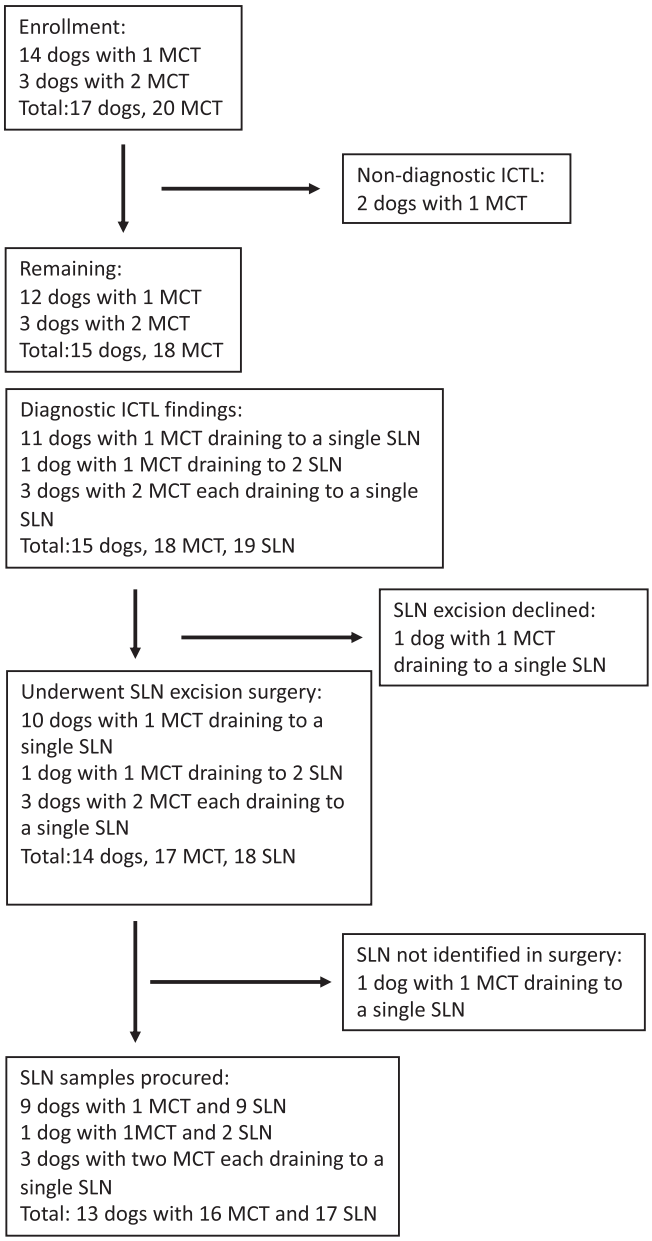

FIGURE 2 Flow diagram illustrating the main study outcomes. ICTL, indirect computed tomographic lymphangiography; MCT, mast cell tumor; SLN, sentinel lymph node

3 | RESULTS

3.1 | Study population

Nineteen owners of dogs that met the study criteria were interviewed, and their dogs were assessed for enrollment. Among these, one declined participation, and one was excluded because the tumor represented recurrence of a previously excised MCT. Seventeen dogs were enrolled, 14 with solitary MCT and three with two MCT (total of 20 MCT in 17 dogs; Figure 2). Population characteristics were age, 7.4 ± 2.6 years; bodyweight, 28.9 ± 13 kg; and body condition score (where 1=lean and 9=obese), 6 of 9 (±1.5). Seven dogs were neutered males, and 10 dogs were spayed females. Breeds represented more than once were Labrador retriever (n = 4), boxer (n = 2), and Staffordshire bull terrier (n = 2). One dog had received treatment with prednisone, and 11 dogs had received treatment with diphenhydramine in the week prior to presentation.

