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ORIGINAL ARTICLE

Sentinel lymph node mapping with computed tomography lymphography for mast cell tumours and a comparison between regional and sentinel lymph node histological status: Sixty-two cases

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Abstract

It is known that the regional lymph node (RLN) may not correspond to the sentinel lymph node (SLN) (the first lymph node draining the tumour), and many diagnostic techniques have recently been aimed at its detection. Although lymphoscintigraphy is the gold standard in both human and veterinary medicine for SLN mapping, it is relatively unavailable in veterinary medicine due to costs and difficult management of the radiotracer. This prospective study evaluated, as a first aim, the feasibility and sensitivity of the computed tomography lymphography (CTL) in detecting the SLN in 62 mast cell tumours (MCTs). The second aim was to evaluate the accuracy of the CTL in identifying the most representative lymph node of the patient's lymphatic status; the histological status of the SNL was compared with that of the RLN, to see in how many cases the patient's stage would have changed according to the RLN. When the RLN turned out to be also the SLN it was decided to excise, as a control LN, the one localised in the neighbourhood of the MCT (neighbouring lymph node; NLN). The detection rate was 90%, with failure of SLN identification in six cases. In 18 (32%) of 56 MCTs with a diagnostic CTL, the SLN did not correspond to the RLN. Forty-five MCTs were surgically removed, together with their corresponding SLN and RLN/NLN. Since the clinical stage of the patient would have changed in only 7% of cases, CTL is a reliable method of detecting the SLN and, for staging purposes, there is no need to remove other LNs.

KEYWORDS
computed tomographic lymphography, dog, lymphadenectomy, mast cell tumour, regional lymph node, sentinel lymph node

1 | INTRODUCTION

Lymph node (LN) involvement represents an important prognostic feature for mast cell tumours (MCTs).[1] A MCT metastasises primarily to the LNs and, if the LN is metastatic, additional adjuvant treatment after surgery may be necessary.

It has been shown that palpation and cytology via fine needle aspiration (FNA) have low sensitivity and specificity for LN metastasis in many tumours,[2–4] and histopathology following LN excision remains the gold standard for its assessment.[5] Regarding MCTs, it is known that LN cytology may be uncertain.[6] The first purpose of a lymphadenectomy is therefore diagnostic[7,8]; however, it may also be therapeutic as additional potential sources of tumour cells are eliminated along with the primary tumour.[9] As far as we know, no study has compared RLN and SLN histological status in canine MCT based on the Weishaar et al. classification.[1]

The concept that an RLN may not be the draining lymph node (SLN) is well established, both in human and veterinary medicine.[10–13]

As the histology of the RLN may not be representative of the actual LN status, SNL mapping and selective lymphadenectomy have become the gold standard in human medicine beginning in the 1990s.[11,12] Selective lymphadenectomy results in fewer postoperative complications, and less pain and lymphoedema.[14] In the past 20 years, the SLN issue has become important even in veterinary medicine, and several studies have been undertaken regarding this.[13,15–22]

At present, lymphoscintigraphy for SLN detection is considered to be the gold standard in human medicine,[23] as well as in veterinary medicine.[21] Due to lack of equipment in the veterinary field, enhanced indirect computed tomography lymphography (CTL) represents a valid second option.[24,25]

The aims of this study were (1) to report the feasibility and sensitivity of CTL in detecting SLNs for cutaneous and subcutaneous MCTs, and (2) to evaluate the accuracy of the CTL in identifying the most representative lymph node of the patient's lymphatic status. This was obtained by comparing the histopathologic status of the RLN with that of the SLN for each solitary MCT.

This comparison evaluated the number of cases in which the patient would have been underdiagnosed and would have been denied adjuvant therapy by removing only the SLN.

2 | MATERIALS AND METHODS

Client-owned dogs with either cutaneous or subcutaneous MCTs were prospectively enrolled at the Veterinary Teaching Hospital (VTH) of the University of Turin from May 2020 to September 2021. Dogs having (a) a new and untreated cytologically diagnosed MCT, (b) a surgical scar resulting from a previously incompletely resected MCT, or (c) a local recurrence of a previously surgically excised MCT, all still amenable to surgical excision, were included. The exclusion criteria were distant metastases according to ultrasonographic-guided fine needle aspiration (FNA) of both the spleen and the liver,[26] and previous surgical excision of a regional LN when the original MCT had already been excised.

The preliminary work-up included physical examination, a complete blood count (CBC), serum biochemistry and a coagulation panel. When enlarged, the LNs were cytologically evaluated by means of FNA. Staging (abdominal ultrasound, liver and spleen FNA) and sentinel lymph node (SLN) mapping using CTL were performed during a single anaesthetic procedure. A CT evaluation of the thorax was also performed during the SLN mapping procedure. Written owner consent was obtained for the anaesthesia, the CTL mapping, and the liver and spleen FNAs.

The dogs underwent general anaesthesia using different protocols based on the preoperative clinical and laboratory work-up, and were first placed in sternal recumbency. A total body CT scan (16 MDCT unit, Siemens Somaton Emotion) without intravenous contrast medium (CM) was first performed in sternal recumbency to evaluate the thorax. The dogs were then moved to dorsal or lateral recumbency according to tumour location in order to avoid any compression on the lymphatics of interest. The skin over and around the tumour bed or the surgical scar was clipped and disinfected, and 0.8–2.0 mL of iodinated CM (Iomeron 300 mg/mL, Iomeprolo, Bracco) were injected into the subcutis near the MCT/scar (a few millimetres away from the lesion) using the four quadrants technique. Either the tumour or the surgical scar area was divided into four quadrants, and one fourth of the CM was injected into each quadrant. As reported by Rossi et al., the amount of CM was chosen based on the dog's weight (<10 kg: 0.8 mL, 10–20 kg: 1.2 mL, >20 kg: 2 mL).[24]

The CT scans were acquired immediately after CM injection, 1 min later and then every 3 min until the first draining LN(s) was/were visualised. The CTL was considered positive if a CM uptake was noted in one or more LNs within 12 min after injecting the CM.[24] The timing of the CM uptake and the number of CT scans was recorded. The CTL was considered negative if no LNs demonstrated a CM uptake 12 min after injecting the CM.

