317 (2026) 106651

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

# Nodal lymphatic mapping in 22 dogs bearing solid malignant tumors and enlarged regional lymph nodes: A descriptive study (2022–2025)

Elisa Maria Gariboldi [1,*], Alessandra Ubiali [1], Roberta Ferrari, Luigi Auletta, Donatella De Zani, Davide Danilo Zani, Francesco Ferrari, Federica Alessandra Brioschi, Paola Roccabianca, Chiara Giudice, Valeria Grieco, Camilla Recordati, Damiano Stefanello

*Dipartimento di Medicina Veterinaria e Scienze Animali, Università degli Studi di Milano, Lodi, Italy*

ARTICLE INFO

*Keywords:*
Enlarged lymph node
Sentinel lymph node
Indocyanine green
Lymphoscintigraphy

ABSTRACT

No data are available about the sentinel lymph node (SLN) mapping in presence of enlarged regional lymph nodes (eRLN), due to metastatic or inflammatory processes. The present study aims to assess the influence of eRLN clinically suspicious for nodal metastasis in SLN mapping in canine malignancies and describe possible alterations of the nodal lymphatic drainage and tracer uptake.

Dogs with malignancies and eRLN were included and underwent to SLN mapping with lymphoscintigraphy and/or near-infrared fluorescence (NIRF). Findings in SLN mapping, distribution of nodal tracer uptake and histological nodal status were recorded.

Twenty-two dogs with eRLN were included. During the lymphographies 2 patterns of nodal tracer distribution were observed: in 9 lymphographies (41%) only the eRLN (single or multiple) was identified (Pattern 1); in 13 (59%), beside the eRLN, at least one non-palpable/normal-sized SLN was identified (Pattern 2). The adjunctive SLNs were detected in the same (25%) or in a different (75%) lymphocentrum of the eRLN. The 54% of the adjunctive non-palpable/normal-sized SLN were metastatic. Among the pattern 2, in 3 lymphographies the eRLN had incomplete or absent nodal distribution of tracer uptake and the tracers were rerouted to one or more non-palpable/normal-sized SLN becoming the neo-SLN.

Neoplastic or inflammatory status of the lymph node may alter the lymphatic drainage in example leading to find non-palpable/normal-sized SLN and various distribution of tracers' uptakes were recorded in eRLN. In presence of eRLN the SLN mapping is strongly suggested rather than limiting lymphadenectomy of only enlarged node, to avoid missing potentially residual microscopic neoplastic nodal disease in additional SLN.

## Introduction

The occurrence of a malignant tumor with an enlarged regional lymph node (eRLN) may be suggestive of clinically suspicious for nodal metastasis and prompts clinicians to enhance preoperative staging to rule out both nodal and distant metastases. If distant metastases are excluded, excision of the eRLN – regardless of its neoplastic or non-neoplastic status – is performed to achieve definitive staging and may improve the effectiveness of adjuvant treatment. (Alvarez-Sanchez et al., 2023; Baginski et al., 2014; Chiti et al., 2021; Collivignarelli et al., 2021; Grimes et al., 2017; Liptak and Boston, 2019; Stefanello et al., 2024; Wan et al., 2021). While the mapping non-palpable and normal-sized sentinel lymph nodes (SLNs) has been extensively investigated in canine surgical oncology, (Annoni et al., 2023; Chiti et al., 2025; Collivignarelli et al., 2021; Ferrari et al., 2020, 2021; Ferraris et al., 2023) no data are currently available on SLN mapping in the presence of regional lymph node enlargement. In human oncology, lymph node enlargement—caused by neoplastic infiltration or inflammatory lymphatic disorders— can alter in the lymphatic flow. These alterations potentially interfere with the SLN mapping: the metastatic spread or inflammatory disease may disrupt the normal lymphatic drainage, blocking the tracers flow (Bassi et al., 2006; Goyal et al., 2005; Lam et al., 2009; Leijte et al., 2009; Liao and Von Der Weid, 2014; Monaco et al., 2012). This can cause incomplete or absent tracer uptake in the

* Corresponding author.
*E-mail address:* elisamaria.gariboldi@unimi.it (E.M. Gariboldi).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.tvjl.2026.106651
Accepted 18 March 2026
Available online 20 March 2026

enlarged node/s and, in some cases, the rerouting of the tracer to a "neo-sentinel lymph node" (neo-SLN) (Goyal et al., 2005; Leijte et al., 2009). A neo-SLN refers to a new alternative lymph node that assumes this role after the original SLN pathway has been disrupted or altered. Understanding and identifying neo-sentinel lymph nodes is important issue in human oncology because metastatic spread may not follow the original pathway (Bassi et al., 2006; Goyal et al., 2005; Leijte et al., 2009; Proulx et al., 2013).

The influence of regional lymph node enlargement, clinically suspicious for metastasis in lymphatic drainage has been investigate in human medicine (Goyal et al., 2005; Leijte et al., 2009; Monaco et al., 2012) and remains unexplored in canine veterinary surgical oncology. In this context, the present study aims to describe the SLN mapping of dogs with solid malignancies and regional lymph node enlargement clinically suspicious for metastasis (with neoplastic or non-neoplastic involvement) on nodal mapping and staging of SLN lymphography in dogs with solid malignancies. In addition, to describe the possible alterations of the nodal tracer distribution in this clinical setting.

## Materials and methods

For this cross-sectional study, data from client-owned dogs bearing malignancies, referred to the Veterinary Teaching Hospital from May 2022 to May 2025 for tumor removal and sentinel lymph nodes excision, were retrospectively collected. All dogs had a cytological or histological diagnosis of a solid malignant tumor. Tumor at first presentation, recurrent tumor, or scars from previous tumor excision, in the absence of any lymphadenectomy, were included if they presented at least a clinically eRLN. The lymph node was defined "enlarged'' if suspicious for neoplastic involvement based on clinical palpation and/or imaging measurement (ultrasound and/or computed tomography). If available, the cytological status before surgery of the enlarged lymph node was recorded. The enlarged lymph node was considered the regional one according to Suami (Suami et al., 2013). In cases of tumors at or close to the intersections of the lymphosomes, all associated regional lymph nodes were evaluated through clinical and imaging examinations.