3.2 | Primary MCT characteristics and nodal status based on physical examination and FNA cytology of the LRLN

Mast cell tumors measured 1.7 ± 0.7 cm (range, 1.1-3.2) in diameter at presentation. All presumptive MCT (n = 20) were diagnosed as cutaneous on the basis of palpation and were confirmed as MCT by FNA cytology. Nineteen MCT were classified cytologically as low grade, and one was classified as high grade. The single high-grade MCT was subsequently identified by histopathology as a subcutaneous MCT, and no histologic grade was assigned. Mast cell tumors were located on the forelimb (n = 4), hind limb (n = 4), trunk (n = 4), head and neck (n = 3), abdomen (n = 2), perineum (n = 2), and prepuce/scrotum (n = 1). Nineteen of 20 LRLN as assigned by the supervising surgeon were aspirated guided either by palpation (n = 7) or by ultrasound guidance (n = 12), resulting in 6 of 19 diagnostic samples, of which 1 of 19 was assessed as consistent with metastatic disease. The nondiagnostic samples were designated as such because of poor cellularity.

3.3 | Indirect computed tomographic lymphography: contrast flow patterns, optimal timing, and effect on sentinel node designation

Indirect computed tomographic lymphangiography was performed for all MCT (n = 20) and was successful in identifying the SLN in 18 of 20 (90%) tumors. Four patterns of contrast dispersal were identified: (1) contrast draining from the tumor site direct to a lymph node within the adjacent lymphatic drainage fields (n = 13/18 [72%]), (2) contrast draining from the tumor site in a pathway sufficient to indicate a lymph node within adjacent lymphatic drainage fields without ever reaching that node (n = 3/18 [17%]), (3) contrast draining from the tumor site direct to two lymph nodes in different

directions simultaneously with both within adjacent lymphatic drainage fields (n = 1/18 [6%]), and (4) contrast draining to a solitary distant lymph node with closer regional lymph nodes bypassed (n = 1/18 [6%]). In the two cases in which the scans were nondiagnostic, no contrast dispersal was identified over the time period evaluated (1-15 minutes). The scan time point that most consistently resulted in a diagnostic scan was 10 minutes after contrast injection (n = 17/18); one tumor required an additional scan at 15 minutes. Indirect computed tomographic lymphangiography implementation resulted in the identification of a sentinel lymph center that differed from the designated locoregional lymph center in five of 18 diagnostic scans (27%; 95% CI = 10%–53%). Among the 18 SLN identified, four of 18 were enlarged according to CT compared with anatomic reference normal values,[16] while 13 of 18 were asymmetrically enlarged compared with the contralateral node. Node size did not change precontrast and postcontrast. No local reactions or degranulation events were observed after peritumoral injection.

3.4 | Surgical excision of SLN and associated morbidity

Eighteen primary tumors received diagnostic ICTL, resulting in the identification of 19 SLN (one node per tumor in 17 scans and two nodes per tumor in one scan). Lymphadenectomy of the SLN was attempted for 18 of the SLN identified; for the remaining case, the single SLN identified was in the iliosacral lymph center, and abdominal celiotomy for lymph center excision was declined by the owners. Lymphadenectomy of the SLN was successful for 17 SLN from 16 MCT; one node (the axillary) could not be located intraoperatively. Surgical time for attempted SLN identification and excision (n = 18) was 38.6 ± 23.4 minutes, representing 40% (±27%) of total surgical time. Surgeon perception of the ease of lymph node identification was 55 ± 24 on a visual analog scale, with the axillary nodes considered the most challenging (scores of 75 and 85) because of the complexity of the approach and limited visualization window. The lymph nodes successfully identified and excised (n = 17) were distributed as follows: prescapular (n = 6), superficial inguinal (n = 6), popliteal (n = 2), axillary (n = 2), and superficial axillary (n = 1). Two dogs exhibited mild, self-limiting clinical signs consistent with lymphedema of the forelimb after excision of forelimb primary tumors and prescapular lymph nodes at 48 hours postoperatively, which resolved by 14 days postoperatively. No incisional complications were noted.