Immediately after CTL, abdominal ultrasound and FNA of both the liver and the spleen were performed. No corticosteroids or antihistamines were administered during and after the CTL. The dogs were discharged from the hospital within 6 h from the procedure. The owners were contacted by phone 24 h after the procedure to ascertain any potential complications (such as swelling or redness of the area of injection, vomiting, etc.). Patient signalment, MCT size (maximum diameter, cm), and the localisation of the corresponding SLNs and RLNs were recorded.

The CTL study data included: type of recumbency on the CT table at the time of the CM injection, time and type of contrast uptake, and distance between the tumour and the SLN; in addition, SLN size (maximum diameter, cm), shape (elongated vs. round) and number were recorded. When present, LN enhancement, was defined as "complete" if the CM was uniformly distributed in the LN, "incomplete" if the opacification was present only in some central areas of the LN or at the periphery, or "null".[24]

Approximately one-week later, according to the cytological result of the liver and the spleen FNAs, and SLN mapping, the MCT (primary or recurrent) or the scar were surgically removed based on the

proportional excision margin system for the lateral margin and a deep fascial plane.[27,28]

The SLN was removed together with the RLN (when they did not match). When the SLN was also the RLN, it was decided to excise the LN in the neighbourhood of the MCT (neighbouring lymph node; NLN) in order to compare it with the SLN.

When the RLN could not be defined given the position of the MCT on the medial sagittal plane, the RLN contralateral to the SLN was also surgically removed.

In the case of a lymphocentrum (LC) (defined as a lymph node or a group of lymph nodes occurring in the same region of the body and receiving afferent vessels from approximately the same regions of the body[29]) with more than one LN (on CT images), all the LNs were removed. The distinction between the SLN and the others of the LC was made based on the position of each of the others and their relationship with the surrounding structures on the CT images. The RLN was identified based on its regional proximity to the tumour and the physiologic lymphatic circulation described.[29,30]

Written owner consent was obtained before each surgical procedure.

After surgical preparation of the area, sterile methylene blue dye 1% (Salf S.p.A; Cenate Sotto, Bergamo, Italia) was injected into the subcutis around the tumour/scar at the surgeon's discretion in order to highlight the non-palpable LNs. Methylene blue dye was injected subcutis peritumourally into four quadrants (0,1 mL/quadrant), approximatively 10 min before surgical incision.[13]

The lymphadenectomy was performed prior to the removal of the MCT. Surgical gloves and instruments were changed after each MCT excision.

After surgery, the dogs were hospitalised in the intensive care unit; recovery time depended on the type of surgery and the reconstructive surgical technique used. All the dogs received non-steroidal anti-inflammatory drugs (NSAIDs) orally for approximately 7 days, postoperatively. Antibiotics were administered based on the extent of the surgery and the type of surgical reconstruction performed. After discharge, the dogs were re-evaluated at 7, 14 and 30 postoperative days to check for any complications in wound healing. All the patients underwent follow-up abdominal ultrasound and a clinical check-up every 3 months during the first year postsurgery. After the first year, the dogs were re-evaluated every 6 months. No subsequent checks were performed except in the case of high grade or HN3 MCTs.

All the surgical samples and the excised lymph nodes were measured using a calliper (cm) after excision; for all the primary and recurrent MCTs, and the scar, the excision margins were spatially identified with sutures and inked; all the samples, including the excised LNs, were described placed in 10% formalin solution and identified for a final histopathologic evaluation.

The surgically excised LN data (size, number, [R or S or N] LN status), surgical time for LN excision (from skin incision to closure) and surgical/postsurgical complications (based on the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events [VCOG-CTCAE v2[31]]) were recorded for each dog.

The histopathological data concerning the tumour/scar and the LNs included: MCT type (cutaneous vs. subcutaneous), MCT histologic grade (according to both the Patnaik 3-tier grading and the Kiupel 2-tier system for cutaneous MCTs,[32,33] and Bellamy[34] and Thompson[35,36] for subcutaneous MCTs), excision margin status (infiltrated vs. not infiltrated) and LN status (according to Weishaar's classification[1]). Each LN was first cut longitudinally. Its evaluation was carried out on two 3-micron thick slices with both eosin/haematoxylin and toluidine blue staining.[1]

The HN0 and HN1 LNs were clustered together and defined as "negative" for metastases; the HN2 and HN3 were clustered together and defined as positive for "metastases".

The SLNs were defined as negative or positive, and were compared with the R/NLNs to see in how many cases the latter was positive with a negative SNL. The same comparison was made when the SNL was part of an LC with multiple LNs.

2.1 | Statistical analysis

Descriptive statistics were calculated for each variable of interest.

For the continuous variables, mean, median and range were calculated. Statistics were carried out using GraphPad Prism (version 9.3.1 for Windows, GraphPad Software, San Diego, California, www.graphpad.com), with statistical significance set at a $p < .05$. For statistical purposes, the Shapiro–Wilk test was used to assess the normality of distribution of the variables (age, weight, dimension). The Spearman test was carried out to assess any possible correlation between the CT scans and tumour-SLN distance. The Chi^2-test for trend and the Chi^2-test were used to test possible associations between SLN histological status, SLN shape and type of contrast uptake. Differences in the medians of the SLN dimensions with respect to the SLN histological status were analysed using the Kruskal–Wallis test followed by a post-hoc Dunn's test.

3 | RESULTS

Fifty-nine dogs, for a total of 62 MCTs, were eligible for inclusion in the study. Breeds included mixed ($n = 14$), Labrador Retriever ($n = 11$), Golden Retriever ($n = 9$), Boxer ($n = 5$), American Pitbull ($n = 3$), English Setter ($n = 3$), American Staffordshire terrier ($n = 2$), Shar-pei ($n = 2$), Argentinian Dogo ($n = 2$) and one each of Jack Russel terrier, Dachshund, Great Swiss Mountain dog, French bulldog, Alaskan Malamute, Kurzaar, Weimaraner and Bolognese. There were 31 males (of which six were neutered) and 28 females (of which 22 were spayed). Mean and median age at diagnosis was 7.5 years (range 1–15.5 years). Mean body weight was 29 kg (median 31 kg, range 7–58 kg).