To be admitted to surgery a preoperative staging had to be performed based on tumor diagnosis and consisted of: dogs with mast cell tumors were staged with thoracic radiographs, abdominal ultrasonography, and cytological evaluation of the spleen and liver, whereas dogs with other tumor types underwent whole-body computed tomography or thoracic radiographs plus abdominal ultrasound. Only dogs without distant metastasis were enrolled. At the time of the surgery, all owners signed a written informed consent for the procedures and data collection. Dogs included in the present study underwent SLN mapping and removal guided by lymphoscintigraphy with Technetium-99 and/or near-infrared fluorescence with indocyanine green (NIRF-ICG). The SLN was defined as the first lymph node/s linked to primary tumor by lymphatic mapping and was thus expected to be first site of tracer uptake from afferent lymphatic vessels.

Lymphography with NIRF and/or lymphoscintigraphy was performed as previously described in literature (Beer et al., 2022; Gariboldi et al., 2025; Manfredi et al., 2021). Briefly, NIRF-lymphography was performed immediately before the surgery with indocyanine green peritumorally injected, and the SLN was intraoperatively identified with a NIRF camera (SPY-PHI QP system, Stryker, MIDA Tecnologia medica S.p.A) (Gariboldi et al. 2025), while lymphoscintigraphy was performed after peritumoral injection of technetium-99m–labeled human serum albumin colloid, and associating a presurgical planar lymphoscintigraphy (Picker Prism 200XP, Picker International, Highland Heights, OH, USA) with an handheld intraoperative gamma probe (Crystal probe SG04; Crystal Photonic GmbH, Berlin, Germany) (Ferrari et al., 2020; Gariboldi et al., 2025; Manfredi et al., 2021).

The lymphocentrum of the enlarged node was always explored, and all enlarged lymph nodes were excised regardless of tracer uptake. Moreover, any additional lymphocentrum detected by tracers were considered sentinel (SLC) and the SLNs were excised. Surgical exploration of the SLC was stopped when no more enlarged nodes were detected by palpation and no additional radioactive or fluorescent nodes were identified. Lymphadenectomy, if feasible, was performed before tumor excision and gloves and instruments were changed after the enlarged nodes manipulation, to avoid possible neoplastic contamination and seeding.

All the lymph nodes (enlarged and non-palpable/normal-sized) extirpated from SLC identified by NIRF and/or lymphoscintigraphy mapping were evaluated ex-vivo to estimate qualitative distribution of radioactivity and fluorescence uptake. The qualitative nodal distribution of tracer uptake was classified as: total uptake (if the tracers were uniformly detectable in the whole lymph node), incomplete uptake (if a heterogeneous tracers' uptake was detected into the lymph node), and no uptake (if not any uptake was detected in the lymph node). In addition, nodal tracer rerouting was recorded (Goyal et al., 2005; Lam et al., 2009; Lützen et al., 2016). Rerouting was defined as the phenomenon in which the tracer is redirected to another lymph node (''neo-sentinel lymph node'') within the same or a different SLC, resulting in incomplete or absent uptake in the enlarged lymph node and uptake in an additional sentinel lymph node (SLN). (Goyal et al., 2005; Lam et al., 2009; Lützen et al., 2016)

Excised tumors and all excised lymph nodes were formalin-fixed for histopathological analysis.

Patient signalment (breed, sex, age, bodyweight); clinical presentation (first presentation, recurrence, scar) of the tumor, anatomical location, size (major axis in mm), presence of ulceration, and histotype (with grading, if applicable) were collected. The following lymph nodes data were recorded: anatomical location of the lymphocentrums of the enlarged lymph node; whether the enlarged lymph node was fixed or not fixed to surrounding normal tissue; the number of the SLCs and the number of the SLNs detected by the tracers. The presence and description of possible rerouting and lymphatic flow alterations were recorded, as well. For each extirpated lymph node (both eRLN and non-palpable/normal-sized SLNs) dimension of the major axis (mm), tracer uptake and nodal histological diagnosis were collected. Histological nodal status was classified as: metastatic from the local tumor (for mast cell tumor – MCT early metastases and overtly metastasis) and non-metastatic (lymph nodes reactive or normal or bearing other lymphoproliferative malignancies). The occurrence of intra-operative complications or post-operative complications related to the lymphadenectomy was also recorded and classified according to LeBlanc et al., 2021.

The retrieved variables were summarized with descriptive statistics reporting the number of patients and the % of the total cases within each category (breed, sex, eRLN identification, lymphographic technique, and lymphatic pattern). Similarly, the number of SLC, the number of SLN, as well as the number of SLN within the enlarged and non-palpable/normal-sized categories were reported. Normality for continuous variables was assessed with Shapiro-Wilk's W test and data were expressed as mean $\pm$ standard deviation for normally distributed variables and median (range) for non-normally distributed variables. Statistical analysis was performed with dedicated software (SPSS 29 for MacOS, SPSS Inc., Headquarters, Chicago, IL, USA) and significance was set at $p \leq 0.05$.

## Results

Among the 210 dogs with solid malignant tumors treated with surgery plus SLN mapping and removal, 22 presented eRLN suggestive of possible metastasis and met the inclusion criteria. Data about patient dogs, tumors, eRLN, lymphatic mapping results, nodal pattern of tracer uptake are summarized in Table 1.

Among the 22 included tumors, 12 (55%) were at first presentation, 6 (27%) were recurrence and 4 (18%) were scars from previous tumor excision. In 11 (50%) cases the tumors/scars were located in head and neck regions, in 4 (18%) in limbs, 3 (14%) in digit, 2 (9%) in trunk, 2

**Table 1**
Comprehensive data about patient dogs, tumors, eRLN, lymphatic mapping results, nodal pattern of tracer uptake.