3.5 | Histopathology of SLN identified by ICTL and effect on nodal metastatic status designation compared with FNA cytology of LRLN

Seventeen lymph nodes were assessed histologically for metastasis from 16 tumors in 13 dogs. According to results of histopathology of the excised SLN, 9 of 16 tumors were positive for metastatic disease (score > HN1) vs 1 of 19 that were positive according to FNA cytology of the LRLN. When tumors with missed or nondiagnostic lymph node samples, nondiagnostic scans, and declined or unsuccessful lymphadenectomy procedures were included, the implementation of ICTL-guided nodal intervention resulted in identification of nodal metastasis (score > HN1) in 9 of 20 (45%) vs 1 of 20 tumors (5%; relative risk = 9.0, 95% CI = 1.3-64.6, P = .01) for tumors assigned to LRLN aspiration. This subsequently changed treatment recommendations (addition of chemotherapy) for 8 of 20 tumors, corresponding to 7 of 14 dogs (50%; 95% CI = 23-76.9, P = .01).

3.6 | Histopathology of the primary tumor and metastatic behavior

Among the excised tumors (n = 20), 13 were assigned a Patnaik grade 2; seven were described as subcutaneous, and no grade was assigned. Within the grade 2 group, 11 tumors were Kiupel low grade, and two tumors were Kiupel high grade. Both of the high-grade tumors had been previously designated as low grade according to cytological assessment. For the 16 tumors in which histopathology of the sentinel node was available, 5 of 10 of the grade 2 Patnaik Kiupel low group were scored as >HN1 metastatic at the node, one of one of the grade 2 Patnaik Kiupel high group was >HN1 metastatic, and three of five of the subcutaneous tumors were > HN1 metastatic. Surgical margins were complete in 17 of 20 tumors, with a tumor-free margin of 7.4 ± 5 mm, and incomplete in 3 of 20 tumors.

4 | DISCUSSION

Dogs presenting with cutaneous and subcutaneous MCT underwent assessment for nodal metastasis by FNA cytology of the LRLN, which was the hospital standard of care at the time of the study, followed by ICTL and histopathology of the surgically excised SLN. Histologic metastatic status of the SLN was higher than cytologic metastatic status of the LRLN and changed stage and adjunctive treatment recommendations in 7 of 14 dogs.

In this population, the ICTL study was straightforward to perform with an optimal scan time of 10 minutes postinjection with iopamidol. The SLN differed from the LRLN in approximately one-quarter of dogs, and surgical excision of the SLN added an average of 40 minutes to the surgical time but otherwise appeared to be a benign intervention. Implementation of ICTL mapping and nodal excision resulted in a larger proportion of animals receiving nodal metastatic information with improved quality of information compared with the preexisting standard of care.

Technical difficulties were encountered with both methods. The nodal FNA cytology method was fraught with nondiagnostic samples despite having been performed by experienced clinicians and with ultrasound guidance in >60% of sampling events. The reason for the high failure rate (13/19) is unknown; previous studies have reported failure rates of 25% to 29%[8,19]; one contributing factor may be that most nodes were normal in size. Unfortunately, there may be a tendency to optimistically equate nondiagnostic cytology of normal-sized lymph nodes with metastasis negative rather than pursue biopsy. The combination of low sampling success together with a previously reported 75% sensitivity of FNA cytology for identifying positive nodes in MCT disease[8] brings in to question the advisability of using this technique in guiding treatment decisions in view of the likely high number of false negatives.

Implementation of the ICTL technique identified several technically challenging areas. Although the ICTL study was easy to perform, 2 of 20 studies were nondiagnostic due to lack of contrast movement away from the injection site despite local massage and having ensured that there was no local compression of the area. After receipt of mapping results, one client declined surgical excision of the node because it was located within a body cavity, and one node (an axillary) could not be located intraoperatively. Success of ICTL for identification of an SLN has been variably reported at 60% to 92%.[12,22] Among the three axillary lymph nodes identified, two were successfully located intraoperatively via a caudal approach to the axilla with the limb abducted, internally rotated, and advanced cranially. After surgical planning based on CT results, an avenue of dissection was established between the deep pectoral and latissimus dorsi muscles, and the nodes were identified and excised without complication with a procedural time of 27 minutes and 53 minutes, respectively.

Conducting this study provided the opportunity to reflect on the potential utility of intraoperative dye techniques (methylene blue/indocyanine green) for guiding lymphadenectomy. Although the authors have no direct experience with these techniques in this context, our impression was that the technique might be very helpful for selecting a single SLN from among a chain of possible candidates under direct visualization after surgical exposure. The technique did not seem likely to be helpful in assisting initial surgical localization of a challenging node or determining which node or nodal group should be surgically exposed for further interrogation. The technique may be complimentary to ICTL or useful in the setting of several candidate nodes adjacent and within the surgical field of the primary tumor.