Only one dog had regional lymphadenomegaly at presentation. A preoperative FNA of this enlarged LN was performed revealing the presence of neoplastic mast cells at cytology.

Of the 62 MCTs included, four were scars from previous incomplete excisions and three were recurrences occurring at 27, 45, and 330 days from previous excisions. Three dogs had two independent MCTs at the same time. The MCT (scars excluded from the evaluation) mean and median diameter were 2.4 and 2 cm, respectively (range 0.4–5 cm). One dog underwent neoadjuvant chemotherapy before surgery to reduce the tumour burden. The MCTs (scars included) were located on the trunk, head and neck, distal limbs, scrotum and perineum, and tail in 28, 13, 11, 9, and 1 case, respectively.

The RLNs for each localisation are reported in Table 1.

For the CTL, 27 dogs were positioned in dorsal recumbency, nine in left or right lateral recumbency, and 17 in sternal recumbency. The three dogs having two MCTs were repositioned in dorsal (from sternal), right lateral (from left lateral) and sternal (from dorsal) decubitus, to map the second MCT.

Of the 59 dogs, 53 showed a positive CTL (90%) while, in six (10%), no LN CM uptake was observed at T12. Overall, a positive CTL was noted in 56 of the 62 MCTs (90.3%). In six MCTs, given the lack of progression of the CM at T12, a second dose of 0.5 mL of CM was injected; however, the CTL was still null. In two dogs, the initial positioning was also changed after two scans due to the non-progression of the CM; however, it was null even at T12.

Of the four scars, three showed a positive CTL (two at T5, 1 at T12) without the need for additional CM injection. For one scar, the CTL study was null.

In 21 tumours (37.5%), one or more SLNs were identified immediately after the peritumoural CM injection (Figure 1) while, in the remaining MCTs (n = 35, 62.5%), the median uptake time was 3 min (range 1–12 min; mean 2.5 min).

The LN enhancement was considered to be complete and incomplete in 37 and 28 SLNs, respectively (Figure 2). In one of the 28 LNs with incomplete enhancement, a ring-like pattern was observed. Forty-two SLNs were classified as elongated in shape, while 23 were round.

The mean and median distances between the tumour and the SLN were 13.5 and 11.0 cm, respectively (range 0.6–40.0 cm).

Forty-nine MCTs had a single SLN while two and three SLNs were identified in five and two MCTs, respectively, for a total of 65 SLNs found.

Six (11%) MCTs displayed SLNs belonging to different lymph-centres (Table 1). Two were localised on the lower thigh while the others were on the stifle, on the caudal abdomen, in the ventral perineum and on the lateral neck.

Of the 56 MCTs with a positive CTL, 26 had the SLN corresponding to the RLN (46%) while, in 18 MCTs, the primary draining LN did not correspond to the RLN (32%). In particular, in 46% of the MCTs localised on the trunk, the SLN was found to be different from the RLN. For seven MCT (12.5%), localised in the perianal region, the correspondence was only partial as not all the RLNs were also the SLNs (Table 1). Five MCTs (9%) were localised at the level of the medial sagittal plane (four on the scrotum and one on the ventral neck) (Table 1); the right inguinal and the right mandibular LNs were identified as SLNs for all the scrotal and ventral neck MCTs, respectively. Due to their position, the contralateral LCs were considered to be RLNs and were, therefore, surgically excised.

Of the 53 dogs with a positive CTL, 11 dogs did not undergo surgery. Five of them had splenic cytology indicative of mast cell infiltration, and chemotherapy was proposed; in three dogs, radiotherapy was preferred to surgery by the owners. Three dogs with a positive CTL were lost to follow-up after staging.

Forty-two dogs with 45 tumours (42 primary MCTs, two scars and one recurrence) with a positive CTL underwent surgery for MCT, SLN and RLN/NLN removal.

Eight MCTs had an LC corresponding to the SLN (six mandibular; two inguinal) with multiple LNs (Figure 3); six MCTs had the regional or neighbouring LC (five mandibular; one inguinal) with more than one LN (Table 2).

At histopathology, 35 MCTs were cutaneous ($n = 34$ grade II Patnaik-low grade Kiupel; $n = 1$ grade II Patnaik-high grade Kiupel) and eight were subcutaneous.

No tumour cells were found in the excised scars. Histological margins were complete in 42 cases (including the two scars) and infiltrated in three cases (Table 2).

The median and mean times for lymphadenectomy were 30 and 34 min, respectively (range 13–90 min). The median and the mean LN diameters were 1.4 and 1 cm respectively.

No statistically significant correlation was found between SLN histological status and LN CM uptake, shape and size. Similarly, the number of CT scans required to highlight the SLN was not significantly correlated with the distance between the MCT and its SLN.

The SLNs were negative in 23 s MCT and positive in 20 MCTs. Of the 23 MCTs with negative SLNs, in three MCTs, the R/NLN was positive. Of the 20 with positive SLNs, no R/NLN was found to have a higher histological grade than the SNL. In two tumours ($n = 44$ and $n = 21$), it was not possible to make a comparison as the SLN and/or the RLN was not found. The overall number of positive and negative SLNs with respect to R / NLNs is shown in Table 3.

Seven MCTs had multiple LNs in the SLN LC. In two MCTs ($n = 52$ and $n = 53$), the non-sentinel LN of the sentinel LC were positive with a negative SLN.

In one MCT (at the level of the fourth mammary gland), the SLNs (inguinal and axillary) appeared to be both the RLN and the NLN; therefore, only the two SLNs were removed.

For one MCT ($n = 43$), the RLN (parotid) was not found, despite the use of methylene blue, and it was decided to remove the ipsilateral mandibular LNs ($n = 2$) as control LNs, due to their proximity to the tumour.

Of the two patients with abdominal SLNs or R/NLNs, only in one case was the exploratory laparotomy for their extirpation accepted by the owners.

Methylene blue dye was used in three dogs, to highlight the medial iliac, axillary and parotid LNs. In the latter two cases, as already mentioned above, they were not identified.

No complications were observed during the CTL or were reported by the owners when contacted by phone, except for a mild swelling

TABLE 1 Signalment, MCT diameter and localization, regional and sentinel lymph node, number of SLN and type (if >1SLN) of LC (same LC vs. different LCs) for each of the 56 MCTs included in the study.