| Signalment | Histological diagnosis local tumor | Tumor anatomical location | Anatomical location of enlarged lymph node | Histological diagnosis eRLN | eRLN Uptake | Adjunctive non-palpable/ normal sized lymph nodes and histological diagnosis | Pattern |
|---|---|---|---|---|---|---|---|
| Mixed breed, intact male, 15 years, 9,8 kg | Fibrosarcoma grade II | Left lower lip | Left mandibular<br>Left mandibular | Metastatic<br>Metastatic | Total uptake<br>Total uptake | - | 1 A |
| Labrador Retriever, intact male, 5 years, 42 kg | Cutaneous MCT Patnaik Grade 2/ Kiupel Low Grade | Right lumbar region | Right medium iliac | HN3 | Total uptake | - | 1 A |
| Labrador Retriever, intact male, 7 years, 48 kg | SCC | Nasal planum | Left mandibular | Non-metastatic (reactive) | Total uptake | - | 1 A |
| Schnauzer, intact male, 7 years, 16,4 kg | SCC | Third digit of the right forelimb | Right superficial cervical | Non metastatic (reactive) | Total uptake | - | 1 A |
| Whippet, spayed female, 8 years, 3,8 kg | Undifferentiated subcutaneous STS grade III | Third/fourth digit of the left forelimb | Left superficial cervical | Non-metastatic (reactive) | Total uptake | - | 1 A |
| Boxer, intact male, 6 years, 33,8 kg | Cutaneous MCT Patnaik Grade 2 Kiupel High Grade | Left shoulder region | Left superficial cervical | HN1 + small cell lymphoma | Incomplete uptake | - | 1B |
| Mixed breed, neutered male, 14 years, 28,7 kg | Rectal plasmacytoma | Rectal mucosae | Right medium iliac | Metastatic | No uptake | - | 1 C |
| Mixed breed, spayed female, 12 years, 14 kg | OMM | Right caudal maxilla | Right mandibular | Metastatic | No uptake | - | 1 C |
| Cavalier King Charles Spaniel, spayed female, 8 years, 7,3 kg | AGASAC | Right anal sac | Sacral<br>Sacral<br>Right medium iliac | Metastatic<br>Metastatic<br>Metastatic | Total uptake<br>Incomplete uptake<br>No uptake | - | 1 A<br>1B<br>1 C |
| Labrador Retriever, intact male, 7 years, 30,5 kg | Cutaneous MCT Patnaik Grade 2 Kiupel Low Grade | Third and fourth digit of the right forelimb | Right superficial cervical | HN2 | Total uptake | Right superficial cervical (HN1) | 2 A |
| French bouledogue, intact male, 7 years, 13,8 kg | Cutaneous MCT Patnaik Grade 2 Kiupel Low Grade | Left hock | Left popliteal | HN2 | Total uptake | Left inguinal (HN2) | 2 A |
| Mixed breed, spayed female, 13 years, 34 kg | Subcutaneous MCT | Left caudal thigh | Left inguinal<br>Left inguinal | HN3<br>HN3 | Total uptake<br>Total uptake | Left popliteal (HN2) | 2 A |
| Mixed breed, neutered male, 11 years, 22,6 kg | Subcutaneous MCT | Right upper lip | Right mandibular | HN3 | Total uptake | Right mandibular (HN2)<br>Right retropharyngeal (HN2) | 2 A |
| French bouledogue, intact male, 9 years, 16 kg | Cutaneous MCT Patnaik Grade 3/ Kiupel High Grade | Medial chin | Left mandibular | HN3 | Total uptake | Left mandibular (HN2)<br>Left retropharyngeal (HN2) | 2 A |
| French bouledogue, intact male, 11 years, 14,5 kg | Cutaneous MCT Patnaik Grade 2 Kiupel Low Grade | Left cheek | Left retropharyngeal<br>Right retropharyngeal | HN0 + T zone lymphoma<br>HN0 + T zone lymphoma | Total uptake<br>Total uptake | Left mandibular (HN0 + T zone lymphoma) | 2 A |
| Cocker Spaniel, intact male, 14 years, 28,5 kg | OMM | Left upper lip | Left mandibular | Metastatic | Total uptake | Left mandibular (Metastatic)<br>Left mandibular (Metastatic) | 2 A |
| French bouledogue, spayed female, 10 years, 11,4 kg | Cutaneous MCT Patnaik Grade 3 Kiupel High Grade | Left hock | Left popliteal | HN2 | Total uptake | Left inguinal (HN0) | 2 A |
| Jack Russel Terrier, neutered male, 15 years, 8,9 kg | Subcutaneous myxosarcoma Grade II | Right forelimb | Right superficial cervical | Metastatic | Total uptake | Right superficial cervical (Metastatic) | 2 A |
| Golden retriever, spayed female,10 years, 27,6 kg | OMM | Right maxilla | Right mandibular | Non-metastatic (reactive) | Total uptake | Right mandibular (Non-metastatic - reactive)<br>Right mandibular (Non-metastatic -reactive)<br>Right retropharyngeal (Non-metastatic - reactive)<br>Left retropharyngeal (Non-metastatic - reactive) | 2 A |
| French bouledogue, spayed female, 8 years, 9,9 kg | Cutaneous MCT Patnaik Grade 3 Kiupel High Grade | Left lower eyelid | Left mandibular | HN3 | Incomplete uptake | Left retropharyngeal (HN1) | 2B* |
| Mixed breed, neutered male, 12 years, 30,7 kg | Subcutaneous MCT | Right upper lip | Right mandibular<br>Right mandibular<br>Left mandibular | HN0<br>HN0<br>HN1 | Incomplete uptake<br>Total uptake<br>Total uptake | Right retropharyngeal (HN0)<br>Left mandibular (HN2)<br>Left mandibular (HN2) | 2B* |

(*continued on next page*)

**Table 1** (*continued*)

| Signalment | Histological diagnosis local tumor | Tumor anatomical location | Anatomical location of enlarged lymph node | Histological diagnosis eRLN | eRLN Uptake | Adjunctive non-palpable/ normal sized lymph nodes and histological diagnosis | Pattern |
|---|---|---|---|---|---|---|---|
| Mixed breed, spayed female, 13 years, 11 kg | Cutaneous MCT Patnaik Grade 2 Kiupel High Grade | Right upper lip | Right mandibular<br>Right mandibular | HN3<br>HN3 | No uptake<br>No uptake | Right retropharyngeal (HN3)<br>Left mandibular (HN2)<br>Left mandibular (HN0)<br>Left retropharyngeal (HN0) | 2 C* |

Legend: MCT= mast cell tumor; SCC=squamous cell carcinoma; STS= soft tissue sarcoma; OMM= oral malignant melanoma; AGASAC= apocrine gland anal sac adenocarcinoma; HN0: non-metastatic according to Weishaar et al. 2014; HN1: pre-metastatic; HN2: early nodal metastasis; HN3 overtly metastasis. *Rerouting (according to Goyal et al., 2005; Leijte et al., 2009; Lutzen et al., 2016)

(9%) in perineal region. The tumors had a median size of 20 mm (10–100 mm). In 11 macroscopic tumors ulceration was present. The eRLN was assessed by clinical examination and palpation in 19 dogs (86%). In the remaining 3 dogs (14%) the enlarged lymph nodes were endocavitary and were detected by imaging techniques (abdominal ultrasound and/or total-body computed tomography). All eRLN were considered not fixed to surrounding tissue based either on clinical palpation and/or during surgical excision. The eRLN cytological diagnosis was available before surgery in 12 dogs (56%): 7 of them were metastatic (4 cutaneous mast cell tumors, 1 subcutaneous mast cell tumor, 1 rectal plasmacytoma and 1 oral malignant melanoma) while 5 of them were negative for metastasis (2 cutaneous mast cell tumors, 1 subcutaneous myxosarcoma, 1 soft tissue sarcoma, 1 squamous cell carcinoma). Three out of the latter resulted metastatic at histological exam.