The optimal time for scanning during ICTL in this study (10 minutes after injection of iopamidol) was longer than the timeframe (0-4 minutes[13,22]) reported in other studies in which water soluble iohexol was used but was within the range of 1 to 20 minutes reported by Majeski et al,[12] who used iopamidol. Both agents are nonionic and low osmolality, but iopamidol has a slightly higher viscosity (10.4 vs 9.4 cP),[23] which may explain this effect.

In this study, seven tumors considered as cutaneous MCT after palpation were subsequently classified as subcutaneous according to histopathology results. The appropriate approach to management of subcutaneous MCT is controversial. Researchers in one study[24] reported reduced tumor related deaths, with low metastatic and local recurrence rates compared with cutaneous MCT, although the study did not include a concurrently assessed cutaneous MCT group, and the authors suggested that subcutaneous MCT be considered less aggressive in their behavior. However, other researchers have failed to identify a more benign disease course in this MCT subgroup and do not recommend a modified clinical approach compared with cutaneous MCT.[4] In the study reported here, although the sample was small, in three of the five subcutaneous MCT for which lymph node histology results were available, metastasis with HN = 2 or higher was identified by histology.

This study had several limitations. The main limitation was the low diagnostic rate of the FNA, affecting direct comparisons of LRLN FNA cytology results with SLN histology results. This may have been worsened because the exact technique used was not standardized but rather was at the clinicians' discretion. However, these same technical challenges also reflect the real world difficulties involved in obtaining diagnostic samples consistently with this technique. In addition, we did not use any intraoperative imaging methods (methylene blue or fluorescence techniques) to further confirm the identity of the excised node as the SLN; we instead relied on localization from CT results. Additional limitations included a small sample, a heterogenous group of both cutaneous and subcutaneous MCT from multiple anatomic sites, and data that reflected the experience of a single center. When any new technique is implemented, a learning curve may be expected; this learning curve is

highly influenced by the individuals involved, so results may not be readily extrapolated to other populations.

Future research focused on making this and other SLN mapping tools as accessible as possible would be helpful in improving treatment for all dogs with MCT. As we strive to achieve enhanced care for our veterinary cancer animals, we must continue to incorporate simple, inexpensive, accurate, and safe techniques into our routine practice.

Whether SLN excision and histopathology confer a survival advantage over LRLN excision or cytology that compensates for the imaging costs and increased surgical time remains to be determined. However, ICTL was straightforward to incorporate into our clinical practice, and, in this animal population, the gain in information value of ICTL-guided SLN excision was substantial enough that we suggest altering our institution's standard of care for dogs with MCT.

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Author contributions: Lapsley J, DVM: Study design; data acquisition including case enrollment, surgical care and follow-up, and data collation; data analysis and interpretation; manuscript draft; and final approval of the manuscript; Hayes GM,, DACVS, DACVECC: Study design and write-up, IACUC and grant approval, case enrollment, surgical samples and data acquisition, data analysis, manuscript draft and revisions, and final approval of the manuscript; Janvier V, DACVR: imaging data acquisition, manuscript drafting, and final approval of the manuscript; Newman AW, DACVP: Study design for the cytology analysis, cytological data acquisition and interpretation and the manuscript draft for this segment; and final approval of the manuscript; Peters-Kennedy J, DACVP, DACVD: Study design for the histopathology analysis, histopathological data acquisition and interpretation and the manuscript draft for this segment; and final approval of the manuscript; Balkman C, DACVIM (Onco): Blinded case review, interpretation of results and formulation of plans for adjunctive treatment, manuscript review, and final approval of the manuscript; Sumner JP, DACVS: Surgical case enrollment, acquisition of surgical samples and data, manuscript review, and final approval of the manuscript; Johnson P, DACVR: Study design for the imaging portion of the study, data acquisition and oversight for computed tomographic imaging, manuscript review, and final approval of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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