Dog	Signalament	MCT diameter (cm)	Mct localization	RLN	SLN	SLN no. and type of LC	Surgery (Y or N)
1	Mixed breed, Fn, 15,5 years	3	Lateral neck-L	Prescapular-L	Mandibular-L	1 SLN	Y
2	Golden, Fn, 8,5 years	2	4th mammary gland-L	Inguinal-L	Inguinal-L; axillary-L	2 SLNs; different LCs	Y
3	Jackrussel, F, 10 years	2	Stifle-L	Inguinal-L	Inguinal-L; medial iliac-L	2 SLNs; different LCs	Y
4	Labrador, Fn,10 years	0,6	Stifle-R	Inguinal-R	Inguinal-R	1 SLN	Y
5	Mixed Breed, Mn, 13,5 years	4	Forearm-L	Prescapular-L	Prescapular-L	1 SLN	N
6	Amstaff, M, 7 years	0,7	Lateral neck-L	Mandibular-L	Prescapular-L	1 SLN	Y
		1	Scrotum	na	Inguinal-R	1 SLN	Y
7	Golden, M, 4 years	1	Dorsal neck-R	Prescapular-R	Prescapular-R	1 SLN	Y
8	Golden, M, 6 years	1,5	Lateral neck-L	Prescapular-L	Prescapular-L	1 SLN	Y
9	Labrador, M, 11 years	2	Pinna (ventral base)-R	Parotid-R	Prescapular-R	1 SLN	Y
10	Pittbull, Fn, 9 years	0,5	Tail-L	Inguinal-L	Medial iliac-L	1 SLN	Y
11	Boxer, M, 8 years	1,5	Scrotum	na	Inguinal-R	1 SLN	Y
12	Dachshund, M, 11,5 years	4	Elbow-R	Axillary-R	Axillary-R	1 SLN	Y
13	Golden, Fn, 5 years	3 (s)	Lateral thorax-R	Axillary-R	Axillary-R	1 SLN	Y
14	Labrador, M, 5,5 years	1	Supraorbital-R	Parotid-R	Mandibular-R	1 SLN	Y
15	Dogo Argentino, Fn, 5 years	5	Thigh lateral-L	Inguinal-L	Inguinal-L	1 SLN	Y
16	Mixed Breed, Fn, 11,5 years	5 (s)	Leg lateral-R	Inguinal-R	Inguinal-R	1 SLN	Y
17	Golden, Fn, 6 years	2	Foot pad, forefoot-R	Prescapular-R	Prescapular-R	1 SLN	Y
18	Mixed Breed, M, 8 years	2,5	Ventral perineum- L	Sacral-L; medial iliac-L; inguinal-L	Medial iliac-L; internal iliac L and R	3 SLNs; different LC	N
19	Bernese Mountain dog, M, 3,5 years	3 (r)	Foot pad, forefoot-R	Prescapular-R	Axillary-R	1 SLN	Y
20	Labrador, F, 7,5 years	2,5	Vulva-L	Inguinal-L; sacral-L; medial iliac-L	Inguinal-L	1 SLN	Y
21	Mixed Breed, M, 9 years	2	Sternum-L	Axillary-L	Axillary-R	1 SLN	Y
22	Mixed Breed, Fn, 8 years	2	Thigh caudal-R	Inguinal-R	Inguinal-R	1 SLN	Y
23	Golden, Fn, 9 years	1,9	Lower thigh-L	Popliteal-L	Popliteal-L	1 SLN	N
24	Mixed Breed, Fn, 10 years	2,1	Thigh lateral-L	Inguinal-L	Inguinal-L	1 SLN	N
25	Labrador, Fn, 7,5 years	7	Flank-R	Axillary-R	Axillary-R	1 SLN	Y
		3	Flank-L	Axillary-L	Prescapular-L	1 SLN	Y
26	Mixed Breed, Fn, 6,5 years	2,3	Thigh caudal-L	Inguinal-L	Inguinal-L	1 SLN	Y
27	Boxer, Fn, 5 years	0,9	Sternum-L	Axillary-L	Prescapular-L	1 SLN	Y
28	Golden, Mn, 7 years	2	Ischiatic tuberosity region-R	Inguinal-R	Medial iliac-R	1 SLN	N

(Continues)

TABLE 1 (Continued)

Dog	Signalament	MCT diameter (cm)	Mct localization	RLN	SLN	SLN no. and type of LC	Surgery (Y or N)
29	French bull dog, Fn, 7 years	3,8	Lower thigh-L	Popliteal-L	Popliteal-L; medial Iliac-L; internal Iiiac-L	3 SLNs; different LCs	N
30	Alaskan malamute, M, 5 years	3,7	Lateral thorax-R	Prescapular-R	Axillary-R	1 SLN	Y
31	Mixed breed, M, 6 years	2,3	Inferior lid-L	Parotid-L	Parotid-L	1 SLN	Y
32	Mixed breed, M, 9 years	0,9	Lumbar region	Inguinal-L	Axillary-L	1 SLN	Y
33	Pittbull, Mn, 3,5 years	0,8(s)	Scrotum	na	Iinguinal-R	1 SLN	N
34	Labrador, M, 8 years	2,6	Lower thigh-L	Popliteal-L	Popliteal-L	1 SLN	N
35	Mixed breed, M, 6,5 years	3	Lateral arm	Prescapular-L	Axillary-L	1 SLN	Y
36	Kurzaar, Mn, 9,5 years	4(r)	Lower thigh-L	Popliteal-L	Inguinal-L; popliteal-L	2 SLNs; different LCs	N
37	Boxer, M, 8,5 years	1,3	Foot pad, forefoot-L	Prescapular-L	Prescapular-L	1 SLN	N
38	Sharpei, M, 6 years	3	Flank fold-L	Inguinal-L	Inguinal-L	1 SLN	Y
39	Labrador, F, 8 years	1	Frontal region-R	Parotid-R	Parotid-R	1 SLN	Y
40	Labrador, Fn, 8 years	5	Perineal ventral-L	Inguinal-L; sacral-L; medial Iliac-L	Inguinal-L	1 SLN	N
41	Boxer, M, 8,5 years	1	Flank fold-L	Inguinal-L	Inguinal-L (2)	2 SLNs; same LC	Y
42	Golden, Fn, 6 years	1	Mandibular-R	Mandibular-R	Mandibular-R	1 SLN	Y
43	English Setter, F, 9 months	0,4	Pinna, lateral-L	Parotid-L	Prescapular-L	1 SLN	Y
44	Labrador, F, 10,5 years	2	Sternum cranial-L	Prescapular-L	Axillary-L	1 SLN	Y
45	English Setter, M, 11 years	2,5	Prepuce-L	Inguinal-L	Inguinal-L	1 SLN	Y
46	Amstaff, M, 8 years	4,5	1st–2nd digit, forefoot-L	Prescapular-L	Prescapular-L	1 SLN	Y
47	Boxer, M, 6 years	1,7	Forearm-L	Prescapular-L	Axillary-L	1 SLN	Y
48	Pittbull, Mn, 9, 5 years	5	Scrotum	na	Inguinal-R	1 SLN	Y
49	Mixed Breed, M, 12 years	4	Lateral thorax-L	Axillary-L	Axillary-L	1 SLN	Y
50	Weimaraner, Fn, 7 years	1,8	Flank-L	Axillary-L	Axillary-L	1 SLN	Y
		1,6	Dorsum-L	Axillary-L	Prescapular-L	1 SLN	Y
51	Bolognese, Fn, 8 years	1,4	Labial commissure-R	Mandibular-R	Mandibular-R	1 SLN	Y
52	English Setter, Fn, 6,5 months	2	Ventral neck-middle	na	Mandibular-R	1 SLN	Y
53	Golden, Fn, 6,5 years	1	Lateral neck- L	Prescapular-L	Mandibular-L (2): mandibular-R (2)	2 SLNs; different LCs	Y