For the lymphographies, NIRF alone was used in 13 (59%), lymphoscintigraphy alone in 6 (27%), and both techniques in 3 (14%). The SLC detection was achieved within the first 10–15 min after the tracers' injection regardless of the tracer used. Eleven lymphographies (50%) identified a single SLC, while in the remaining 11 (50%) multiple (range, 2–4) SLCs were detected, for a total of 38 SLCs that were surgically explored.

During exploration, 25 SLCs (66%) contained a single SLN while 13 SLCs (34%) contained multiple SLN (from 2 to 3). A total of 54 lymph nodes were removed: 30 (56%) were enlarged and 24 (44%) were non-palpable/normal sized. The eRLN and SLN tracer uptake is reported in Tables 1–2. Notably, 5 out of 30 eRLNs (17%) showed no tracer uptake; all were metastatic, resulting in false-negative findings (Table2).

Based on the lymphatic mapping results observed, nodal pattern were categorized as follow (Figs. 1–2; Table1):

- Nodal pattern 1: In 9 lymphographies (41%), only the eRLNs (single or multiple) were identified by the tracers, and no adjunctive non-palpable/normal-sized SLNs were detected. In 5 lymphographies, the eRLNs showed total tracer uptake (pattern 1 A); in 1, it showed incomplete uptake (pattern 1B); in 2, no uptake was observed (pattern 1 C); and in 1 case with multiple eRLNs, tracer uptake varied among the nodes (including total, incomplete, and no uptake).
- Nodal pattern 2: In 13 lymphographies (59%), beyond the enlarged lymph nodes (single or multiple), at least one adjunctive non-palpable/normal-sized SLN was detected. In 10 of them, the eRLN had a total tracer uptake (pattern 2 A), while in 3 of them the eRLN tracer uptake was incomplete (pattern 2B) or absent (pattern 2 C) and the tracers were rerouted to a neo-SLN

**Table 2**
Histological nodal status of excised lymph nodes and their tracers' uptake.

| Nodal tracer uptake | Tot | Histological status | |
|---|---|---|---|
| | | Metastatic | Non metastatic |
| **Enlarged lymph nodes removed:** | **30** | **21** | **9** |
| Total uptake | 21 | 14 | 7 |
| Incomplete uptake | 4 | 2 | 2 |
| No uptake | 5 | 5 | - |
| **Adjunctive non-palpable/normal sized** | **24** | **13** | **11** |
| **SLNs removed:** | 18 | 12 | 6 |
| Total uptake | 6 | 1 | 5 |
| Incomplete uptake | | | |

Among the 24 non-palpable/normal-sized extirpated SLNs, 6 SLNs (25%) were in the same SLC as the enlarged one, and 18 (75%) belonged to different adjunctive SLCs, (Table 3). The overall median dimension of extirpated SLNs was 20 (range, 4 – 50) mm. The median size was 25 (range, 15 – 50) mm for the eRLNs, and 15 (range, 4 – 40) mm for non-palpable/normal-sized SLNs.

Out of the 54 extirpated lymph nodes, 34 (63%) were metastatic and 20 (37%) were non-metastatic. Among the latter, 4 were incidentally diagnosed with lymphoma at histology (3 T-zone lymphoma and in 1 small-cell lymphoma).

Considering only the 30 eRLN, 21 (70%) were metastatic, and 9 (30%) were not (4 were reported as reactive, 2 of them were diagnosed with nodal T-zone lymphoma, 1 with small-cell lymphoma, 1 was HN0 and 1 HN1). Of the 24 non-palpable/normal-sized SLNs, 13 (54%) were metastatic, and 11 (46%) were not (1 was diagnosed as T-zone lymphoma). Among the 13 metastatic non-palpable/normal-sized SLNs (10 SLNs metastatic for MCT, 2 for oral malignant melanoma and 1 for STS), 10 (77%) were detected in a different SLC from the one of the eRLN. In the present study population, retrieval of an adjunctive SLN did not change the staging of the dog (compared to the staging obtained by the eRLN). Presence or absence of nodal metastasis of enlarged and non-palpable/normal sized SLNs and nodal tracer uptake are summarized in Table 2.

No intra-operative complications were registered at the sites of lymphadenectomy, while post-operative complications were recorded in 10 dogs (45%), and included: seroma (8 dogs), abscess (1 dog), and transient lymphedema (1 dog). Complications were classified as grade I in 7 cases (6 seroma and 1 transient lymphedema), and as grade II in 3 cases (2 seroma that required anti-inflammatory drugs and one case of wound infection treated with oral antibiotics, as indicated by bacterial culture examination).

## Discussion

The primary finding of the present study is that 59% of the included dogs had a flow alteration and the tracer was directed also to another lymph node (pattern 2). Among them only 3 cases had re-routing according to definition (pattern 2B-2C) (Goyal et al., 2005; Lam et al., 2009; Lützen et al., 2016). In all cases of pattern 2, mapping with ICG and/or Technetium-99 identified at least one non-palpable/normal-sized SLN in addition to the eRLN, regardless of whether the latter was metastatic or non-metastatic. Based on histopathological evaluation, 54% of the additional non-palpable/normal-sized SLNs were metastatic. As previously reported in literature these lymph nodes would not have been removed without mapping (Worley, 2014; Alvarez Sanchez et al., 2023), and this indicates that metastatic spread may not be confined to the solely enlarged lymph nodes. (Goyal et al., 2005; Leijte et al., 2009; Lützen et al., 2016). Even if among the 22 cases included in the study the