Abbreviations: F, female; Fn, female neutered; L, left; LC, lymph center; Male, male; MCT, mast cell tumour; Mn, male neutered; na, not available; R, right; RLN, regional lymph node; SLN, sentinel lymph node; sx, surgery.

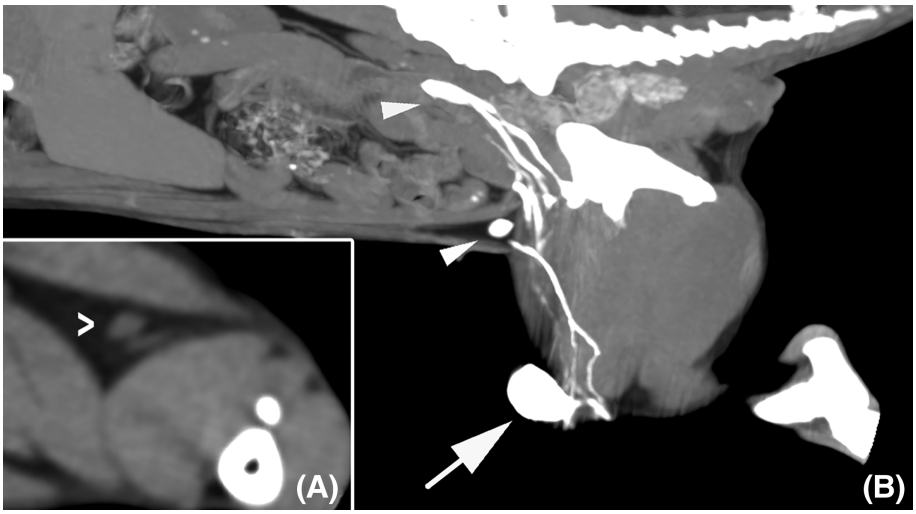

FIGURE 1 Computed tomography parasagittal multiplanar reconstruction image showing the peri-injectional site around a mast cell tumour (arrow) localised on the stifle with (A) its ipsilateral popliteal neighbouring lymph node and (B) the two-sentinel contrast-enhanced lymph nodes (ipsilateral inguinal and medial iliac) (arrowhead).

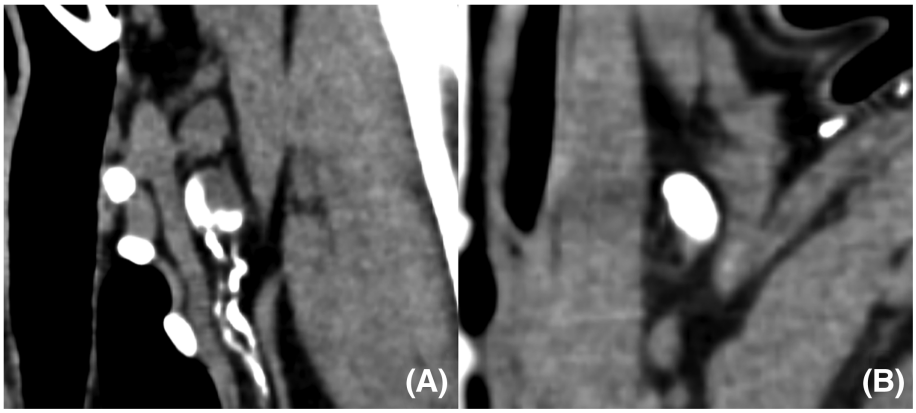

FIGURE 2 Computed tomography dorsal multiplanar reconstruction image showing (A) a prescapular sentinel lymph node with incomplete contrast medium enhancement; (B) a prescapular sentinel lymph node with a complete contrast medium enhancement.

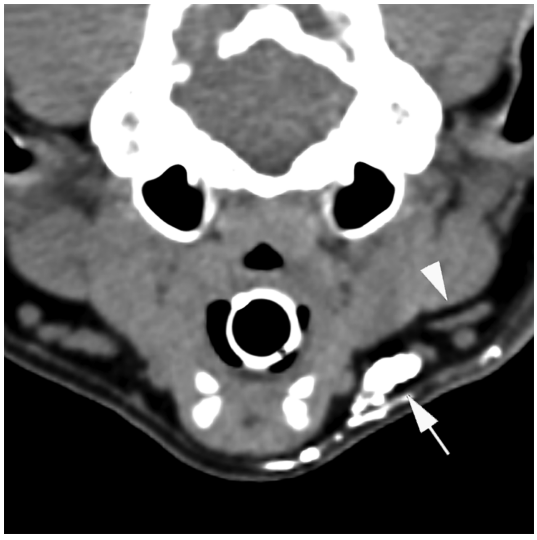

FIGURE 3 Computed tomography image showing a mandibular lymphocentrum with multiple lymph nodes. The contrast uptake is noted only in the medial lymph node (sentinel lymph node) (arrow) while the lateral one is not enhanced (arrowhead).