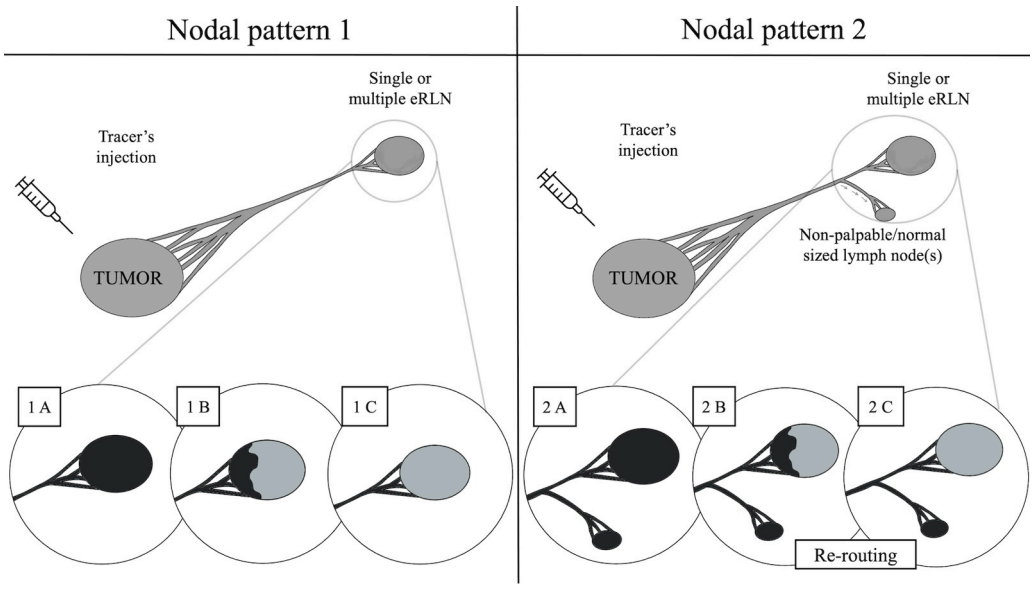


**Fig. 1.** Nodal pattern and distribution of tracer uptake with technetium and/or indocyanine green observed in the study. Pattern 1: the lymphography detected only the eRLN(s) from one or multiple lymphocentrum and no adjunctive non-palpable/normal-sized SLNs. The eRLN (single or multiple) presented different tracer uptake (total and/or incomplete and/or absence of tracer uptake). Pattern 2: beside the eRLN(s) the lymphography detected at least one non-palpable/normal-sized SLN in the same or in different SLC from the enlarged one. The eRLN presented one or different tracer uptake (total and/or incomplete and/or absence of tracer uptake).

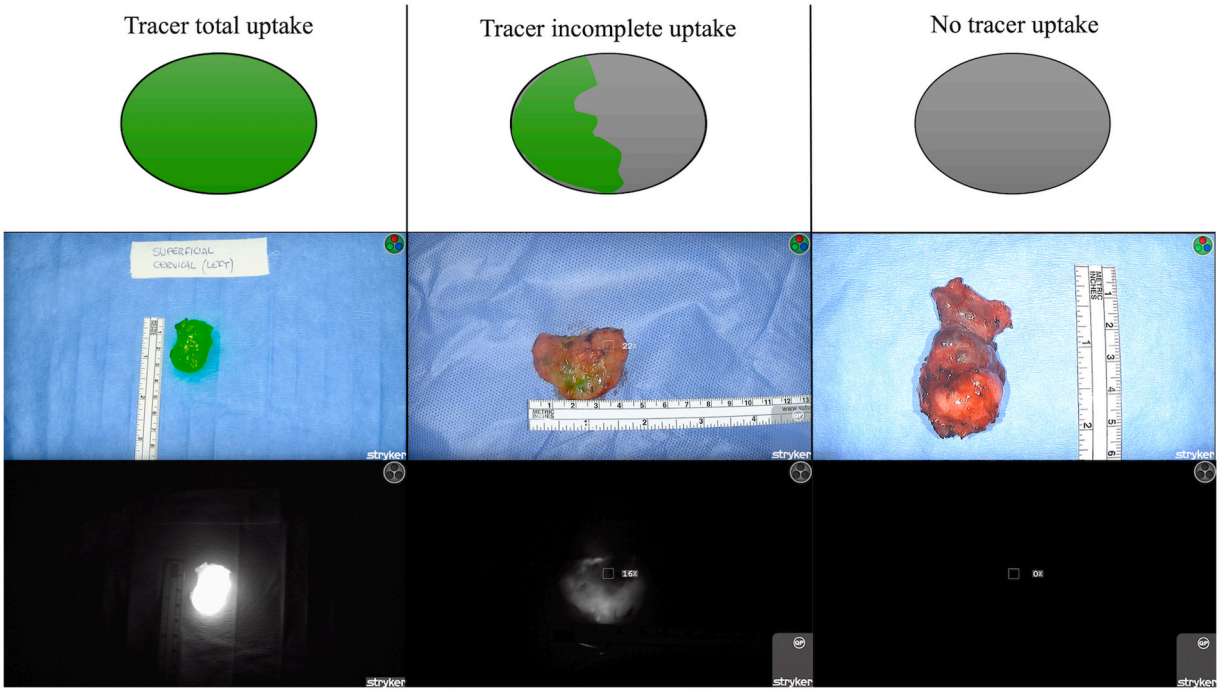


**Fig. 2.** An example of tracer uptake in lymph nodes with NIRF-ICG. Example of 3 enlarged lymph nodes with different uptake patterns for NIRF-ICG (SPY-PHI overlay and monochromatic visualization mode).

oncologic stage did not change, these results highlight the relevance of lymph node mapping in assisting microscopic residual nodal metastatic disease identification in non-palpable/normal-sized SLN in dogs with solid malignancies and eRLN. Moreover, 75% of the adjunctive non-palpable/normal-sized SLNs were retrieved in different SLCs than the one of the eRLNs and most of them were metastatic (77%). This data supports the hypothesis that, in presence of malignancies, the lymphatic drainage may be unpredictable, and Technetium-99 and ICG can spread also to multiple SLCs, leading to the identification of more than one SLN linked with the tumor. The occurrence of multiple lymphatic drainage has drawn attention in veterinary oncology, as a potential risk factor for nodal metastasis and as a possible prognostic indicator. (Ferrari et al., 2021; Stefanello et al., 2024; Alvarez-Sanchez et al., 2023). These findings should be carefully considered even for the 25% of cases in which an additional non-palpable/normal-sized SLN belonged to the same lymphocentrum of the eRLN. While pre-operative mapping might identify one or more SLC, the possibility to surgically identify and follow the same tracer (respectively Technetium-99 and ICG) during SLC exploration enhances the probability of removing multiple SLN with a high detection rate, as previously reported (Chiti et al., 2025; Gariboldi