TABLE 2 MCT localization, histologic grade, surgical margins status, SLN number and histologic status according to Weishaar 2014,[1] other LNs in the sentinel LC (and its histologic status), RLN number and histologic status according to Weishaar 2014[1] for each MCT treated with surgery.

Case number	Localization	MCT histology	Surgical margins	SLN number and status	Other LNs in the SLN LC	RLN number and status	NLN number and status
1	Lateral neck-L	P-2 K-LG	Clean	1; HN0	1; (HN0)	1; HN0	
2	4th mammary gland-L	Subcutaneous, well diff.	Clean	2; HN2 (inguinal); HN0 (axillary)	0		
3	Stifle-L	Subcutaneous, well diff.	Clean	2; HN3 (medial Iliac-L); HN3 (inguinal-L)	0		1; HN3 (popliteal)
4	Stifle-R	P-2 K-LG	Clean	1; HN3 (inguinal)	0		1; HN3 (popliteal-R)
6	Lateral neck-L	P-2 K-LG	Clean	1; HN0 (prescapular-L)	0	2; HN0 (mandibular-L)	
6	Scrotum	P-2 K-LG	Clean	1; HN0 (inguinal-R)	0		1; HN0 (inguinal-L)
7	Dorsal neck-R	Subcutaneous, well diff.	Clean	1; HN0 (prescapular-R)	0		1; HN0 (mandibular-R)
8	Lateral neck-L	P-2 K-LG	Clean	1; HN1 (prescapular-L)	0		1; HN1 (prescapular-R)
9	Pinna (ventral base)-R	P-2 K-LG	Clean	1; HN2 (prescapular-R)	0	1; HN2 (parotid-R)	
10	Tail-L	P-2 K-LG	Clean	1; HN0 (medial Iliac-L)	0	1; HN0 (inguinal-L)	
11	Scrotum	P-2 K-LG	Clean	1; HN3 (inguinal-R)	0		1; HN1(inguinal-L)
12	Elbow-R	P-2 K-LG	Clean	1; HN1 (axillary-R)	0		1; HN1 (prescapular-R)
13	Lateral thorax-R	P-2 K-LG[a]	Clean	1; HN1 (axillary-R)	0		1; HN1 (prescapular-R)
14	Supraorbital-R	P-2 K-LG	Clean	1; HN1 (mandibular-R)	1; (HN1)	1; HN1 (parotid-R)	
15	Thigh lateral-L	P-2 K-LG	Clean	1; HN1 (inguinal-L)	0		1; HN1 (popliteal-L)
16	Thigh lateral-R	P-2 K-LG[a]	Clean	1; HN2 (inguinal-R)	0		1; HN2 (popliteal-R)
17	Foot pad, forefoot-R	P-2 K-LG	Infiltrated	1; HN3 (prescapular-R)	0		1; HN2 (axillary-R)
19	Foot pad, forefoot-R	P-2 K-LG	Clean	1; HN1 (axillary-R)	0	1; HN0 (prescapular-R)	
20	Vulva-L	P-2 K-LG	Infiltrated	1; HN3 (inguinal-L)	0		1; HN2 (inguinal-R)
21	Sternum-L	P-2 K-LG	Clean	not found (axillary-R)	na	not found	
22	Thigh caudal-R	P-2 K-LG	Clean	1; HN2 (inguinal-R)	0		1; HN0 (popliteal-R)
25	Flank-R	P-2 K-LG	Clean	1; HN0 (axillary-R)	0		1; HN0 (prescapular-R)
25	Flank-L	P-2 K-LG	Clean	1; HN0 (prescapular-L)	0	1; HN0 (axillary-L)	
26	Thigh caudal-L	P-2 K-LG	Clean	1; HN1 (inguinal-L)	0		1; HN1 (popliteal-L)
27	Sternum-L	P-2 K-LG	Clean	1; HN2 (prescapular-L)	0	1; HN1 (axillary-L)	
30	Lateral thorax-R	P-2 K-LG	Clean	1; HN2 (axillary-R)	0	1; HN0 (prescapular-R)	
31	Inferior lid-L	P-2 K-LG	Clean	1; HN2 (parotid-L)	0		3; HN2 (mandibular-L)
32	Lumbar region	P-2 K-LG	Clean	1; HN1 (axillary-L)	0	1; HN0 (inguinal-L)	
35	Lateral arm-L	Subcutaneous, well diff.	Clean	1; HN0 (axillary-L)	0	1; HN0 (prescapular-L)	
38	Flank fold-L	P-2 K-LG	Clean	1; HN1 (Inguinal-L)	0		1; HN1 (popliteal-L)
39	Frontal region-R	P-2 K-LG	Clean	1; HN1 (parotid-R)	0		1; HN1 (mandibular-R)
41	Flank fold-L	P-2 K-LG	Clean	2; HN2 (inguinal-L); HN2 (inguinal-L)	0		1; HN0 (popliteal-L)
42	Mandibular-R	Subcutaneous, well diff.	Clean	1; HN1 (mandibular-R)	1; (HN1)		2; HN2 (mandibular-L)

TABLE 2 (Continued)