**Table 3**
The SLC distribution of the 24 adjunctive non-palpable/normal-sized SLNs and their histological status.

| | N°/total (%) | Histological status of the lymph nodes | |
|---|---|---|---|
| | | Metastatic | Non metastatic |
| **TOT** | **24** | **13** | **11** |
| **In the same SLC of the eRLN** | 6 (25%) | 3 (23%) | 3 (30%) |
| **In a different SLC of the eRLN** | 18 (75%) | 10 (77%) | 8 (70%) |

et al., 2023; Stefanello et al., 2024). In addition, intraoperative gamma probe and NIRF camera allowed the measurement of residual radioactivity/fluorescence that led to objective decision making during the dissection of the SLCs, since their absence indicates that there are no more SLN to be removed within the SLC (Chiti et al., 2025; Gariboldi et al., 2023; Stefanello et al., 2024). This approach may help avoid missing metastatic non-palpable/normal-sized SLNs associated with the tumor, whether located in the same or a different SLC from the eRLN, thereby preventing incomplete staging and underestimation of metastatic disease extent. However, it remains unclear whether the additional lymph nodes removed can be considered second-tier nodes. Unlike human oncology, veterinary medicine currently lacks standardized criteria to accurately differentiate first- from second-tier lymph nodes in dogs. We can also hypothesize — although it needs to be confirmed in further studies — that the removal of additional non-palpable or normal-sized SLNs may be associated with a clinical benefit, reducing the tumor burden. In addition, considering that the surgical complications when using a tracer that allows for an intraoperative guidance, such as Technetium-99 or NIRF-ICG, were mild and in line with previous publications (Chiti et al., 2023; Mattioli et al., 2025), the surgeons might be encouraged to search for the additional SLN, also exploring more than one SLC in the same dog.

Regarding the second aim of the study, we were able to describe different nodal patterns and different qualitative tracers' uptakes in the removed lymph nodes. This confirms that the findings of altered nodal uptake reported in humans could also be observed in dogs in the presence of eRLN. Indeed, in several human cancers, like cutaneous melanoma, penile cancer and breast cancer, in the presence of enlarged lymph nodes and possible metastatic invasion of lymphatic vessels, the lymphatic flow may be altered (Goyal et al., 2005; Lam et al., 2009; Leijte et al., 2009; Lützen et al., 2016; Nathanson and Mahan, 2011). Neoplastic invasion in the lymph node with related microscopic or macroscopic alteration or normal architecture may be associated with higher intranodal pressure leading to deviation of lymphatic flow (''rerouting'') (Goyal et al., 2005; Nathanson and Mahan, 2011). Rerouting was not frequently observed in this sample population (3 cases all MCTs out of 22 lymphographies), and this seems to reflect what is reported in human literature (Bassi et al., 2006; Goyal et al., 2005; Leijte et al., 2009; Proulx et al., 2013). In the cases of rerouting, the enlarged lymph node was metastatic, and the tracer drainage was rerouted to another SLN. This was likely due to obstruction of the afferent lymphatic vessels by tumor embolization, mimicking the typical rerouting phenomenon described in the human literature. However, rerouting or altered lymphatic drainage may also occur in non-neoplastic lymph nodes in association with inflammatory flow abnormalities and lymphatic dysfunction (Kandeel et al., 2013; Liao and Von Der Weid, 2014; Monaco et al., 2012).

It should be noted that in 10 cases, even if the eRLN exhibited a full tracer uptake, an additional SLN was identified. This could possibly represent simultaneous lymphatic drainage to multiple SLN (e.g., one enlarged and the other normal sized), although a rerouting scenario cannot be excluded. In such instances, increased intranodal pressure caused by metastatic burden may still allow tracer accumulation in the affected lymph node, while also promoting tracer diversion to a secondary, neo-sentinel lymph node (Nathanson and Mahan, 2011).

Five enlarged lymph nodes lacked uptake and were therefore considered false negatives as they were all metastatic. To explain the absence of tracer uptake, it might be hypothesized, according to the literature, that when the microscopical architecture of the lymph node is fulfilled by the tumor, the tracer may fail to reach the lymph node, leading to false negatives results (Bassi et al., 2006; Goyal et al., 2005; Leijte et al., 2009; Nathanson and Mahan, 2011). In one study of Goyal et al. (2005) involving lymph nodes with breast cancer metastases, it was observed that radioactive tracer uptake decreased with increasing metastatic involvement. This could suggest that the absence of the tracer uptake in an enlarged lymph node may be related to the presence of metastasis. However, based on our results and the few cases included, we cannot establish a reliable correlation between tracer uptake and the presence or absence of metastasis. Further prospective studies are required to integrate data with quantification of tracers' uptake and association with neoplastic invasion of the lymph nodes.

The main limitation of this study is the small sample size, which nonetheless reflects the frequency of enlarged lymph nodes, that in the present study were observed in 22 out of the 210 dogs during daily clinical practice in the last 3 years at our VTH. This is the first study in canine surgical oncology that explores the influence of SLN mapping in the presence of eRLN, and lymph node tracers' uptake alteration. Therefore, further research involving a larger, more homogeneous population is needed to better understand the relationship between histotype, tumor characteristics, nodal pattern and tracer's uptake. Furthermore, collecting data about follow-up might define the prognostic inference of additional sentinel lymph nodes removal in presence of eRLN in different canine malignancies.

In conclusion, based on this sample population, SLN mapping in the presence of eRLN suspicious for neoplastic involvement in dogs with solid malignant tumors may represent a useful adjunct to surgical excision of the enlarged lymph node. Additionally, lymphatic mapping with ICG and Technetium-99 appears to facilitate the identification of additional sentinel lymph nodes and may reduce the risk of overlooking residual microscopic nodal disease.

## Funding statement

No funding was received for this study.

## Data availability statement

All data generated or analyzed during this study are included in this article. Additional datasets are available from the corresponding author upon reasonable request.

## Ethical statement

This retrospective study was conducted at our Veterinary Teaching Hospital of authors' Department during daily clinical practice, where we managed owner's dogs with spontaneous cancers referred for consultation and surgical treatment. We want to declare that the opinion of the Research Ethics Committee was not required before to submit the paper. Dogs underwent standard procedures for curative intent treatments and before clinical, diagnostic or treatment procedures, all owners provided to sign a written consent for all procedures and data collection. The procedure and technologies adopted in this study have been previously described in several published research.

## CRediT authorship contribution statement

**Damiano Stefanello:** Writing – original draft, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Luigi Auletta:** Writing – review & editing, Resources. **Roberta Ferrari:**

Writing – review & editing, Resources. **Davide Danilo Zani:** Writing – review & editing, Resources, Conceptualization. **Donatella De Zani:** Writing – review & editing, Resources. **Federica Alessandra Brioschi:** Writing – review & editing. **Francesco Ferrari:** Writing – review & editing. **Chiara Giudice:** Writing – review & editing. **Paola Roccabianca:** Writing – review & editing. **Camilla Recordati:** Writing – review & editing. **Elisa Maria Gariboldi:** Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Valeria Grieco:** Writing – review & editing. **Alessandra Ubiali:** Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization.

## Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they have nothing to disclose.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

No acknowledgments for this study.

## References

Alvarez-Sanchez, A., Townsend, K.L., Newsom, L., Milovancev, M., Gorman, E., Russell, D.S., 2023. Comparison of indirect computed tomographic lymphography and near-infrared fluorescence sentinel lymph node mapping for integumentary canine mast cell tumors. Veterinary Surgery 52, 416–427. https://doi.org/10.1111/vsu.13929.

Annoni, M., Borgonovo, S., Aralla, M., 2023. Sentinel lymph node mapping in canine mast cell tumours using a preoperative radiographic indirect lymphography: technique description and results in 138 cases. Vet Comp Oncol 21, 469–481. https://doi.org/10.1111/vco.12906.

Baginski, H., Davis, G., Bastian, R.P., 2014. The prognostic value of lymph node metastasis with grade 2 MCTs in dogs: 55 cases (2001-2010). J Am Anim Hosp Assoc 50, 89–95. https://doi.org/10.5326/JAAHA-MS-5997.

Bassi, K.K., Seenu, V., Ballehaninna, U.K., Parshad, R., Chumber, S., Dhar, A., Gupta, S. D., Kumar, R., Srivastava, A., 2006. Second echelon node predicts metastatic involvement of additional axillary nodes following sentinel node biopsy in early breast cancer. Indian J Cancer 43 (3), 103–109. https://doi.org/10.4103/0019-509x.27931.

Beer, P., Rohrer-Bley, C., Nolff, M.C., 2022. Near-infrared fluorescent image-guided lymph node dissection compared with locoregional lymphadenectomies in dogs with mast cell tumours. J Small Anim Pract 63, 670–678. https://doi.org/10.1111/jsap.13529.

Chiti, L.E., Beer, P., Ohlerth, S.M., Hartnack, S., Nolff, M.C., 2025. SHINE – validation of near infrared fluorescence lymphography against lymphoscintigraphy for sentinel lymph node biopsy in dogs with mast cell tumours. Vet Comp Oncol 23 (2), 320–329. https://doi.org/10.1111/vco.13058.

Chiti, L.E., Gariboldi, E.M., Ferrari, R., Luconi, E., Boracchi, P., De Zani, Donatella, Zani, Davide, Manfredi, M., Spediacci, C., Grieco, V., Giudice, C., Recordati, C., Ferrari, F., Stefanello, D., 2023. Surgical complications following sentinel lymph node biopsy guided by γ-probe and methylene blue in 113 tumour-bearing dogs. Vet Comp Oncol 21, 62–72. https://doi.org/10.1111/vco.12861.

Chiti, L.E., Stefanello, D., Manfredi, M., Zani, D.D., De Zani, D., Boracchi, P., Giudice, C., Grieco, V., Di Giancamillo, M., Ferrari, R., 2021. To map or not to map the cN0 neck: Impact of sentinel lymph node biopsy in canine head and neck tumours. Vet Comp Oncol 19, 661–670. https://doi.org/10.1111/vco.12697.

Collivignarelli, F., Tamburro, R., Aste, G., Falerno, I., Signore, F.Del, Simeoni, F., Patsikas, M., Gianfelici, J., Terragni, R., Attorri, V., Carluccio, A., Vignoli, M., 2021. Lymphatic drainage mapping with indirect lymphography for canine mammary tumors. Animals 11 (4), 1115. https://doi.org/10.3390/ani11041115.

Ferrari, R., Boracchi, P., Chiti, L.E., Manfredi, M., Giudice, C., De Zani, D., Spediacci, C., Recordati, C., Grieco, V., Gariboldi, E.M., Stefanello, D., 2021. Assessing the risk of nodal metastases in canine integumentary mast cell tumors: is sentinel lymph node biopsy always necessary? Animals 11 (8), 2373. https://doi.org/10.3390/ani11082373.

Ferrari, R., Chiti, L.E., Manfredi, M., Ravasio, G., De Zani, D., Zani, D.D., Giudice, C., Gambini, M., Stefanello, D., 2020. Biopsy of sentinel lymph nodes after injection of methylene blue and lymphoscintigraphic guidance in 30 dogs with mast cell tumors. Vet Surg 49, 1099–1108. https://doi.org/10.1111/vsu.13483.

Ferraris, E.I., Olimpo, M., Giacobino, D., Manassero, L., Iussich, S., Lardone, E., Camerino, M., Buracco, P., Morello, E.M., 2023. 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. Vet Comp Oncol 21, 208–220. https://doi.org/10.1111/vco.12878.

Gariboldi, E.M., Ubiali, A., Chiti, L.E., Ferrari, R., De Zani, D., Zani, D.D., Grieco, V., Giudice, C., Recordati, C., Stefanello, D., Auletta, L., 2023. Evaluation of surgical aid of methylene blue in addition to intraoperative gamma probe for sentinel lymph node extirpation in 116 canine mast cell tumors (2017–2022). Animals 13 (11), 1854. https://doi.org/10.3390/ani13111854.

Gariboldi, E.M., Ubiali, A., Boracchi, P., Luconi, E., Ferrari, R., Auletta, L., De Zani, D., Zani, D.D., Roccabianca, P., Grieco, V., Giudice, C., Recordati, C., Stefanello, D., 2025. Intrapatient application of lymphoscintigraphy and near-infrared fluorescence in canine and feline sentinel lymph node mapping and removal. BMC Vet Res 21, 590. https://doi.org/10.1186/s12917-025-05013-2.

Goyal, A., Douglas-Jones, A.G., Newcombe, R.G., Mansel, R.E., 2005. Effect of lymphatic tumor burden on sentinel lymph node biopsy in breast cancer. Breast J 11, 188–194. https://doi.org/10.1111/j.1075-122X.2005.21591.x.