Case number	Localization	MCT histology	Surgical margins	SLN number and status	Other LNs in the SLN LC	RLN number and status	NLN number and status
43	Pinna, lateral-L	P-2 K-LG	Clean	1; HN0 (prescapular-L)	0	2; HN0 (mandibular-L)	
44	Sternum cranial-L	Subcutaneous, well diff.	Clean	not found (axillary-L)	na	1; HN0 (prescapular-L)	
45	Prepuce-L	P-2 K-LG	Clean	1; HN2 (inguinal-L)	0		1; HN2 (inguinal-R)
46	1st–2nd digit, forefoot-L	P-2 K-LG	Infiltrated	1; HN2 (prescapular-L)	0		1; HN0 (axillary-L)
47	Forearm-L	P-2 K-LG	Clean	1; HN2 (axillary-L)	0	1; HN0 (prescapular-L)	
48	Scrotum	P-2 K-HG	Clean	1; HN2 (inguinal-R)	2; (HN0)		3; HN2 (inguinal-L)
49	Lateral thorax-L	P-2 K-LG	Clean	1; HN2 (axillary-L)	0		1; HN0 (inguinal-L)
50	Flank-L	P-2 K-LG	Clean	1; HN2 (axillary-L)	0		1; HN2 (inguinal-L)
50	Dorsum-L	P-2 K-LG	Clean	1; HN1 (prescapular-L)	0	1; HN2 (axillary-L)	
51	Labial commissure-R	Subcutaneous, well diff.	Clean	1; HN2 (mandibular R)	2; (HN2)		1; HN0 (retropharyngeal-R)
52	Ventral neck-middle	Subcutaneous, well diff.	Clean	1; HN0 (mandibular-R)	2; (HN2)		3; HN0 (mandibular-L)
53	Lateral neck- L	P-2 K-LG	Clean	2; HN0 (mandibular-R); HN0 (mandibular-L)	2; (HN2)	1; HN2 (prescapular-L)	

Abbreviations: L, left; LC, lymph center; LN, lymph node; MCT, mast cell. tumour; na, not available; P-2 K-LG, Patnaik second grade-Kiupel low grade; P-2 L-HG, Patnaik second grade-Kiupel high grade; R, right; RLN, regional lymph node; SLN, sentinel lymph node; sx, surgery; well diff., well differentiated.

[a]Scar; the histological grade reported in the table refers to the first surgery.

and irritation of the injection area in three dogs, completely and spontaneously resolved within 24 h.

Regarding postsurgical complication, one patient experienced mild and transient two-days lameness after an axillary lymphadenectomy which resolved spontaneously.

Nine dogs experienced grade 1 seroma at the site of the lymphadenectomy.

One dog experienced grade 2 wound dehiscence, with complete failure of the local flap used to close the labial defect. The wound was treated with local care and left to heal by second intention.

One dog developed a grade 2 seroma after a bilateral mandibular and retropharyngeal lymphadenectomy. Simple aspiration was carried out and prednisolone (Prednicortone, Dechra, Italia) was administered initially at 0.5 mg/kg q24h for 7 days PO and then progressively reduced.

Grade 3 post-surgical complications were observed in two dogs. In the first one, partial wound dehiscence at the site of an axillary lymphadenectomy occurred 7 days after surgery, and sedation and suture apposition were required. The second patient experienced a grade 3 seroma at the surgical site of the MCT removal 2 weeks after surgery. The fluid collection, localised at the level of the right thoracic wall, was sampled and cytologically evaluated to exclude infection; an active skin drain was positioned and then removed 6 days later.

4 | DISCUSSION

In this study, indirect CTL allowed the detection of at least one SLN in 56 of the 62 MCTs included, leading to a detection rate of 90.3%, in line with the literature.[3,37,38] The procedure failed to identify the SLN in six of the 62 MCTs. CTscan units are now widely available, and indirect CTL can be easily performed in many veterinary facilities at a relatively low cost. This procedure can identify not only the LC but also the lymphatic vessels, with better spatial resolution than radiology.[39]

The gold standard for an SLN in human medicine is preoperative lymphoscintigraphy with intraoperative gamma probing.[40,41] The high detection rate and the possibility of localising the SLN intraoperatively suggests that this is undoubtedly the preferable technique for SLN mapping which seems likely, even in veterinary medicine.[21,25,42]

In fact, excellent results in terms of the number of SLNs detected and then surgically excised have been reached by combining lymphoscintigraphy and peritumoural intraoperative methylene blue injection, with a detection rate of 91%.[21] The dye allows better visualisation and therefore easier isolation of the SLN from the surrounding tissues, especially in overweight patients or in regions characterised by the presence of many delicate structures, such as the axilla and the neck. Once more, it should be emphasised that less aggressive dissections are correlated with fewer postoperative complications.[43] Nevertheless, lymphoscintigraphy, in the veterinary field, is not widely used due to the need for radioactive materials, relatively high costs and poor availability of the equipment. Therefore, considering the importance of evaluating an SLN, various other techniques have been developed for its detection. They include indirect lymphography with

TABLE 3 number of MCT with SLN+ versus − compared with R/NLN+ versus −.

	SLN+	SLN−
R/NLN+	$n = 10$	$n = 3$
R/NLN−	$n = 10$	$n = 20$

radiography,[17,44] contrast-enhanced ultrasound (CEUS),[45] near-infrared imaging (NIR)[46] and CTL.[3,24,25,37,47]

In the six MCTs with an inconclusive indirect CTL, the CM remained stationary at the level of the injection sites. Massaging the organ involved has been reported to facilitate the progression of the CM.[24] This was avoided in all cases except in those in which no CM progression occurred due to the possibility of causing MCT degranulation.

The choice of patient's recumbency is important, and care must be taken to avoid any external pressure on the lymphatic vessels which is capable of slow down or arrest the CM progression.[25] In two cases in which no progression was observed, the decubitus was changed after two scans; however, despite this, the CM did not reach any LN at T12. Four dogs with MCTs on the trunk and a negative CTL were all overweight or obese. The presence of abundant adipose tissue could play a role in slowing down the lymphatic drainage; in fact, this issue is also still under debate in human medicine.[48,49] Another dog with a null CTL was a Shar-pei having an MCT localised in the hock region. This breed has a higher percentage of subcutaneous collagen than other breeds, and it can be speculated that this could affect the lymphatic drainage. On the other hand, it should be also noted that this study also included another Shar-Pei having a 3-cm MCT localised in the flank fold which showed a positive CTL within 3 min.

In the majority of the CTLs in the present study, the CM was identified within the lymphatics immediately after the injection while, as has already been reported,[25] the study had a high probability of being unsuccessful if the CM remained peritumourally for more than 3 min. In the Authors' experience, the addition of the CM a few minutes after a negative scan did not change the result.