Grimes, J.A., Secrest, S.A., Northrup, N.C., Saba, C.F., Schmiedt, C.W., 2017. Indirect computed tomography lymphangiography with aqueous contrast for evaluation of sentinel lymph nodes in dogs with tumors of the head. Vet Radiol and Ultrasound 58, 559–564. https://doi.org/10.1111/vru.12514.

Kandeel, A.A.S., Younes, J., Zaher, A.M., 2013. Significance of popliteal lymph nodes visualization during radionuclide lymphoscintigraphy for lower limb lymphedema. Indian J Nucl Med 28, 134–137. https://doi.org/10.4103/0972-3919.119540.

Lam, T.K., Uren, R.F., Scolyer, R.A., Quinn, M.J., Shannon, K.F., Thompson, J.F., 2009. False-negative sentinel node biopsy because of obstruction of lymphatics by metastatic melanoma: the value of ultrasound in conjunction with preoperative lymphoscintigraphy. Melanoma Res 19, 94–99. https://doi.org/10.1097/CMR.0b013e32832166b7.

LeBlanc, A.K., Atherton, M., Bentley, R.T., Boudreau, C.E., Burton, J.H., Curran, K.M., Dow, S., Giuffrida, M.A., Kellihan, H.B., Mason, N.J., Oblak, M., Selmic, L.E., Selting, K.A., Singh, A., Tjostheim, S., Vail, D.M., Weishaar, K.M., Berger, E.P., Rossmeisl, J.H., Mazcko, C., 2021. Veterinary cooperative oncology group—common terminology criteria for adverse events (VCOG-CTCAE v2) following investigational therapy in dogs and cats. Vet Comp Oncol 19, 311–352. https://doi.org/10.1111/vco.12677.

Leijte, J.A.P., Van Derploeg, I.M.C., Valdés Olmos, R.A., Meweg, O.E., Horenblas, S., 2009. Visualization of tumor blockage and rerouting of lymphatic drainage in penile cancer patients by use of SPECT/CT. J Nucl Med 50, 364–367. https://doi.org/10.2967/jnumed.108.059733.

Liao, S., Von Der Weid, P.Y., 2014. Inflammation-induced lymphangiogenesis and lymphatic dysfunction. Angiogenesis. https://doi.org/10.1007/s10456-014-9416-7.

Liptak, J.M., Boston, S.E., 2019. Nonselective lymph node dissection and sentinel lymph node mapping and biopsy. Vet Clin North Am Small Anim Pract. https://doi.org/10.1016/j.cvsm.2019.04.003.

Lützen, U., Zuhayra, M., Marx, M., Zhao, Y., Colberg, C., Knüpfer, S., Baumann, R., Kähler, K.C., Jünemann, K.P., Naumann, C.M., 2016. Value and efficiency of sentinel lymph node diagnostics in patients with penile carcinoma with palpable inguinal lymph nodes as a new multimodal, minimally invasive approach. Eur J Nucl Med Mol Imaging 43, 2313–2323. https://doi.org/10.1007/s00259-016-3482-6.

Manfredi, M., De Zani, D., Chiti, L.E., Ferrari, R., Stefanello, D., Giudice, C., Pettinato, V., Longo, M., Di Giancamillo, M., Zani, D.D., 2021. Preoperative planar lymphoscintigraphy allows for sentinel lymph node detection in 51 dogs improving staging accuracy: Feasibility and pitfalls. Vet Radiol and Ultrasound 62, 602–609. https://doi.org/10.1111/vru.12995.

Mattioli, G., Cino, M., Stefanello, D., Drudi, D., Morello, E.M., Pisani, G., Chiti, L.E., Pierini, A., Gariboldi, E.M., De Zani, D., Massari, F., Giacobino, D., Martano, M., 2025. Peripheral sentinel lymphadenectomy in 163 dogs: postoperative surgical complications and comparison between intraoperative dissection techniques. Vet Surg 54, 766–776. https://doi.org/10.1111/vsu.14246.

Monaco, S.E., Khalbuss, W.E., Pantanowitz, L., 2012. Benign non-infectious causes of lymphadenopathy: a review of cytomorphology and differential diagnosis. Diagn Cytopathol 40 (10), 925–938. https://doi.org/10.1002/dc.21767.

Nathanson, S.D., Mahan, M., 2011. Sentinel lymph node pressure in breast cancer. Ann Surg Oncol 18, 3791–3796. https://doi.org/10.1245/s10434-011-1796-y.

Proulx, S.T., Luciani, P., Christiansen, A., Karaman, S., Blum, K.S., Rinderknecht, M., Leroux, J.C., Detmar, M., 2013. Use of a PEG-conjugated bright near-infrared dye for functional imaging of rerouting of tumor lymphatic drainage after sentinel lymph node metastasis. Biomaterials 34, 5128–5137. https://doi.org/10.1016/j.biomaterials.2013.03.034.

Stefanello, D., Gariboldi, E.M., Boracchi, P., Ferrari, R., Ubiali, A., De Zani, D., Zani, D.D., Grieco, V., Giudice, C., Recordati, C., Caniatti, M., Auletta, L., Chiti, L.E., 2024. Weishaar's classification system for nodal metastasis in sentinel lymph nodes: clinical outcome in 94 dogs with mast cell tumor. J Vet Intern Med 38, 1675–1685. https://doi.org/10.1111/jvim.16997.

Suami, H., Yamashita, S., Soto-Miranda, M.A., Chang, D.W., 2013. Lymphatic territories (Lymphosomes) in a Canine: an animal model for investigation of postoperative lymphatic alterations. PLoS One 8 (7), e69222. https://doi.org/10.1371/journal.pone.0069222.

Wan, J., Oblak, M.L., Ram, A., Singh, A., Nykamp, S., 2021. Determining agreement between preoperative computed tomography lymphography and indocyanine green near infrared fluorescence intraoperative imaging for sentinel lymph node mapping

in dogs with oral tumours. Vet Comp Oncol 19, 295–303. https://doi.org/10.1111/vco.12675.
Weishaar, K.M., Thamm, D.H., Worley, D.R., Kamstock, D.A., 2014. Correlation of nodal mast cells with clinical outcome in dogs with mast cell tumour and a proposed classification system for the evaluation of node metastasis. J Comp Pathol 151, 329–338. https://doi.org/10.1016/j.jcpa.2014.07.004.
Worley, D.R., 2014. Incorporation of sentinel lymph node mapping in dogs with mast cell tumours: 20 consecutive procedures. Vet Comp Oncol 12, 215–226. https://doi.org/10.1111/j.1476-5829.2012.00354.x.