Of the four scars included, three showed a positive CTL, although in none of them was the LN visualised within the first or the second scan. As also reported in humans, SLN mapping is problematic in the case of scars due to the lymphatic drainage disruption caused by the first surgery.[50,51] Even though, for this study, it was decided to also include dogs which had already undergone incomplete surgery, the exploitability of these results should be considered low, even when an SLN was identified.

The lack of statistical significance between the SLN CM uptake time and the MCT-SLN distance could be due to external factors, such as lymphatic compression or the abundance of subcutis. Based on these results, the only reasonable recommendation is to perform the first CT scan immediately after the CM injection and then 1 min later, regardless of the distance between the MCT and the SLN.

The lack of statistical correlation between the histological status of the SLN and the variables considered (LN size, shape, contrast uptake) was in line with the fact that, in the present study, mainly pre-metastatic (HN1) and early metastatic (HN2) LNs were included. In fact, it is foreseeable that these conditions may not result in morphological and structural changes capable of influencing the uptake pattern of the LN itself. Further studies, also including the evaluation of overt metastatic LNs, are warranted.

The SLN mapping was particularly useful in truncal MCTs, for which it is more difficult to establish which is the RLN, and, in general, for MCTs localised at an equal distance from different LCs. In a high percentage (46%) of MCTs localised in such areas, the SLN was found to be different from the RLN. In the same way, it appeared very useful for MCTs localised on the median sagittal plane.

The secondary aim of the study was to determine the diagnostic reliability of CTL in determining the SLNs of canine patients with MCTs, considering the method not reliable (intended as identifying the wrong SLN) when the SLN was negative with a positive R/NLN. In fact, considering the SLN as the first LN which drains the lymph coming from the tumour, it is not possible to have a positive R/NLN with a negative SLN. In the few cases in which this happened, the Authors hypothesised that, during the CTL, the CM migrated to the wrong SLN.

For each MCT, the histology of the SLNs and the R/NLNs was compared, and the number of cases in which R/NLN status would have changed the patient's stage (I vs. II) was evaluated.

As shown in Table 3, this situation (negative SLN + positive RLN) occurred in only three cases (7%) in which the SLN was HN0 or HN1 and the R/NLN was HN2. These patients, with the sole evaluation of the SLN would have been considered negative for lymphatic metastases. Being diagnosed as an early metastatic LN, no adjuvant therapy would have been indicated; however, the prognosis presented to the owner would have changed. In the present study no HN3 RLN with a negative SLN was detected. This situation would have been more serious as based on the SLN defined by CTL, the patient would have been denied additional therapy.

The removal of all the LNs present in the SLN LC, which goes against the principles of SLN mapping, was performed to see whether there were positive non-SLNs in the LC with negative SLN, indicating inaccuracy of the CTL. This situation occurred in two MCTs, both located in the neck, a region with a complex lymphatic network.[52,53] Therefore the dye should be considered as a tool for increasing SNL identification.[21,22,45] It is possible that combining a vital dye with CTL, more LNs of the LC would also have been considered SLN. Intraoperative methylene blue dye was rarely used in this cases series because CTL allowed defining preoperatively the number and position of LNs as well as the relationship with the surrounding structures. However, it is possible that these relationships would change when changing the patient's position in surgery. Methylene blue dye was useful for a rapid identification of the iliac and sacral LNs as well as for the parotid LN in one MCT. In two cases, the axillary and the parotid LNs were not found, even with the aid of methylene blue dye which, unfortunately, did not progress after injection. However, no conclusions can be drawn from its use as it has been used in only a few cases, even if this dye has been reported to be a reliable

intraoperative tool allowing the surgeon to easily identify the SLN and to dissect the tissue more conservatively.[13]

The CTL was safe and well tolerated in all the cases in the present study with very few minor complications. The concern regarding the increase in MCT size due to degranulation induced by the perilesional CM injection, led the Authors to postpone surgery. Complications following LN excision were observed in 28% of the lymph node excision; in all but two cases (4.5%), no treatment was required. In humans, an extensive lymphadenectomy may be correlated with a higher risk of complications as compared to a limited lymphadenectomy (19.8% vs. 8.2%).[54] Regarding the operative time, the lymphadenectomy procedure almost always lasted less than 1 h. The progressive surgical skills acquired also helped in progressively reducing the operative time.

As compared to lymphoscintigraphy, indirect CTL has several limitations and should be seen as a fallback and not as an alternative. In fact, in addition to being characterised by lower sensitivity in detecting SLNs, it does not allow easily distinguishing the LNs involved within the LC, unless combined with an intraoperative technique, such as methylene blue dye.[21] In the present study, the differentiation of the SLN from the others LNs of the LC was carried out with the help of the CT images, evaluating the location of each LN relative to the others and the neighbouring anatomical structure s, such as the salivary gland and the vessels. However, this method is not accurate as the appearance and the relationships between the LNs of the LC can change if the patient's positioning during surgery differs from CTL positioning. This could potentially lead to choosing the wrong LN as the SLN.

The limitation of the present study, although a substantial number of dogs with MCTs was included, is the unbalanced number of low-grade MCTs as compared to high-grade ones; however, this reflected what normally occurs in clinics. Additional studies are needed to evaluate whether the percentages of positive R/NLNs with negative SLNs are the same in aggressive MCTs, and how many overt metastatic SLNs and RLNs are present.

Another possible limitation of the present study is represented by the fact that the histological LN status was evaluated on two 3-micron thick slices with both eosin/haematoxylin and toluidine blue staining, not in accordance with the recently published article by Sabattini et al.[55] which recommended the execution of two 2-micron slices for each of the two halves of the lymph node, sectioned longitudinally, in order to achieve high sensitivity. This is because the current study was carried out at the same time.

In conclusion, indirect CTL should be considered to be a valid tool for LN mapping when lymphoscintigraphy is not available, especially when combined with a vital dye.

The low percentage of negative SLNs and positive RLNs suggests that, when removing the only SLN, there is a high probability (93%) that its status reflects the patient's actual lymphatic status. Therefore, from a diagnostic point of view, it is enough to remove the SLN as the removal of additional LNs may increase surgical time and complications. Additional studies are required to assess whether these conclusions are also valid for high-grade MCTs and whether a more extensive lymphadenectomy could have some clinical benefit.

CONFLICT OF INTEREST STATEMENT

The Authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

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

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